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Quercus robur and *pyrenaica*: The Potential of Wild Edible Plants for Novel Kombuchas

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Abstract: Wild edible plants (WEPs) can be utilised as a substrate to develop novel types of fermented beverages. The aim of this study was to investigate the potential of incorporating *Quercus* spp. leaves as a substrate to develop new varieties of kombuchas. The physicochemical properties of kombuchas elaborated with *Quercus robur* (KQR) and *Quercus pyrenaica* (KQP) leaves' infusion were compared with traditional black tea kombuchas (KBT). Total acidity (TA), pH, total soluble solids (TSSs), instrumental colour, total phenolic compounds (TPCs), sucrose, fructose, and glucose were analysed for the three types of infusions and kombuchas at 0, 3, 7, 10, and 14 fermentation days. The results revealed that the type of substrate and the fermentation time significantly influenced the biochemical changes that occurred during 14 days. KQP and KQR turned out to be significantly brighter (L^*: 53.91 ± 0.12 and 55.66 ± 0.23, respectively) than KBT (L^*: 48.79 ± 0.34) and had significantly lower sucrose content (22.06 ± 0.79 g L^{-1} and 45.69 ± 1.61, respectively) than KBT (59.28 ± 2.25 g L^{-1}). KBT showed significantly higher content of total polyphenols (1.50 ± 0.05 g GAE L^{-1}) than KQR (0.76 ± 0.09 g GAE L^{-1}) and KQP (0.51 ± 0.04 g GAE L^{-1}) after 14 days of fermentation. Regarding the kinetics of sugars, sucrose reduction was significantly lower in KBT samples (11.36 g L^{-1}) than in KQP and KQR samples (47.01 and 28.31 g L^{-1}, respectively) at the end of fermentation. These results suggest that higher content of TPC may slow down the fermentation process. *Quercus* spp. leaves may be a viable alternative substrate for developing analogues of kombucha with WEPs and for adding gastronomic and sustainable value.

Keywords: new fermented tea; wild edible plants; *Quercus* spp.; fermented methods; sugar kinetics

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1. Introduction

Kombucha is the name of the beverage obtained from the fermentation of sugary tea using a symbiotic culture of bacteria and yeasts (SCOBY) [1]. The symbiotic culture responsible for the fermentation process is composed of acetic acid bacteria (AAB), lactic acid bacteria (LAB), and yeast embedded in a floating biofilm and used as a backslop for successive fermentation processes [2–4]. After the brewing process, a fruity–sour, refreshing, and sparkling beverage is obtained due to the multiple organic acids and CO_2 that are released during the process [5].

In the last few decades, the kombucha market has witnessed a significant increase in demand. Currently, this drink has become one of the most popular low-alcoholic fermented beverages [6]. In the context of economic data, the global kombucha market size was valued at USD 2.64 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 15.6% from 2022 to 2030 [7].

The increase in kombucha consumption has been associated with healthy benefits attributed to the synergistic effect of their composition in bioactive compounds produced during fermentation [1]. As the literature suggests, the antioxidant properties of polyphenols and products of fermentation, including glucuronic acid, acetic acid, DSL, phenolic

compounds, and B-complex vitamins, among others are responsible for the health benefits associated with tea and kombucha [8–11]. Hepatoprotective and antimicrobial effects; improvement in the immune system; and antioxidant, anticancer, hypoglycaemic, and antihypertensive activity are some of the potential health benefits described in the literature [1,9,12]. However, evidence of health properties is mostly limited to chemical analyses and animal and cell culture models. Thus, clinical studies are needed to reveal the potential benefits of kombucha in human health [5,13,14].

While black and green tea varieties have traditionally served as substrates for preparing kombucha, a wide range of alternative kombuchas are nowadays available commercially. Recent studies have explored the suitability of these substrates in yielding novel kombucha analogues, leading to interesting results [15,16]. Consequently, diverse tea varieties such as oolong or plant infusions, like jasmine and mulberry leaves [17], and rooibos [3], have also been tested. Medicinal herbs (*Thymus vulgaris* L., *Lippia citriodora*, *Rosmarinus officinalis*, *Foeniculum vulgare*, and *Mentha piperita*) [18], as well as seaweed like *Porphyra dentata* [19], have also been used for this purpose. Even fruits like some varieties of apples [20], pitanga and umbu-caja [21], Indian gooseberry [22], "snake fruit" (salak) [23], and coconut [24] have been used for kombucha elaboration. Thus, wild edible plants (WEPs) can be utilised as substrates to develop innovative varieties of kombuchas.

WEPs have been an essential food source for human beings since before agriculture, indeed, they have been gathered and consumed even after domestication. In the Mediterranean region, they have been considered a traditional component of the diet. In addition, they are still an integral part of the food culture in some developing countries, especially in times of food scarcity [25]. These plants may play an important role in environmental sustainability as they grow wildly and can be used as functional ingredients to develop new food products. This sustainable character is persuading more and more consumers, chefs, and nutritionists to introduce WEPs in their dishes and the diets they prescribe [26]. International organisms and institutions have recognised the cultural and ecological value of WEPs [27]. Accordingly, traditional knowledge regarding WEPs was included in The Convention on Biological Diversity (CBD) by the United Nations (UN), which provides a key reference for the recognition of their value and importance for the preservation of biodiversity [28].

Quercus is a genus of the Fagaceae family, encompassing approximately 500 species worldwide across Europe, East Asia, North America, and South America [29]. *Quercus* forests are not only economically and ecologically important but also culturally significant, as the species is considered a sacred tree species in many countries and cultures [30].

The human consumption of acorns is an ancestral practice that has been observed in prehistoric archaeological sites, as well as in some of the first written texts of antiquity [31]. This practice has endured, maintaining a vital role in the traditional wisdom of many societies [32]. Furthermore, oak leaves have been utilised in the preparation of pickles and infusions [33,34]. While some studies have focused on the development of kombuchas using leaves from various oak tree species [35–38], to the best of our knowledge, none of the studies have assessed the species *Quercus robur* and *Quercus pyrenaica*.

Situated within this context, the objective of this study was to evaluate the effect of two species of *Quercus* spp. (*Q. robur* and *Q. pyrenaica*) as potential substrates to develop new fermented beverages incorporating WEPs. Changes in the composition of new kombuchas produced from *Quercus* spp. were evaluated during 14 days of fermentation, in terms of pH; total acidity; total soluble solids; total phenolic content; instrumental colour; and sucrose, glucose, and fructose content.

2. Materials and Methods

2.1. Plant Materials

Q. robur and *Q. pyrenaica* green leaves were foraged in one location in north Spain (Getaria, Basque Country). Leaves were collected in July 2021 and gathered from trees in the same plot. They were transported to the laboratory and conditioned immediately.

Green leaves were soaked in tap water and rinsed until the water was clear. They were steamed in an oven (Rational SCC 61, Landsberg am Lech, Germany) at 100 °C and 100% humidity for 1 min. Then, they were oxidised in the same oven at 30 °C and 90% humidity for 2 h. Consecutively, leaves were dried at 30 °C for 48 h. Temperature below 32 °C was selected to preserve the quality of the leaves [39]. *Q. robur* and *Q. pyrenaica* leaves were processed separately. Commercial bags of black tea (Darjeeling tea, Baqué café) were used to prepare black tea infusions as control.

2.2. Preparation of Infusions and Fermented Beverages

Kombuchas were elaborated with 3 types of leaves, namely *Quercus robur* (KQR), *Quercus pyrenaica* (KQP), and black tea (KBT), following the process described in Figure 1. Infusions were prepared by adding 30 g of dry leaves to 3 L of boiled water (1%, w/v), and the mixture was left for 10 min, filtered through a sterile cloth, and transferred to cylindrical jars (15 cm diameter and 25 cm height). Then, sucrose was added at a concentration of 70 g L^{-1} to each infusion and mixed until dissolved. After the infusions were cooled at room temperature, they were inoculated with 10% (v/v) of commercial starter culture with pH value 3.25 ± 0.05 to boost fermentation, and SCOBY cellulose layer (1.4%, w/v) (Kirandia Probioticos naturales, Madrid, Spain) under aseptic conditions. The same batch of starter culture and SCOBY was used for all kombuchas, and three independent batches of each kombucha were prepared under the same conditions. Each jar was covered with cheese clothes and kept at 23 ± 2 °C for 14 days.

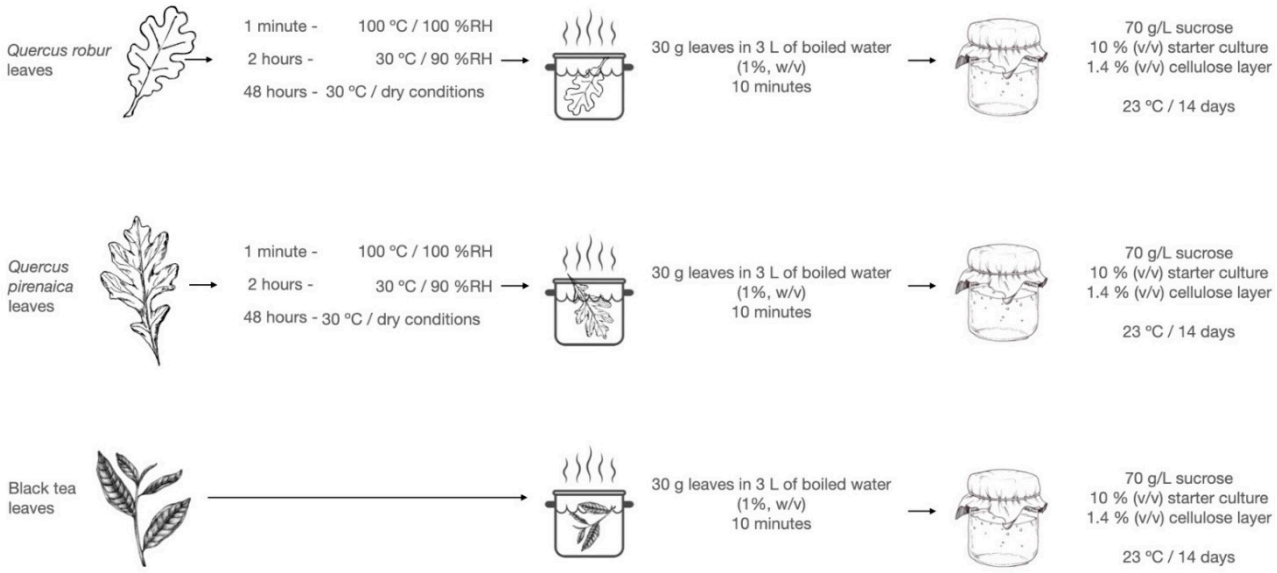

Figure 1. Schematic diagram of kombucha preparation procedure.

2.3. Sampling

Aliquot samples (150 mL) were collected after sucrose addition to infusion and at 0 (immediately after inoculation), 3, 7, 10, and 14 fermentation days. Samples were degassed to remove the carbon dioxide using a vacuum machine (Sammic SU 316, Azkoitia, Spain), centrifuged (Heraeus Megafuge 8, Thermo Scientific, Dreieich, Germany) at 9000× g for 10 min, and filtered through qualitative filter paper (Branchia, Barcelona, Spain). Then, pH, total soluble solids, titratable acidity, and instrumental colour were analysed on the same follow-up day. Aliquots of each sample were frozen (−20 °C) until further analysis. A total of 54 samples (3 types of leaves × 3 batches × 6 fermentation times) were analysed.

2.4. Determination of pH, Total Soluble Solids, and Total Acidity

The pH of the kombucha samples was measured with an electronic pH meter Crison Basic 20 (Crison Instruments SA, Barcelona, Spain) [40]. Total soluble solids (TSSs) were determined using the refractometric method with a manual hand refractometer (VWR Inc.,

Leuven, Belgium), and the results were expressed in °Brix [41]. The total acidity (TA) of samples was analysed using a titration method with a standard solution of sodium hydroxide (0.1 N) [41]. Briefly, 10 mL of each sample and 40 mL of distilled water were titrated with 0.1 mol L^{-1} of NaOH solution until pH 8.10 ± 0.02. The results were expressed as grams of acetic acid per L of the sample. All procedures were conducted in duplicate.

2.5. Instrumental Colour

Colour was measured directly with a CR-400 colorimeter (Minolta, Tokyo, Japan) coupled to a CM-A131 cuvette (10 mm). The equipment was set up for illuminant D65 and calibrated using a standard white reflector plate. The results were expressed in CIE $L^*a^*b^*$ units of L^*, a^*, and b^*. Hue angle (H) and chroma (C) were calculated according to Equations (1) and (2), respectively. The colour difference between fermented beverages and infusions (ΔE^*) was calculated using Equation (3) [42]. All measurements were carried out in quintuplicate.

$$H = tg^{-1}\ (b^*/a^*) \quad (1)$$

$$C = [(a^*)^2 + (b^*)^2]^{1/2} \quad (2)$$

$$\Delta E^* = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \quad (3)$$

2.6. Total Phenolic Content

The total phenolic content was determined by the Folin–Ciocalteu assay using gallic acid as a representative standard and expressing the results as gallic acid equivalents (g GAE L^{-1}) of the kombucha samples.

The Folin–Ciocalteu method adapted from Singleton et al. [43] was used to determine the total phenolic content of kombucha samples. The reaction mixture was prepared by mixing 0.1 mL of the sample, 7.9 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent, and 1.5 mL of sodium carbonate (20%, *w*/*w*). After incubation at room temperature for 2 h in the dark, absorbance was measured at 765 nm with a Hitachi spectrophotometer (Hitachi, UH5300, Tokio, Japan). The blank was prepared by replacing the sample with distilled water. Gallic acid was used as a calibration standard, and the results were expressed as gallic acid equivalents per L of the sample (g GAE L^{-1}).

2.7. Sugar Content

Sucrose, glucose, and fructose were determined using high-performance liquid chromatography and a pulsed amperometric detector (HPLC-PAD) according to the method of Deba-Rementeria (2021) [44], with some modifications. Briefly, kombucha samples were diluted 1:50 (*v*/*v*) with ultrapure water at 60 °C and then shaken for 30 min and centrifuged at 4200 rpm for 10 min. The supernatant was filtered using a 25 mm, 0.45 µm nylon VWR® Syringe Filter (VWR, Inc., Leuven, Belgium). Then, 25 µL of each sample were injected in a Knauer HPLC system (Knauer Scientific Devices GmbH, Berlin, Germany) equipped with a low-pressure gradient pump (Knauer Azura® P 6.1L, Knauer Scientific Devices GmbH, Berlin, Germany), using an automatic injector (Knauer Azura® AS 6.1L) with a partial loop (100 µL loop). A Metrosep Carb 2 150/4.0 column (15 cm long and 4 mm internal diameter), placed in a CT 2.1 column thermostat programmed at 35 ± 0.1 °C, was used for the separation of compounds. An isocratic mobile phase of 300 mmol L^{-1} NaOH and 1.0 mmol L^{-1} NaAc (sodium acetate), at a flow rate of 0.5 mL min^{-1} and 80 bar backpressure, was used. The equipment was coupled to an amperometric detector 945 Professional Detector Vario (Metrohm AG, Herisau, Switzerland) in pulsed amperometric mode. Standards for glucose, fructose, and sucrose were used to quantify the presence of the different compounds. The standard curves for each type of sugar ranged from 2 to 50 mg L^{-1}.

2.8. Statistical Analysis

All the values are expressed as mean ± standard deviation (SD) of three batches. Physicochemical and instrumental data of kombuchas were analysed by two-way analysis of variance (ANOVA) using "type of kombucha" and "fermentation time" as factors, followed by Tukey (HDS) Post Hoc test (p value < 0.05). All statistical analyses were performed using XLSTAT Version 2022.5.1 (Addinsoft, Paris, France).

3. Results and Discussion

3.1. pH Values, Total Acidity, and Total Soluble Solids

The pH of the three studied kombuchas decreased sharply with a concomitant increase in the titratable acidity during fermentation. The evolution of the pH and acidity of the kombucha samples during fermentation time are shown in Figures 2 and 3, respectively. Significant differences (p < 0.05) were found in pH and acidity considering the type of kombucha and the fermentation time.

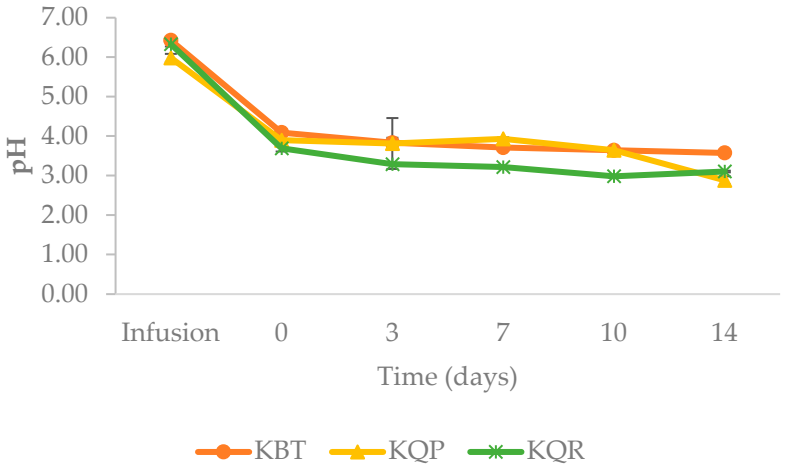

Figure 2. Evolution of pH values ± SD of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

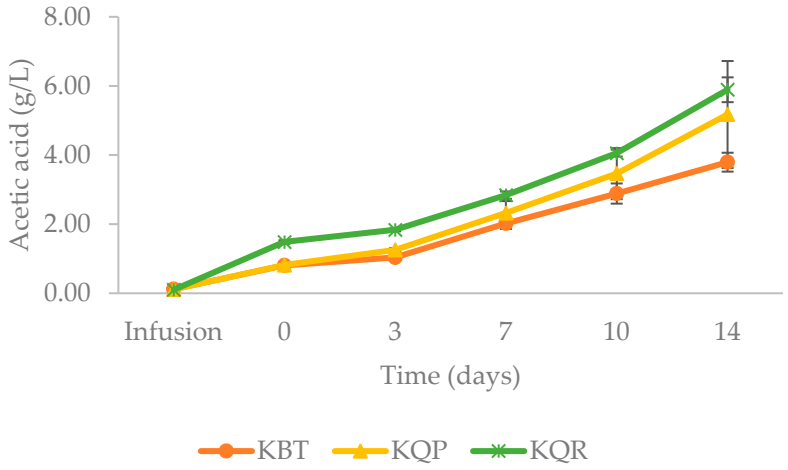

Figure 3. Evolution of total acidity (TA) (g acetic acid L^{-1} ± SD) of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

The sweetened infusions of black tea, *Q. robur*, and *Q. pyrenaica* presented pH values around 6. These values of pH (KBT 6.42 ± 0.02 ^{a}; pH KQP 5.98 ± 0.10 ^{c}; pH KQR 6.33 ± 0.66 ^{b}) showed significant differences (p < 0.05) probably associated with leaves' composition, as well as the brew and extraction processes of black tea and *Quercus* leaves, as reported by Ang et al. [13]. During infusion, several phenolic acids and organic acids

were dissolved in the extracts, lowering the pH [13]. After starter culture addition (day 0), a strong drop in pH was observed in KBT, KQR, and KQP samples (4.09 ± 0.0 ^{a}, 3.69 ± 0.03 ^{c}, and 3.90 ± 0.08 ^{b}, respectively). Nevertheless, during the following 14 fermentation days, pH values slightly decreased for KBT, KQR, and KQP until reaching values of 3.57 ± 0.01 ^{a}, 3.10 ± 0.10 ^{c}, and 2.88 ± 0.02 ^{b}, respectively.

The pH of all kombucha beverages fell into the pH range of 2.5 to 4.2 after starter inoculation, which is considered safe for human consumption [45]. The main cause of the decrease in pH is related to the production of some organic acids. Bacteria and yeast metabolise sucrose into a number of organic acids, such as acetic acid, glucuronic acid, and gluconic acid, during fermentation [11,46,47]. Although the individual quantification of each organic acid produced is preferable for a better understanding of fermentation, acetic acid is the most common acid produced during kombucha fermentation [19,42,48,49].

Recently, acetic acid has generated a strong interest due to its beneficial qualities, such as its function as a precursor to the manufacture of vitamin C, its ability to transform into glucosamine, and its capacity to remove toxins from the body [50].

In our study, acidity values significantly increased in all kombucha samples during fermentation (Figure 3). Acetic acid increased linearly with time (days) at 0.25, 0.34, and 0.37 g d^{-1} L^{-1} in KBT, KQP, and KQR, respectively (R^2 > 0.96). Although acidity values of the three types of kombucha presented values close to 2–3 g L^{-1} during the initial stages of fermentation (7 days), and then the total acidity value in KQR reached 5.89 ± 0.36 ^{a} (g acetic acid L^{-1}), similar to that in KQP (5.18 ± 1.55 ^{ab}) but significantly higher than that in KBT (3.79 ± 0.27 ^{b}) at the end of the studied period. A similar increase in acetic acid during fermentation was also observed in other studies [11,51].

Considering the fact that there was no difference in pH and acidity among the three batches for the same kind of kombucha, and all batches were prepared with the same divided SCOBY, our results suggest that the infusion composition can have an effect on microbiological activity during fermentation. These results are in agreement with the findings of Coton et al. [2], who reported that tea composition had a clear impact on microbiological diversity in green tea and black tea kombuchas prepared from the same divided biofilm. In addition, other authors [11,36,49] have also found differences in pH and acidity associated with the type of infusion.

Regarding the total soluble solid (TSS) content, the two-way ANOVA results indicated that fermentation time and the type of substrate had a significant effect. TSS values decreased significantly (p < 0.001) with fermentation time for all fermented kombucha beverages (Figure 4). The kombuchas prepared from infusions of *Q. pyrenaica* had a significantly lower content (p < 0.001) of TSS than those prepared from black tea and *Q. robur* infusions.

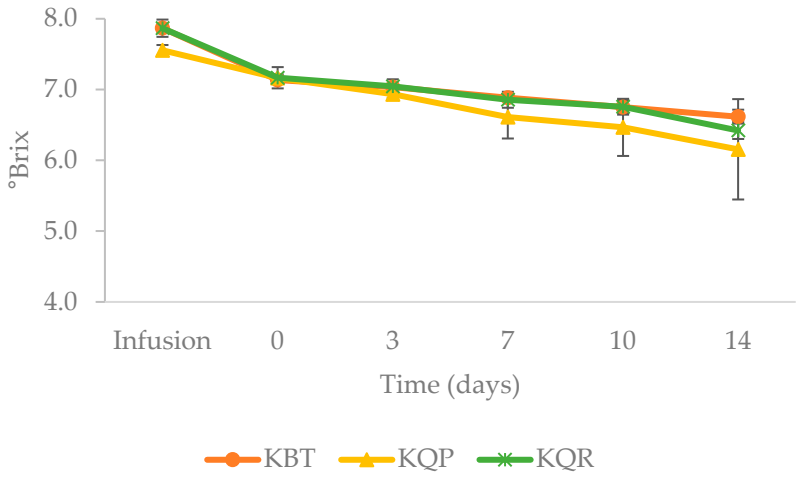

Figure 4. Evolution of total soluble solids (TSS) (°Brix ± SD) of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

This reduction is related to the consumption of soluble sugar (sucrose) by microorganisms during fermentation [51]. A similar observation was reported in other studies. Moreover, Yıkmış et al. [52] observed a reduction in TSS during 30 days of storage at 4 °C (after 10 days of fermentation at 24 ± 3 °C), which suggests that consumption of sugars by microorganisms could continue under refrigerated conditions.

3.2. Instrumental Colour

The results of colour parameters are shown in Table 1. The colour of beverages is a key characteristic for their better acceptance by consumers. In general, the beverage colour is the first organoleptic property perceived by consumers [53].

Table 1. Evolution of colour parameters (L^*, a^*, b^*, C, H, and ΔE^*) of black tea kombucha (KBT), as well as *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas.

		L^*	a^*	b^*	C	H	ΔE^*
KBT	Infusion	44.14 ± 0.55 a	8.15 ± 0.33 a	31.56 ± 0.25 a	32.59 ± 0.29 a	1.32 ± 0.01 a	
	0	48.88 ± 0.29 b	3.03 ± 0.24 b	29.86 ± 0.28 b	30.01 ± 0.30 b	1.47 ± 0.01 b	7.18
	3	48.78 ± 0.25 b	2.99 ± 0.20 b	28.83 ± 0.11 c	28.99 ± 0.13 c	1.47 ± 0.01 b	7.45
	7	49.32 ± 0.18 c	2.38 ± 0.31 c	27.09 ± 0.32 d	27.19 ± 0.34 d	1.48 ± 0.01 c	8.95
	10	49.67 ± 0.26 c	2.25 ± 0.29 c	26.56 ± 0.39 e	26.66 ± 0.41 e	1.49 ± 0.01 c	9.50
	14	48.79 ± 0.34 b	1.60 ± 0.28 d	24.92 ± 0.50 f	24.98 ± 0.51 f	1.51 ± 0.01 d	10.41
KQP	Infusion	51.11 ± 0.06 a	−0.48 ± 0.15 a	20.90 ± 0.52 a	20.91 ± 0.52 a	−1.55 ± 0.01 a	
	0	53.95 ± 0.17 b	−1.54 ± 0.05 b	16.70 ± 0.96 b	16.77 ± 0.95 b	−1.48 ± 0.01 b	5.18
	3	54.29 ± 0.31 c	−1.78 ± 0.05 d	15.72 ± 1.45 bc	15.82 ± 1.44 bc	−1.46 ± 0.01 c	6.21
	7	54.00 ± 0.15 b	−1.69 ± 0.08 c	15.47 ± 1.14 c	15.56 ± 1.15 c	−1.46 ± 0.00 c	6.27
	10	54.06 ± 0.13 b	−1.84 ± 0.08 d	14.93 ± 1.04 cd	15.04 ± 1.04 cd	−1.45 ± 0.01 d	6.80
	14	53.91 ± 0.12 b	−1.60 ± 0.05 bc	14.37 ± 0.66 d	14.46 ± 0.66 d	−1.46 ± 0.00 c	7.20
KQR	Infusion	50.78 ± 1.21 a	−0.19 ± 0.12 a	20.59 ± 0.80 a	20.60 ± 0.80 a	−1.56 ± 0.01 a	
	0	55.16 ± 0.42 bc	−1.78 ± 0.12 d	15.58 ± 0.15 b	15.69 ± 0.14 b	−1.46 ± 0.01 b	6.85
	3	54.52 ± 0.54 b	−1.77 ± 0.10 cd	14.16 ± 0.55 c	14.28 ± 0.55 c	−1.45 ± 0.01 c	7.60
	7	55.15 ± 0.21 bc	−1.72 ± 0.07 cd	14.29 ± 0.18 c	14.39 ± 0.19 c	−1.45 ± 0.00 bc	7.82
	10	54.77 ± 0.46 b	−1.59 ± 0.09 b	14.29 ± 0.36 c	14.38 ± 0.35 c	−1.46 ± 0.01 b	7.59
	14	55.66 ± 0.23 c	−1.65 ± 0.09 bc	13.94 ± 0.36 c	14.04 ± 0.35 c	−1.45 ± 0.01 bc	8.38

Values followed by different letters within the same column in each kombucha (KBT, KQP, and KQR) are significantly different ($p < 0.05$).

Fermentation time and plant material had a significant effect ($p < 0.001$) in all the colour parameters studied. There was a noticeable contrast in colour between the black tea infusion and the two kinds of *Quercus* infusions, although KQP and KQR seemed very similar in appearance.

ΔE^* indicates the extent of colour difference between the preinoculation infusion and fermented samples. The colour difference can range from imperceptible (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0), and great (>6.0) [37]. In this study, the main colour modifications (ΔE) occurred after the addition of starter culture, and ΔE^* values immediately after inoculation (day 0) were 7.18, 5.18, and 6.85 for KBT, KQP, and KQR, respectively. Then, the colour of the three studied kombuchas changed to a lesser extent during the fermentation time, reaching ΔE^* values of 10.41, 7.20, and 8.38 for KBT, KQP, and KQR, respectively. Similar to pH value changes, the most significant changes occurred after inoculation. The level of pH has an important impact on the beverages, as it influences colour variations [35].

Regarding lightness, it increased after the inoculation of the three kinds of infusions. Kombuchas prepared from *Quercus* spp. leaves (KQP and KQR) showed significantly greater lightness than those produced from black tea (KBT) ($p < 0.001$). According to Chu et al. [54], the changes in lightness are associated with the transformation of polyphenols produced by microorganisms. In our study, KBT samples had higher polyphenol content than KQP and KQR and were the darkest samples.

In terms of the a^* values (redness–greenness) and b^* (yellowness–blueness) values, a clear trend was observed. These colour parameters decreased over time due to the fermentation of kombucha ($p < 0.001$) and the reduction in black tea was significantly higher than in *Quercus* kombuchas during fermentation. KQR and KQP samples showed

lower *a** and *b** values than kombuchas prepared with black tea infusions, indicating that KQP and KQR samples were less red and yellow than KBT samples. Kayisoglu et al. and Zou et al. also reported a reduction in *a** and *b** during the fermentation of black tea kombuchas [42,55]. However, other authors have already reported differences in the colour changes of kombuchas depending on the plant material [19,38,52,56] or processing conditions [35].

Previous studies have reported changes in colour (especially in terms of lightning) because of fermentation [38,42,56]. In our study, the sugared infusions and kombuchas prepared from *Quercus* spp. leaves showed greater luminosity than those produced from black tea ($p < 0.001$). Luminosity is one of the most important organoleptic properties perceived by consumers. Therefore, kombuchas prepared with *Quercus* leaves might be considered attractive by consumers.

In terms of the *a** and *b** values, kombuchas prepared with *Quercus* spp. leaves showed lower *a** and *b** values than kombuchas prepared with black tea infusions ($p < 0.001$), indicating that KQP and KQR samples were less red and yellow than KBT samples.

3.3. Total Phenolic Content

The evolution of the total phenolic content (TPC) in KBT, KQP, and KQR during fermentation is shown in Figure 5. ANOVA results indicate that the type of substrate and fermentation time had a significant effect on the total phenolic content. Among the explanatory variables, based on Type III sums of squares, the variable "kombucha type" was the most influential, and the three types of kombuchas showed significant differences ($p < 0.001$). KBT had the highest total phenolic content at all fermentation times followed by KQR and KQP. After day 0 of the fermentation process, no significant changes were observed in the TPC in KBT samples. A similar tendency was observed for KQR and KQP, with no significant changes in the TPC between day 0 and day 14 of the fermentation. These results indicate that phenolic compounds in kombuchas are mainly affected by the type of plant material, but other factors such as hydrolysis under acid conditions, isomerisation, or polymerisation are also determinants in the evolution of phenol concentration during fermentation [52]. On the one hand, several authors have reported that TPC progressively increased during kombucha fermentation, suggesting that complex phenolic compounds might be subjected to degradation in the acidic environment of kombucha due to the enzymes produced by bacteria and yeasts [49,57–59]. On the other hand, other studies have revealed the stability of tea polyphenols during kombucha fermentation [11,60] or a lower phenolic content at the end of fermentation [11,42,52,54,55], which has been associated with the polymerisation of phenolic substances, such as catechins [61]. In addition, Gaggia et al. [3] found that the evolution of phenolic compounds had different trends depending on the type of infusion used as a starting point.

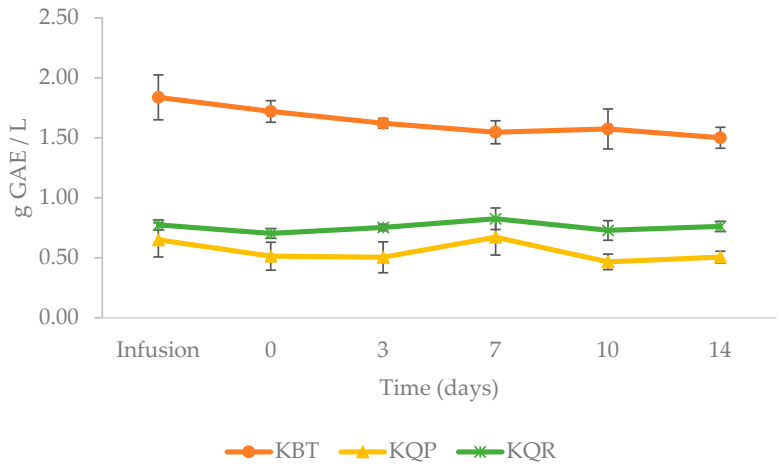

Figure 5. Evolution of total phenolic content (TPC) (mean values ± SD) of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

3.4. Sugars—Kinetics of Sugar Consumption during Fermentation

Kombucha production involves two main processes: alcoholic fermentation and acetic acid fermentation. Alcoholic fermentation is driven by the yeasts present in the SCOBY. Yeast cells hydrolyse sucrose into glucose and fructose through yeast invertase and produce ethanol via glycolysis with a preference for fructose as substrate. Apart from that, the acetic acid bacteria present in the SCOBY produce acetic acid and other organic acids from the oxidation of ethanol, through alcohol dehydrogenase and aldehyde dehydrogenase enzymes. At the end of the process, water and carbon dioxide are also found as final products [5,11,62].

Figures 6–8 show sugar kinetics (sucrose, fructose, and glucose, respectively) during fermentation. The initial sucrose content in the three sweetened infusions from black tea, *Quercus robur*, and *Quercus pyrenaica* were 70.64 ± 1.25, 69.06 ± 2.13, and 74.00 ± 1.51 g L^{-1}, respectively, and no other sugars were detected. As shown in Figure 6, after the addition of the starter, no significant changes in sucrose content were found in any of the samples during 3 days of fermentation. However, after that period, the sucrose consumption presented a different behaviour for each type of kombucha, and the reduction in sucrose content was more moderate in the KBT samples than in the kombuchas prepared with *Quercus* infusions. In KBT samples, sucrose decreased 11.36 g L^{-1} during 14 days of fermentation, while the sucrose consumption in KQP and KQR samples was significantly higher and it was reduced 47.01 and 28.31 g L^{-1}, respectively.

Figure 6. Evolution of sucrose content (mean g L^{-1} ± SD) of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

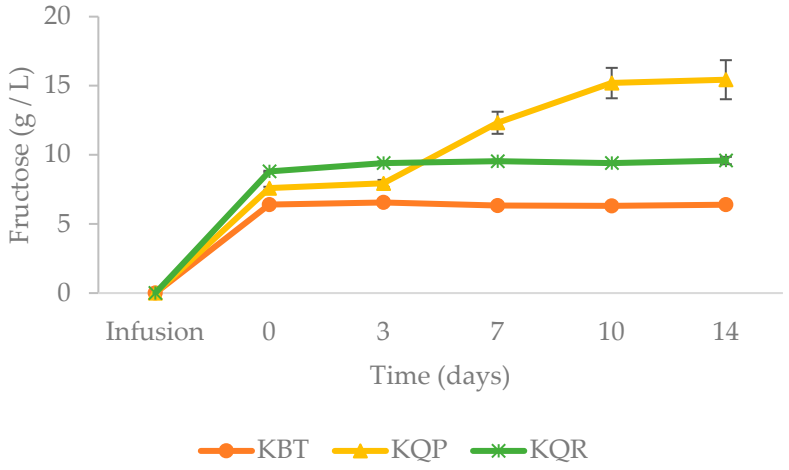

Figure 7. Evolution of fructose content (mean g L^{-1} ± SD) of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

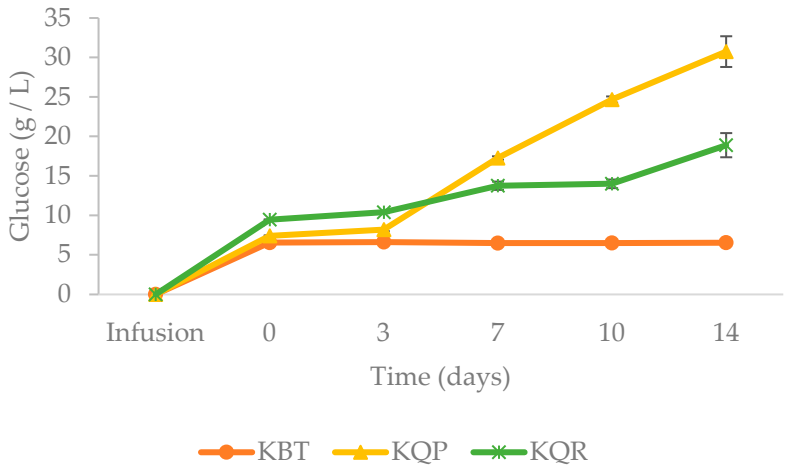

Figure 8. Evolution of glucose content (mean g L^{-1} ± SD) of black tea kombucha (KBT), and *Q. pyrenaica* (KQP) and *Q. robur* (KQR) kombuchas, during fermentation.

In KBT samples, there was a rapid increase in fructose and glucose content (day 0) after the inoculation of the infusion. However, this content remained almost constant for up to 14 days of fermentation, with values around 6 g L^{-1} for each monosaccharide. In comparison with KQR and KQP samples, KBT samples showed a significant reduction in these sugars.

In KQR samples, fructose content remained constant after inoculation with the SCOBY, with values of 9.34 ± 0.32 g L^{-1}. Meanwhile, in KQP samples, fructose content increased from values of 7.85 ± 0.10 g L^{-1} (day 0) to values of 15.43 ± 1.41 g L^{-1} (day 14).

An increase in glucose content was observed in both KQP and KQR samples. In KQR, glucose values increased from 9.45 ± 0.04 (day 0) to 18.89 ± 1.53 (day 14), while in KQP, this increase was more pronounced, increasing from 7.41 ± 0.05 to 30.73 ± 1.94.

A higher sucrose content and a lower total acidity in KBT at the end of fermentation could indicate less microbial activity. These results suggest that the higher content of polyphenols in black tea infusions compared with *Quercus* spp. infusions may slow down the fermentation process. Our results support the previous findings reported by Almajano et al. [63], who investigated the phenolic content and antimicrobial activities of different tea extracts and herbal infusions. Their results showed that the highest antimicrobial activity occurred in samples with the highest total polyphenol concentration and antioxidant activity. Furthermore, our results are consistent with those reported by Cardoso et al. [49], who compared kombuchas prepared with green tea and black tea infusions and found that those with higher polyphenol content consumed less sucrose during kombucha fermentation and produced less acidity. Additionally, samples with the highest polyphenol content also presented lower bacterial and yeast counts, although these differences were not significant. However, other studies indicate that an increase in phenolic content in the extract accelerates the metabolism of microorganisms, resulting in higher consumption of sugar [3,48,64]. Therefore, more studies are needed to understand the effect of individual phenolic compounds in the development or inhibition of microorganisms during the fermentation process.

In the reviewed studies of kombuchas prepared from different plant infusions, it has been confirmed that, during fermentation, the dynamics of sucrose consumption and the formation of glucose and fructose differ depending on the type of herbal plant used [3,42,49,59] or fermentation conditions, such as temperature [50], jar dimensions [65], and oxygen availability [4]. These results are also supported by studies conducted with infusions of different *Quercus* species (*Q. arizonica*, *Q. covallata*, *Q. arizonica*, and *Q. resinosa*) [25,38].

4. Conclusions

The evolution of the different parameters studied depended on the type of initial infusion and the fermentation time. After the fermentation process, the three types of kombuchas exhibited a significant reduction in their pH levels, as well as an increase in total acidity content. The pH of all kombucha beverages fell under the pH range of 2.5 to 4.2 after starter inoculation, which is considered safe for human consumption. Kombuchas produced from *Quercus* spp. leaves showed greater luminosity than those prepared from black tea. Luminosity is one of the most important organoleptic properties perceived by consumers. Furthermore, in *Quercus* spp. kombuchas, with lower TPC, sucrose consumption was significantly higher than in the black tea kombuchas. Comparing the two types of *Quercus* spp. kombuchas, we found that there was a greater reduction in sucrose and a higher increase in fructose and glucose content with *Q. pyrenaica* than with *Q. robur*. Although these results may indicate that the higher content of TPC may slow down the fermentation process, new studies are needed to establish the relationship between TPC and sugar kinetics to support this hypothesis. The composition of the plant material used played an important role during the fermentation process. The use of *Quercus* spp. leaves as a raw material allows the incorporation of alternative ingredients into the diet that have fallen into disuse. Through this study, new fermented products incorporating WEPs were developed and characterised, contributing to the generation and diversification of novel fermented beverages. Although kombucha beverages were successfully produced on *Quercus robur* and *Quercus pyrenaica*, more studies are necessary to reliably determine their beneficial effects on health and consumer acceptance.

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