

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/318514528>

Acid contents and the effect of fermentation condition of Kombucha tea beverages on physicochemical, microbiological and sensory properties

Article in *CyTA - Journal of Food* · July 2017

DOI: 10.1080/19476337.2017.1321588

CITATIONS

4

READS

590

4 authors, including:



Katarzyna Neffe-Skocińska

Warsaw University of Life Sciences - SGGW

12 PUBLICATIONS 35 CITATIONS

[SEE PROFILE](#)



Iwona Scibisz

Warsaw University of Life Sciences - SGGW

34 PUBLICATIONS 305 CITATIONS

[SEE PROFILE](#)



Danuta Kołożyn-Krajewska

Warsaw University of Life Sciences - SGGW

82 PUBLICATIONS 372 CITATIONS

[SEE PROFILE](#)

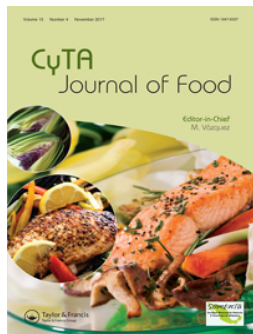
Some of the authors of this publication are also working on these related projects:



fermented plant beverages [View project](#)



Projekt Nr/IS-1/031/NCBR/2014 of the programme Social Innovations, entitled "A Model of Reducing Food Losses and Wastage to the Benefit of the Society" (also known under the acronym MOST [literary: BRIDGE]). [View project](#)



Acid contents and the effect of fermentation condition of Kombucha tea beverages on physicochemical, microbiological and sensory properties

Katarzyna Neffe-Skocińska, Barbara Sionek, Iwona Ścibisz & Danuta Kołożyn-Krajewska

To cite this article: Katarzyna Neffe-Skocińska, Barbara Sionek, Iwona Ścibisz & Danuta Kołożyn-Krajewska (2017) Acid contents and the effect of fermentation condition of Kombucha tea beverages on physicochemical, microbiological and sensory properties, *CyTA - Journal of Food*, 15:4, 601-607, DOI: [10.1080/19476337.2017.1321588](https://doi.org/10.1080/19476337.2017.1321588)

To link to this article: <http://dx.doi.org/10.1080/19476337.2017.1321588>



© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 18 Jul 2017.



Submit your article to this journal [↗](#)



Article views: 230



View related articles [↗](#)



View Crossmark data [↗](#)

Acid contents and the effect of fermentation condition of Kombucha tea beverages on physicochemical, microbiological and sensory properties

Katarzyna Neffe-Skocińska^a, Barbara Sionek^a, Iwona Ścibisz^b and Danuta Kołożyn-Krajewska^a

^aDepartment of Food Gastronomy and Food Hygiene, Warsaw University of Life Sciences, Warszawa, Poland; ^bDepartment of Food Technology, Warsaw University of Life Sciences, Warszawa, Poland

ABSTRACT

Kombucha is a healthy beverage which is a final result of tea fermentation by adding a starter culture of the acetic acid bacteria and yeasts. The effect of fermentation conditions on physicochemical, microbiological and sensory properties of Kombucha tea beverages was evaluated with emphasis placed on determining sugars and organic acids content, including pro-health glucuronic acid. Fermentation process was conducted for 10 days at 20°C, 25°C and 30°C. The optimal conditions for the fermentation of Kombucha tea beverages were a temperature of 25°C and a period of 10 days which allowed to retrieve a product with good physicochemical, microbiological and sensory quality. The content of glucuronic acid increased during fermentation at all temperatures reaching the highest, on the 10th day of fermentation at 25°C. It was observed that all beverages were a good overall quality, whereas Kombucha fermented at 25°C was assessed as the highest.

Contenido de ácido y efectos de las condiciones de fermentación en las propiedades fisicoquímicas, microbiológicas y sensoriales de bebidas de té de Kombucha

RESUMEN

La fermentación de té, mediante la incorporación de cultivo iniciador de bacterias ácidoacéticas y levaduras, produce la saludable bebida llamada Kombucha. Para efectos de este estudio, la valoración del efecto que las condiciones de fermentación tienen en las propiedades fisicoquímicas, microbiológicas y sensoriales de bebidas de té de Kombucha se centró en la determinación de azúcares y de la concentración de ácidos orgánicos, incluyendo el beneficioso ácido glucurónico. El proceso de fermentación se extendió 10 días a temperaturas de 20°C, 25°C y 30°C, constatándose que una temperatura de 25°C y un tiempo de 10 días constituyen condiciones óptimas para fermentar bebidas de té de Kombucha, ya que el producto resultante presentó buena calidad fisicoquímica, microbiológica y sensorial. A todas las temperaturas probadas, se comprobó que durante el proceso de fermentación aumentó la concentración de ácido glucurónico, alcanzando el contenido más elevado en el décimo día de fermentación a una temperatura de 25°C. Aunque se verificó que todas las bebidas presentaban buena calidad general, el té de Kombucha fermentado a 25°C mostró la valoración más alta.

ARTICLE HISTORY

Received 13 January 2017
Accepted 6 April 2017

KEYWORDS

Kombucha tea beverage; fermentation conditions; glucuronic acid; sensory quality

PALABRAS CLAVE

Bebida de té de Kombucha; condiciones de fermentación; ácido glucurónico; calidad sensorial

1. Introduction

As a starter culture, Kombucha is a symbiotic system of viable microorganisms consisting mainly of symbiotic association of acetic acid bacteria (AAB) and yeasts. Kombucha is also a name of a healthy beverage which is a final result of tea fermentation by adding a starter culture of the aforementioned microorganisms (Ismail, Bassyouni, Kamel, & Gabr, 2015; Jayabalan, Malbaša, Lončar, Vitas, & Sathishkumar, 2014). Kombucha beverage contains products of microbiological reactions that play a vital role in biochemical reactions in the organism. There are many reports that Kombucha may help prevent chronic diseases and has beneficial properties for human health such as antihyperglycemic, antimicrobial, antioxidant, anti-carcinogenic and antihyperlipidemic; however, most of the benefits were stated in models (Chakravorty et al., 2016; Dufresne & Farnworth, 2000; Mo, Zhu, & Chen, 2008). Kombucha consists of the following favourable components: organic acids, vitamins, polyphenols,

amino acids, antibiotics and microelements (Jayabalan et al., 2014; Marsh, O'Sullivan, Hill, Ross, & Cotter, 2014). Some scientific publications confirm induction of synthesis of vitamin C, B vitamins and folic acid during the fermentation process of Kombucha (Jayabalan et al., 2014; Malbaša, Lončar, & Kolarov, 2002). The most important organic acids produced during fermentation are glucuronic, gluconic, lactic, malic, citric, tartaric, folic, malonic, oxalic, succinic, pyruvic and usnic acids. Glucuronic acid deserves special attention. It is the result of a microbiological process of glucose oxidation and it is one of the most valuable, healthy Kombucha components. Being produced by the liver in a human body, it exhibits detoxifying effects. This acid has an ability to bind with xenobiotics, including also phenols present in the liver, allowing these substances to be excreted by kidneys more efficiently. Glucuronic acid is also a precursor in the biosynthesis of vitamin C (Jayabalan et al., 2014; Nguyen, Nguyen, Nguyen, & Le, 2015). Furthermore, in the human

body, this acid is converted into glucosamine, which is a chemical compound used in prophylaxis of osteoarthritis (Nguyen et al., 2015; Yavari, Assadi, Moghadam, & Larijani, 2011).

Numerous significant biochemical reactions take place during aerobic fermentation, which occurs during the production of Kombucha beverages. A proper course and selection of parameters for Kombucha fermentation process determines the quality of the obtained beverage, including the content of healthy ingredients (Jayabalan, Marimuthu, & Swaminathan, 2007; Marsh et al., 2014). Mechanism of the production process of Kombucha drinks combines alcoholic fermentation conducted by yeasts and acidification resulting from AAB metabolism. Study results presented in literature often vary. A proper choice of fermentation parameters allows to obtain a product of reproducible high quality remaining at a stable level that is also rich in healthy components (Battikh, Bakhrouf, & Ammar, 2012). Therefore, the aim of the presented study was to select and optimise conditions of the fermentation process and their influence on microbiological, sensory and physicochemical changes with emphasis placed on determining sugar, alcohol and organic acid content, including healthy glucuronic acid in designed Kombucha beverages.

2. Materials and methods

2.1. Kombucha starter culture

Kombucha starter culture otherwise known as 'tea fungus' were obtained from Dr Palisa collection (Topsco company, Czech Republic). The starter culture used in the study was stored in refrigerator ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and consisted of a liquid component (sour broth) and cellulosic layer ('tea fungus' floating on the liquid surface).

2.2. Kombucha beverage preparing and sampling procedures

Study material consisted of three tea beverages fermented in laboratory conditions with Kombucha starter cultures. The following ingredients were used to make the beverage: sterile distilled water, Kombucha starter culture, green tea leaves (LOYD, London UK), black tea leaves (LIPTON, London UK) and beet sugar, sucrose.

Each beverage was prepared from a total quantity of tea addition 6 g L^{-1} (a mixture of 2 g L^{-1} of green tea and 4 g L^{-1} of black tea) and 100 g L^{-1} of sucrose. The tea infusions were placed in three sterile beakers, and 1 L of sterile, distilled water at a temperature of $70\text{--}80^{\circ}\text{C}$ is poured into the beakers. The tea was brewed for 15 min and then poured into sterile glass containers in order to remove tea leaves. Sucrose was added to each tea infusion and then it was stirred until it solved and reached a temperature of 22°C . Afterwards, sugared tea infusions were inoculated with Kombucha starter culture in the amount of 60 ml of sour broth and 50 g of cellulosic layer per 1 L of the infusion. Containers were secured with a sterile gauze to ensure aerobic conditions for the fermentation. The fermentation process was conducted for 10 days in dark incubators (Memmert GmbH & Co. KG, Germany; with cooling system) set for three different temperatures (30°C , 25°C , 20°C).

Microbiological and physicochemical analyses and pH measurements were conducted directly after the addition of a starter culture to tea infusions (day 0) and on the 3rd, 7th and 10th day of the fermentation process. The sensory analysis was carried out on the last, 10th day of the fermentation.

2.3. Physicochemical analyses

2.3.1. Determination of the content of organic acids

The content of organic acids in the fermented Kombucha samples was determined according to a modified method of Jayabalan et al. (2007). A 10 ml sample of fermented Kombucha was centrifuged at 10,000 rpm (RCF approx. $14,000 \times g$) within 10 min at 8°C . Next, a volumetric flask of 10 ml was filled with 1–2 ml of supernatant and filled with redistilled water. Prior to chromatic determination, the obtained extracts were filtered with nylon syringe filters with pore size of $0.45\text{ }\mu\text{m}$. Assays were performed with the use of a liquid chromatograph produced by Shimadzu with a diode detector SPD-M20A, cooperating with the LC10-AtVp pump, SIL 20AHT autosampler and CTO-10ASVp oven. Separation was performed using Luna C-18(2) $5\text{ }\mu\text{m}$ column (Phenomenex, $250\text{ mm} \times 4.6\text{ mm}$) at 30°C . A solution of 20 mmol potassium dihydrogen phosphate (pH 2.4) and methanol (970:30) was used as eluent, using a flow of 1 ml min^{-1} . A wavelength of 210 nm was used to identify all organic acids. The sample dosage volume was 20 μL . Quantitative analysis of certain organic acids (citric, malic, glucuronic, acetic, quinic, lactic, oxalic) was performed using the calibration curve method. The average of at least three parallel assays was assumed as the result.

2.3.2. Determination of the content of sugars

Determination of the content of sugars in the fermented Kombucha samples was performed using the Chen and Liu's (2000) and Agblevor, Murden, and Hames' (2004) method with own modification. A 10 ml sample of fermented Kombucha was centrifuged at 10,000 rpm for 10 min at 8°C . Next, a volumetric flask of 50 ml was filled with 2 ml of supernatant and afterwards with 2 ml 2% $\text{Ca}(\text{OH})_2$ in order to neutralise the environment. The flask was filled with distilled water to the mark. Prior to chromatic determination, the resulting solutions were filtered using a syringe polyamide (PA) filters with pore size of $0.45\text{ }\mu\text{m}$.

The analysis was performed with a high-performance liquid chromatography (HPLC) system manufactured by Shimadzu equipped with a refractometer and Carbohydrate Analysis $10\text{ }\mu\text{m}$ column of $300\text{ mm} \times 3.9\text{ mm}$ produced by Waters. Separation of sugars was conducted in isocratic system at 25°C and with a flow of 1.5 ml min^{-1} . A mixture of acetonitrile and water (800: 200, v/v) was used as an eluent. The sample dosage volume was 20 μL . A quantitative analysis of glucose, fructose and sucrose was performed on the basis of calibration curves for standard solutions via a computer programme LC solution for data collection.

2.3.3. Determination of the content of alcohol

Determination of ethyl alcohol content was performed using an HPLC method according to Rodrigues, Rocha, De Macedo, and Gonçalves (2011) with own modification. A volumetric flask of 10 ml was filled with 0.5–2 ml of the analysed sample and supplemented with redistilled water. Prior to chromatic

determination, the extracts were filtered with syringe PA filters with pore size of 0.45 μm . The analysis was performed with an HPLC system manufactured by Shimadzu equipped with a refractometer and Aminex HPX-87H 25 μm column of 300 mm \times 7.8 mm (Bio-Rad Laboratories, Hercules, CA, USA). A solution of 5 mmol L^{-1} H_2SO_4 dosed with a flow speed of 0.55 ml min^{-1} was used as the eluent. The analysis was performed using the isocratic method at 63°C. Ethyl alcohol was identified on the basis of retention time compared to the standard.

2.3.4. pH measurement

Acidity of the tested product was determined with the use of a calibrated pH metre (ELMETRON CP551, Poland). Three replicate measurements were performed.

2.4. Microbiological analyses

A total of AAB was measured along with a total number of lactic acid bacteria (LAB) and yeast counts. Analyses were performed in the cellulose layer and in the liquid component of the product. In case of the cellulose pellicle, 5 g was homogenised from 45 ml of sterile peptone water (BioMaxima, Poland) and then a decimal dilution series was carried out. In case of the liquid part, 1 ml of the beverage was collected and poured into 9 ml of peptone water after which a decimal dilution series was performed. For determination of AAB and yeasts, the surfacing method was used. In order to determine AAB, two culture media were used: Sabouraud Dextrose Agar (BioMaxima) and Acetobacter Agar with calcium carbonate (HiMedia Laboratories, Mumbai, Maharashtra, India). The differentiating factor of bacteria and yeast colonies was temperature (30°C) and time of incubation (48 h). The growth of AAB on the both media was comparable. Yeast count was determined with the use of a selective medium manufactured by Sabouraud Chloramfenicol Yeast Glucose Agar (BioMaxima). The incubation lasted 120 h and was performed at 25°C. LAB was determined with deep inoculation method using a selective

culture medium manufactured by MRS Agar (de Man, Rogosa and Sharpe Agar; BioMaxima).

The number of acetic bacteria and yeast remained at the same logarithmic level in both the liquid and the cellulose pellicle. Therefore, the obtained results of AAB and yeast number (from cellulose layer and liquid component of the Kombucha beverages) were summed, averaged and presented in $\log \text{CFU ml}^{-1}$ in Table 1.

2.5. Sensory analyses

Sensory analysis was performed with the Quantitative-Descriptive Analysis method (ISO 13299.2:1998) on the last 10th day of the fermentation. Sensory discriminants were selected and defined in a panel discussion by a team of 16 evaluators. They selected six smell discriminants (tea, lemon, acetic, yeast, sour, other), eight taste discriminants (tea, lemon, acetic, yeast, sour, bitter, storage – stale, other) and discriminants such as colour tone and clarity. Afterwards, general quality of the beverage was determined. The assessments were plotted on unstructured graphic scales (0–10 c. u.). The assessment was performed in triplicates.

3. Statistical analysis

Statistical analysis of the obtained results was conducted using Microsoft Excel and Statistica 10 programmes. The two-way analysis of variance was performed, taking into account the influence of temperature and fermentation time.

4. Results and discussion

Measurement of pH is the factor which controls the proper course of fermentation and is used to determine the end of the process (Malbaša, Lončar, & Djurić, 2008). Furthermore, concentration of hydrogen ions is a factor that can activate or inhibit development of each microorganism species in food. AAB tolerates active acidity of environment in the range of 3.6–6.3. The pH of not less than 5.4 facilitates

Table 1. Changes in total count of AAB, yeast, LAB and pH values in Kombucha beverages during 10 days of fermentation at 20°C, 25°C and 30°C.

Tabla 1. Cambios en el conteo total de AAB, levadura, LAB y valores de pH en bebidas de Kombucha durante 10 días de fermentación a temperaturas de 20°C, 25°C y 30°C.

Microbial species (CFU ml^{-1})/pH value	Temperature of fermentation	Days of fermentation			
		0	3	7	10
AAB	20°C	3.57 \pm 0.2 ^{aA}	4.60 \pm 0.3 ^{aB}	6.72 \pm 0.1 ^{aC}	7.39 \pm 0.1 ^{bD}
	25°C	3.93 \pm 0.1 ^{aA}	4.90 \pm 0.2 ^{aB}	6.90 \pm 0.2 ^{aC}	7.61 \pm 0.2 ^{bD}
	30°C	3.65 \pm 0.2 ^{aA}	5.15 \pm 0.1 ^{aB}	7.10 \pm 0.2 ^{aC}	6.77 \pm 0.2 ^{aC}
Yeast	20°C	4.02 \pm 0.1 ^{aA}	5.86 \pm 0.2 ^{bB}	7.00 \pm 0.1 ^{bC}	7.83 \pm 0.2 ^{cD}
	25°C	4.24 \pm 0.1 ^{aA}	7.00 \pm 0.2 ^{cB}	7.22 \pm 0.1 ^{bB}	7.43 \pm 0.1 ^{bB}
	30°C	4.01 \pm 0.2 ^{aA}	4.73 \pm 0.1 ^{aB}	5.48 \pm 0.1 ^{aC}	6.40 \pm 0.2 ^{aD}
LAB	20°C	ND	ND	ND	ND
	25°C	ND	ND	ND	ND
	30°C	ND	ND	ND	ND
pH	20°C	3.08 \pm 0.1 ^{aA}	2.85 \pm 0.1 ^{aA}	2.88 \pm 0.1 ^{aA}	2.67 \pm 0.1 ^{aA}
	25°C	3.07 \pm 0.1 ^{aB}	2.80 \pm 0.1 ^{aA}	2.79 \pm 0.1 ^{aA}	2.77 \pm 0.2 ^{aA}
	30°C	3.04 \pm 0.1 ^{aB}	2.81 \pm 0.1 ^{aA}	2.71 \pm 0.1 ^{aA}	2.63 \pm 0.2 ^{aA}

Data are expressed as mean \pm SD of $n = 3$ samples.

Means in the same column followed by different lowercase letters represent significant differences ($P < 0.05$).

Means in the same row followed by different uppercase letters represent significant differences ($P < 0.05$).

ND: not detected.

Notas: Los datos se expresan como la media \pm DE de $n = 3$ muestras.

Las medias en la misma columna seguidas de distintas letras minúsculas representan diferencias significativas ($P < 0.05$).

Las medias en la misma fila seguidas de distintas letras mayúsculas representan diferencias significativas ($P < 0.05$).

ND: no detectado.

their development. An optimal pH for yeasts depends on species and strain, but on average it falls into a range of 4.5–6.5 (Antolak & Kręgiel, 2015). During the analysed fermentation process, pH in all Kombucha beverages decreased from 3 to 2.60 units on average (Table 1). Low pH can contribute to a decrease of general sensory quality of the beverage to an unacceptable level (Chu & Chen, 2006). However, the general quality on the last study day of the fermentation was high (Figure 1).

The initial pH after inoculation was about 3 and was lower than reported by Kallel, Desseaux, Hamdi, Stocker, and Ajandouz (2012). In the study of sweetened tea, they observed the decrease of pH from 5.5 to 3.8 immediately after inoculation of fermentation broth. In the study performed by Cretović et al. (2008), the pH value of the sweetened tea immediately after inoculation was 4.7. After the third day of fermentation, the concentration of acetic and citric acid starts to increase. Despite the constant rise of organic acids during the process of fermentation, independently of applied different temperatures, no significant changes of pH value was observed. It may be attributed to some buffering effects (capacity) of the fermentation broth (Cretović et al., 2008).

The AAB data are average of results from both media and it was similar. As compared to the remaining two analysed products, the number of AAB was one logarithmic cycle higher on the third and seventh test day (Table 1).

Total yeast count in the prepared beverages amounted on average to 4 log CFU ml⁻¹ prior to fermentation. The fastest increase of yeasts was found in the beverages fermented for 10 days at 25°C. Yeast count reached 7 CFU ml⁻¹ on the third day of the process and it remained stable until its end. In the cases of fermentation in 25°C and 20°C, the logarithmic phase of yeast growth lasted until the seventh day of the fermentation and it remained stable (7 log CFU ml⁻¹ on average) until the end of the process. The lowest yeast count was found in the beverages fermented at 30°C. This number increased throughout the fermentation process reaching the value of 6.40 log CFU ml⁻¹ on the 10th day (Table 1). On the basis of the studies conducted, it can be concluded that the fermentation in case of this product could last longer than the assumed 10 days. A similar

situation was observed in case of the fermentation at 20°C, because an optimal fermentation temperature for Kombucha beverage is 22–28°C (Blanc, 1996). On the other hand, optimal conditions for the growth and development of AAB, yeasts and LAB were not formed at a temperature of 30°C and during a period of 10 days. According to scientific reports, such process can be conducted in a temperature range of 24–28°C and can last for 7, 10, 14 and even 21 days (Battikh et al., 2012; Chakravorty et al., 2016; Teoh, Heard, & Cox, 2004). In their studies, Chen and Liu (2000) fermented Kombucha at 28°C for 60 days. The process parameters – 30°C and 10 days – were selected for the presented study due to easier possibility of LAB growth. Based on own results, LAB was not determined in any of the assumed fermentation temperatures. However, according to Jayabalan et al. (2007), Hrnjez et al. (2014) and Chakravorty et al. (2016), it is possible in such products. These results are compatible with studies conducted by Sievers, Lanini, Weber, and Schuler-Schmid Teuber (1995), in which all bacteria isolated from “tea fungus” were classified to *Acetobacter*. Bacteria of i.a. *Bifidobacterium*, *Enterobacter*, *Lactobacillus* species (Chakravorty et al., 2016) were determined in Kombucha tea in other studies.

During the fermentation process of sugared tea infusion, disaccharides undergo decomposition into monosaccharides under the influence of enzymes and acids, i.e. simple sugars. Afterwards, the role of yeasts in the fermentation process is to convert sugar into alcohol and carbon dioxide. Concurrently, AAB lives on the cellulose part of the Kombucha starter culture. They play a vital role in the fermentation process, because they are responsible for creating new cellulose layers and, moreover, they metabolise alcohol produced by yeasts into organic acids. Apart from organic acids, the basic metabolites in the fermentation process of Kombucha are simple sugars, carbon dioxide and ethanol (Chakravorty et al., 2016). According to Chen and Liu (2000), Lončar, Petrović, Malbaša, and Verac (2000), Malbaša et al. (2002) and Jayabalan et al. (2007), acetic, glucuronic and gluconic acids are the main healthy products of Kombucha drink which is produced from sucrose and black tea in a controlled and optimised fermentation process. According to Nguyen et al. (2015), a right choice of fermentation conditions and selection of Kombucha starter culture enables production of beverages with the highest content of healthy organic acids, including glucuronic acid. Apart from acetic acid, it is one of the main organic acids resulting from fermentation (Jayabalan et al., 2007). A strong interest in this acid in recent years is associated with its valuable properties, including i.a. its role as a precursor to biosynthesis of vitamin C, capability of converting itself into glucosamine and eliminating toxins from the body. The amount of produced glucuronic acid depends on a particular AAB strain and yeasts, that is why selection of right microorganisms and fermentation conditions of Kombucha tea is so important (Malbaša, Lončar, Vitas, & Čanadanović-Brunet, 2011; Nguyen et al., 2015). Moreover, production of glucuronic acid during fermentation can be ineffective as a result of activity of undesirable types of yeasts and moulds, like *Penicillium* sp., *Candida* sp. (Jayabalan et al., 2014; Nguyen et al., 2015).

The content of analysed organic acids during 10 days of Kombucha fermentation in three temperature ranges was variable in time. The main organic acid in the beverage was acetic acid (Table 2). The concentration of acetic acid

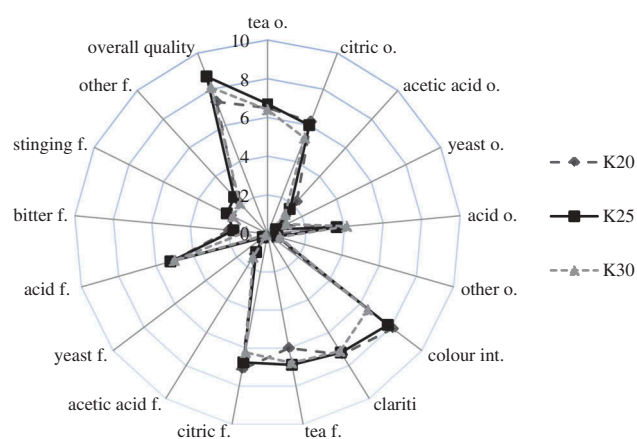


Figure 1. Sensory profiles of the Kombucha beverages after 10 days of fermentation process at 20°C, 25°C and 30°C. o – odor; int.: intensity; f.: flavour.

Figura 1. Perfiles sensoriales de bebidas de Kombucha después de 10 días a 20°C, 25°C y 30°C. o – olor; int. – intensidad; f.: flavor.

Table 2. Changes in the organic acid content during 10 days of Kombucha tea fermentation at 20°C, 25°C and 30°C.**Tabla 2.** Cambios en la concentración de ácido orgánico durante 10 días de fermentación de té de Kombucha a temperaturas de 20°C, 25°C y 30°C.

Kind of organic acids (g·L ⁻¹)	Temperature of fermentation	Days of fermentation			
		0	3	7	10
Quinic acid	20°C	0.44 ± 0.03 ^{aA}	0.51 ± 0.01 ^{aA}	0.46 ± 0.02 ^{aA}	0.47 ± 0.01 ^{aA}
	25°C	0.43 ± 0.04 ^{aA}	0.48 ± 0.01 ^{aA}	0.46 ± 0.02 ^{aA}	0.47 ± 0.04 ^{aA}
	30°C	0.44 ± 0.03 ^{aA}	0.46 ± 0.05 ^{aA}	0.44 ± 0.02 ^{aA}	0.46 ± 0.04 ^{aA}
Oxalic acid	20°C	0.042 ± 0.00 ^{aA}	0.044 ± 0.00 ^{aA}	0.041 ± 0.00 ^{aA}	0.040 ± 0.00 ^{aA}
	25°C	0.040 ± 0.00 ^{aA}	0.050 ± 0.00 ^{aA}	0.046 ± 0.00 ^{aA}	0.044 ± 0.00 ^{aA}
	30°C	0.038 ± 0.01 ^{aA}	0.044 ± 0.01 ^{aA}	0.041 ± 0.00 ^{aA}	0.043 ± 0.00 ^{aA}
Malic acid	20°C	0.031 ± 0.01 ^{aA}	0.027 ± 0.00 ^{aA}	0.029 ± 0.00 ^{aA}	0.029 ± 0.00 ^{aA}
	25°C	0.031 ± 0.00 ^{aA}	0.028 ± 0.00 ^{aA}	0.029 ± 0.00 ^{aA}	0.029 ± 0.00 ^{aA}
	30°C	0.030 ± 0.01 ^{aA}	0.029 ± 0.00 ^{aA}	0.028 ± 0.00 ^{aA}	0.030 ± 0.00 ^{aA}
Citric acid	20°C	0.044 ± 0.02 ^{aA}	0.051 ± 0.00 ^{aA}	0.061 ± 0.00 ^{aB}	0.071 ± 0.00 ^{aC}
	25°C	0.047 ± 0.03 ^{aA}	0.068 ± 0.00 ^{aB}	0.067 ± 0.01 ^{aB}	0.086 ± 0.00 ^{bC}
	30°C	0.043 ± 0.01 ^{aA}	0.050 ± 0.01 ^{aA}	0.057 ± 0.00 ^{aB}	0.071 ± 0.01 ^{aC}
D- Glucuronic acid	20°C	0.003 ± 0.34 ^{aA}	0.020 ± 0.68 ^{aB}	0.0380 ± 1.40 ^{aC}	0.054 ± 2.58 ^{bD}
	25°C	0.003 ± 0.29 ^{aA}	0.0184 ± 0.50 ^{aB}	0.0343 ± 1.43 ^{aC}	0.063 ± 4.11 ^{cD}
	30°C	0.003 ± 0.32 ^{aA}	0.017 ± 0.70 ^{aB}	0.035 ± 1.81 ^{aC}	0.040 ± 1.18 ^{aD}
Acetic acid	20°C	ND	1.04 ± 0.01 ^{aA}	1.16 ± 0.05 ^{aB}	1.52 ± 0.01 ^{bC}
	25°C	ND	0.96 ± 0.03 ^{aA}	1.26 ± 0.03 ^{aB}	1.65 ± 0.04 ^{cC}
	30°C	ND	0.86 ± 0.03 ^{aA}	1.06 ± 0.05 ^{aB}	1.42 ± 0.07 ^{aC}
Lactic acid	20°C	ND	ND	ND	ND
	25°C	ND	ND	ND	ND
	30°C	ND	ND	ND	ND

Data are expressed as mean ± SD of $n = 3$ samples.

Means in the same column followed by different lowercase letters represent significant differences ($P < 0.05$).

Means in the same row followed by different uppercase letters represent significant differences ($P < 0.05$).

ND: not detected.

Notas: Los datos se expresan como la media ± DE de $n = 3$ muestras.

Las medias en la misma columna seguidas de distintas letras minúsculas representan diferencias significativas ($P < 0,05$).

Las medias en la misma fila seguidas de distintas letras mayúsculas representan diferencias significativas ($P < 0,05$).

ND – no detectado.

during the process of fermentation increased in all applied temperatures due to glucose utilization by AAB. Despite the constant rise at the end of the process (10th day), the acetic acid content was relatively low, within the range of 1.42–1.52 g L⁻¹. It can be explained by the insufficient amount of glucose for the acetic acid synthesis. The part of glucose was probably consumed by the synthesis of cellulose during the fermentation. Presented results are lower than those in the study of Kallel et al. (2012) (5.4–8 g L⁻¹) and Jayabalan et al., 2007 (after 2 weeks of fermentation Kombucha). In the analysed Kombucha beverages, the content of D-glucuronic acid increased to approximately 0.054 g, 0.063 g and 0.040 g L⁻¹ on the 10th day of fermentation at a temperature of 20°C, 25°C and 30°C, respectively (Table 2). Similar values were reported in a study conducted by Nguyen et al. (2015) in which the content of glucuronic acid in Kombucha fermented at 29°C for 7 days was from approximately 0.025 to over 0.1 g L⁻¹. Chen and Liu (2000) proved that the content of the analysed acid amounted to 39 g L⁻¹ only on the 60th day of the fermentation process at 24°C. This is a large disparity in the content of glucuronic acid as compared to the results of Jayabalan et al. (2007), in which the obtained maximum content on the 12th day of fermentation of the beverage at 24°C amounted to 2.33 g L⁻¹. Such disparity of results can be mainly associated with a microbiological variety of local Kombucha starter cultures and the beverage recipe itself along with fermentation conditions. In own studies, the beverage was fermented from a mixture of green tea and black tea (2:1) with 10% of sucrose. In the quoted studies of Jayabalan et al. (2007), Kombucha consisted only of black tea and sucrose.

Production of acetic acid, one of the basic metabolites and fermentation processes in Kombucha, increased slowly

during the fermentation time reaching the maximum of 1.65 g L⁻¹ on the 10th day of fermentation at 25°C (Table 2). These changes were correlated with an increase of AAB number. The increase of acetic acid during fermentation was also observed in the studies of Chakravorty et al. (2016) and Jayabalan et al. (2007). The content of quinic, oxalic, malic and citric acids was much lower at the fermentation temperatures analysed. The lactic acid was not found.

Sucrose is one of the most commonly added sugars in Kombucha tea production (Jayabalan et al., 2014). In Kombucha beverages studied, at every fermentation temperature sucrose content decreased, most rapidly at 25°C reached the lowest value of 9.3 g L⁻¹ on the 10th day, which is an expected result of its conversion into glucose and fructose by yeast cells. Metabolic changes at a temperature of 30°C were not intense, which was reflected in minor, though statistically significant ($P < 0.05$), decrease of sucrose content from 99.7 g L⁻¹ before fermentation to 75.4 g L⁻¹ on the last day and the lowest growth of yeast cells in the next days of fermentation (Table 3). The content of reducing sugars increased during fermentation. The greatest amounts of glucose and fructose were produced on each fermentation day at a temperature of 25°C. Afterwards, tests revealed that the content of fructose during fermentation at all temperatures is lower than glucose content (differences are statistically insignificant) (Table 3), which suggests that fructose is preferred as the source of carbon by yeast cells. Microbiological changes that occurred on the 10th day of fermentation of the Kombucha beverages confirm this observation. Studies of Sievers et al. (1995) also indicate that after a 23-h fermentation of Kombucha tea at 20–22°C, 88% of fructose and only 40% of glucose were

Table 3. Changes in sugar and ethanol concentrations during 10 days of Kombucha tea fermentation at 20°C, 25°C and 30°C.**Tabla 3.** Cambios en las concentraciones de azúcar y etanol durante 10 días de fermentación de té de Kombucha a temperaturas de 20°C, 25°C y 30°C.

Kind of sugars and ethanol content (g L ⁻¹)	Temperature of fermentation	Days of fermentation			
		0	3	7	10
Sucrose	20°C	98.7 ± 0.03 ^{aD}	91.6 ± 0.10 ^{bC}	47.9 ± 0.18 ^{bB}	1.97 ± 0.19 ^{bA}
	25°C	97.6 ± 0.06 ^{aD}	70.6 ± 0.17 ^{aC}	41.7 ± 0.16 ^{aB}	0.93 ± 0.17 ^{aA}
	30°C	99.7 ± 0.02 ^{aD}	92.6 ± 0.11 ^{bC}	88.2 ± 0.12 ^{cB}	7.54 ± 0.14 ^{cA}
Fructose	20°C	0.9 ± 0.00 ^{aA}	2.3 ± 0.02 ^{aA}	17.7 ± 0.10 ^{bB}	27.4 ± 0.18 ^{bC}
	25°C	1.2 ± 0.01 ^{aA}	7.3 ± 0.02 ^{bB}	20.2 ± 0.06 ^{cC}	30.9 ± 0.02 ^{cD}
	30°C	0.7 ± 0.01 ^{aA}	2.2 ± 0.01 ^{aB}	3.5 ± 0.02 ^{aC}	8.7 ± 0.04 ^{aD}
Glucose	20°C	0.8 ± 0.00 ^{aA}	2.7 ± 0.03 ^{aA}	19.6 ± 0.13 ^{bB}	33.4 ± 0.14 ^{bC}
	25°C	1.4 ± 0.03 ^{aA}	11.8 ± 0.15 ^{bB}	21.9 ± 0.09 ^{cC}	37.7 ± 0.04 ^{cD}
	30°C	0.9 ± 0.00 ^{aA}	2.6 ± 0.01 ^{aB}	3.8 ± 0.04 ^{aB}	10.5 ± 0.07 ^{aC}
Ethanol	20°C	ND	3.1 ± 0.03 ^{bA}	7.1 ± 0.04 ^{bB}	10.7 ± 0.06 ^{bC}
	25°C	ND	3.1 ± 0.02 ^{bA}	7.8 ± 0.03 ^{bB}	11.0 ± 0.03 ^{bC}
	30°C	ND	2.2 ± 0.03 ^{aA}	4.3 ± 0.01 ^{aB}	6.9 ± 0.03 ^{aC}

Data are expressed as mean ± SD of $n = 3$ samples.

Means in the same column followed by different lowercase letters represent significant differences ($P < 0.05$).

Means in the same row followed by different uppercase letters represent significant differences ($P < 0.05$).

ND: not detected.

Notas: Los datos se expresan como la media ± DE de $n = 3$ muestras.

Las medias en la misma columna seguidas de distintas letras minúsculas representan diferencias significativas ($P < 0,05$).

Las medias en la misma fila seguidas de distintas letras mayúsculas representan diferencias significativas ($P < 0,05$).

ND – no detectado.

metabolised into ethanol. Opposite to yeasts, *Acetobacter* bacteria strains prefer glucose to fructose (Kallel et al., 2012; Seto, Kojima, Tonouchi, Tsuchida, & Yoshinaga, 1997). It seems that the type and metabolic activity of yeasts have a determining influence on conversions of these sugars during fermentation (Sievers et al., 1995). Furthermore, yeast cells and AAB use substrates for fermentation in different and complementary way. Yeast cells hydrolyse sucrose into glucose and fructose and produce ethanol through glycolysis. Acetic bacteria use glucose for the production of gluconic acid and ethanol for the production of acetic acid (Sievers et al., 1995; Jayabalan et al., 2007; Mo et al., 2008). The mechanism of biochemical changes during fermentation of sugared tea with the symbiotic system of yeasts–acetic acid bacteria is complex, whereas order and a detailed image of these changes is not fully understood although a range of studies on chemical composition of Kombucha were performed (Kallel et al., 2012). Fermentation time, initial content of sucrose and black tea, and content of Kombucha culture have an influence on the development of the final metabolism products (Jayabalan et al., 2014).

Along with a decrease in the amount of sucrose, the content of ethanol increased with the fermentation time reaching the maximum value of 1.10% on the 10th day at 25°C (Table 3). The expected decrease of ethanol due to the conversions into acetic acid was not observed (Chakravorty et al., 2016; Chen & Liu, 2000), probably because of the short period of fermentation.

It was observed that following the fermentation process all beverages were of high sensory quality (Figure 1). The general quality was 7–8 c.u. on average, whereas Kombucha fermented at 25°C (8.70 c.u.) was assessed as the highest. The three types of beverages examined had mainly tea, lemon and sour smell, whereas acetic, yeast and other smells were barely detectible. In terms of sensory discriminants that describe the taste profile of the beverages analysed, it was found that lemon, tea and sour tastes were barely noticeable with the highest intensity. High clarity and similar colour tone were typical for all beverages.

5. Conclusions

The results of the study revealed that optimal conditions for the fermentation of Kombucha beverages were a temperature of 25°C and a period of 10 days which allowed to obtain a microbiologically stable product with a good sensory quality. In the Kombucha beverages analysed, the content of glucuronic acid increased during the process at all temperatures; however, the greatest amount was obtained on the 10th day of fermentation at 25°C. At all fermentation temperatures studied, sucrose content decreased in Kombucha beverages, the most rapidly at 25°C, which is an expected result of its conversion into glucose and fructose. The studies showed that during the fermentation process at all temperatures, fructose content was lower than glucose suggesting that fructose is preferred as a source of carbon by yeast cells. Determining optimal time of fermentation and content of starter culture require further research, including possible formation of ingredients beneficial for health during fermentation, such as vitamin C or glucuronic acid.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Agblevor, F.A., Murden, A., & Hames, B.R. (2004). Improved method of analysis of biomass sugars using high-performance liquid chromatography. *Biotechnology Letters*, 26(15), 1207–1211. doi:10.1023/B:BILE.0000036596.95796.42
- Antolak, H., & Kręgiel, D. (2015). Acetic acid bacteria – taxonomy, ecology, and industrial application. *Zywnosc Nauka Technologia Jakosc*, 4 (101), 21–35. doi:10.15193/ZNTJ/2015/101/053
- Battikh, H., Bakhrouf, A., & Ammar, E. (2012). Antimicrobial effect of Kombucha analogues. *LWT – Food Science and Technology*, 47, 71–77. doi:10.1016/j.lwt.2011.12
- Blanc, P.J. (1996). Characterisation of tea fungus metabolites. *Biotechnology Letters*, 18, 139–142. doi:10.1007/BF00128667
- Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation:

- Microbial and biochemical dynamics. *International Journal of Food Microbiology*, 220, 63–72. doi:10.1016/j.ijfoodmicro.2015.12.015
- Chen, C., & Liu, B.Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology*, 89, 834–839. doi:10.1046/j.1365-2672.2000.01188
- Chu, S., & Chen, C. (2006). Effects of origins and fermentation time on the antioxidant activities of Kombucha. *Food Chemistry*, 98, 502–507. doi:10.1016/j.foodchem.2005.05.080
- Cretović, D., Markov, S., Djurić, M., Savić, D., & Velićanski, A. (2008). Specific interfacial area as a key variable in scaling-up Kombucha fermentation. *Journal of Food Engineering*, 85, 387–392. doi:10.1016/j.jfoodeng.2007.07.021
- Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health – a review. *Food Research International*, 33, 409–421. doi:10.1016/S0963-9969(00)00067-3
- Hrnjez, D., Vaštag, Ž., Milanović, S., Vukić, V., Ilić, M., Popović, L., & Kanurić, K. (2014). The biological activity of fermented dairy products obtained by Kombucha and conventional starter cultures during storage. *Journal of Functional Foods*, 10, 336–345. doi:10.1016/j.jff.2014.06.016
- Ismail, A.A., Bassyouni, R.H., Kamel, Z., & Gabr, S.M. (2015). Detoxification of Patulin by Kombucha tea culture. *Cyta – Journal of Food*, 14(2), 271–279. doi:10.1080/19476337.2015.1096828
- ISO. International Organization for Standardization. (1998). *ISO 13299.2:1998. Sensory analysis—methodology—general guidance for establishing a sensory profile*. Switzerland: Geneva.
- Jayabalan, R., Malbaša, R.V., Lončar, E.S., Vitas, J., & Sathishkumar, M. (2014). A review on Kombucha tea – microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, 13, 538–550. doi:10.1111/1541-4337.12073
- Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organics acids and tea polyphenols during Kombucha tea fermentation. *Food Chemistry*, 102, 392–398. doi:10.1016/j.foodchem.2006.05.032
- Kallel, L., Desseaux, V., Hamdi, M., Stocker, P., & Ajandouz, E. (2012). Insights into the fermentation biochemistry of Kombucha teas and potential impacts of Kombucha drinking on starch digestion. *Food Research International*, 49, 226–232. doi:10.1016/j.foodres.2012.08.018
- Lončar, E.S., Petrović, S.E., Malbaša, R.V., & Verac, R.M. (2000). Biosynthesis of glucuronic acid by means of tea fungus. *Die Nahrung*, 44, 138–139.
- Malbaša, R.V., Lončar, E.S., & Djurić, M. (2008). Comparison of the products of Kombucha fermentation on sucrose and molasses. *Food Chemistry*, 106, 1039–1045. doi:10.1016/j.foodchem.2007.07.020
- Malbaša, R.V., Lončar, E.S., & Kolarov, L.J.A. (2002). L-lactic, L-ascorbic, total and volatile acids contents in dietetic Kombucha beverage. *Romanian Biotechnological Letters*, 7, 891–896.
- Malbaša, R.V., Lončar, E.S., Vitas, J.S., & Čanadanović-Brunet, J.M. (2011). Influence of starter cultures on the antioxidant activity of Kombucha beverage. *Food Chemistry*, 127, 178–184. doi:10.1016/j.foodchem.2011.02.048
- Marsh, J.A., O'Sullivan, O.O., Hill, C., Ross, P.R., & Cotter, P.D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple Kombucha (tea fungus) samples. *Food Microbiology*, 38, 171–178. doi:10.1016/j.fm.2013.09.003
- Mo, H., Zhu, Y., & Chen, Z. (2008). Microbial fermented tea – a potential source of natural food preservatives. *Trends in Food Science & Technology*, 19, 124–130. doi:10.1016/j.tifs.2007.10.001
- Nguyen, K.N., Nguyen, P.B., Nguyen, H.T., & Le, P.H. (2015). Screening the optimal ratio of symbiosis between isolated yeast and acetic bacteria strain from traditional Kombucha for high-level production of glucuronic acid. *LWT – Food Science and Technology*, 64, 1149–1155. doi:10.1016/j.lwt.2015.07.018
- Rodrigues, T.H.S., Rocha, M.V.P., De Macedo, G.R., & Gonçalves, L.R.B. (2011). Ethanol production from cashew apple bagasse: Improvement of enzymatic hydrolysis by microwave – assisted alkali pretreatment. *Applied Biochemistry and Biotechnology*, 164, 929–943. doi:10.1007/s12010-011-9185-3
- Seto, A., Kojima, Y., Tonouchi, N., Tsuchida, T., & Yoshinaga, F. (1997). Screening of bacterial cellulose-producing *Acetobacter* strains suitable for sucrose as carbon source. *Bioscience, Biotechnology and Biochemistry*, 61, 735–736. doi:10.1271/bbb.61.735
- Sievers, M., Lanini, C., Weber, A., Schuler-Schmid, U., & Teuber, M. (1995). Microbiology and fermentation balance in Kombucha beverage obtained from a tea fungus fermentation. *Systematic and Applied Microbiology*, 18, 590–594. doi:10.1016/S0723-2020(11)80420-0
- Teoh, A.L., Heard, G., & Cox, J. (2004). Yeast ecology of Kombucha fermentation. *International Journal of Food Microbiology*, 95, 119–126. doi:10.1016/j.ijfoodmicro.2003.12.020
- Yavari, N., Assadi, M.M., Moghadam, M.B., & Larijani, K. (2011). Optimizing glucuronic acid production using tea fungus on grape juice by response surface methodology. *Australian Journal of Basic and Applied Sciences*, 5(11), 1788–1794.