LWT - Food Science and Technology 189 (2023) 115482

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Novel kombucha beverages with antioxidant activity based on fruits as alternative substrates

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ARTICLE INFO

Keywords:
Antioxidant activity
Fermentation
Phenolic compounds
SCOBY
Strawberry

ABSTRACT

The consumption of kombucha drinks has remarkably grown during the last decade and, besides traditional ingredients such as tea leaves, alternative raw materials with interesting functional properties are being proposed. The aim of this study was to obtain alternative kombuchas enriched in phenolic compounds and with a high antioxidant activity using fruits instead of tea leaves, and to determine the optimal fermentation time. The fruit-based kombuchas were prepared using cherry, plum, apricot, strawberry, persimmon, grape, orange, or pomegranate and three different microbial consortia (SCOBYs/SCs). The pH, carbohydrate, ethanol, and phenolic levels, and *in vitro* antioxidant capacity were determined along the fermentation process (21 days). The obtained products showed safe pH values (2.5–4.2) and suitable ethanol levels (<1.2%). Their carbohydrate content was almost half that in commercial tea kombucha. The activity of fermentative microorganisms increased total phenolic content (TPC) and radical scavenging capacity (TEAC) in all preparations, and differences were found depending on the SC used. Maximum TPC and TEAC values were observed in the strawberry kombucha (up to 13.7 mg/100 mL and 2.05 μmol/mL, respectively). The results showed that fruits, mainly strawberries, could be used to obtain alternative kombucha drinks with high *in vitro* antioxidant activity.

1. Introduction

Kombucha beverages have experienced an extraordinary boom during the last decade; however, they are estimated to have originated over 2200 years ago and are geographically linked to northeastern China, particularly in the Manchuria region (Bishop et al., 2022; Kapp & Sumner, 2019). These drinks are traditionally prepared using black and green tea leaves and sugar through the fermentative activity of a diverse and variable mixture of bacteria and yeasts called 'SCOBY' (Symbiotic Consortium of Bacteria and Yeasts). This SCOBY (SC) is usually composed by lactic acid bacteria (LAB, such as *Lactobacillus* and *Oenococcus* spp.), acetic acid bacteria (AAB, *e.g.*, *Acetobacter*, *Gluconobacter,* and *Komagataeibacter* spp.), and a wide range of yeasts, such as *Saccharomyces*, *Debaryomyces, and Kluyveromyces* spp. (Coton et al., 2017; Gaggìa et al., 2019). Its specific composition determines the optimal fermentation time, which is normally set between 7 and 10 days, as well as the nutritional profile and content of bioactive compounds of the resultant kombuchas (Morales, 2020; Torán-Pereg et al., 2021). From a molecular perspective, these tea-derived kombuchas contain, among others, sugars, organic acids, minerals, vitamins, proteins, low ethanol levels, phenolic compounds such as flavonoids, and other specific functional bioactives such as D-saccharic acid-1, 4-lactone (DSL) (De Roos & De Vuyst, 2018).

Despite the widespread use of tea leaves (Teixeira Oliveira et al., 2023; Wang et al., 2022), other raw materials have been tested in recent years to develop alternative kombuchas with different organoleptic and functional properties. In addition to cereals, herbs, mushrooms, and

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Abbreviations: SCOBY, SC (symbiotic consortium of bacteria and yeasts); LAB, (lactic acid bacteria); AAB, (acetic acid bacteria); TCC, (total carbohydrate content); TPC, (total phenolic content); TEAC, (Trolox equivalent antioxidant activity).

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https://doi.org/10.1016/j.lwt.2023.115482
Received 24 July 2023; Received in revised form 23 September 2023; Accepted 28 October 2023

algae, fruits have been used to complement tea leaves in these beverages (Freitas et al., 2022). To date, a great variety of them have been tested, such as grapes (Ayed et al., 2017), coconuts (Watawana et al., 2016), apples (Zubaidah et al., 2018), papaya (Sharifudin et al., 2021), and strawberry tree fruits (Tejedor-Calvo and Morales, 2023). These tea substitutes generate kombuchas with different chemical compositions and sensory attributes than those of tea-derived kombuchas. Although further research and clinical trials are required to validate kombucha bioactivities, promising results have been reported using *in vitro* and animal models in terms of their antioxidant, immunomodulatory, antiproliferative, and antimicrobial activities (Ivanišová et al., 2020; Júnior et al., 2022). Given that their functional properties depend on their bioactive constituents, these kombucha analogues may have different functional properties than the traditional tea-derived kombuchas.

Oxidative stress is a major health concern in modern lifes. This imbalance occurs when biological mechanisms cannot alleviate production and accumulation of reactive oxygen species in cells and tissues. The resultant damage may subsequently lead to disorders associated with cancer and cardiovascular, renal, respiratory, or neurological diseases (Pizzino et al., 2017). To address this issue, dietary antioxidants have been exhaustively investigated and plant species have been subjected to different procedures to obtain antioxidant fractions or compounds (Morales, 2022; Yusoff et al., 2022).

In this sense, since many fruits have an interesting phenolic composition and these compounds have demonstrated great antioxidant potential (Iglesias-Carres et al., 2019; Nayak et al., 2015), they may be used to obtain phenolic-rich kombuchas with antioxidant capacity without the addition of tea leaves. Most of the investigated and commercial kombuchas, which incorporate alternative substrates in their composition, combine them with tea leaves (Klawpiyapamornkun et al., 2023; Leonarski, Guimarães, Cesca, & Poletto, 2022). Therefore, the aim of this study was to obtain fruit-based kombuchas with enriched phenolic content and high antioxidant activity using fruits instead of tea leaves and different SCs and to determine the optimal fermentation time. The assessment of differential SCs effects with specific microbial compositions (Tejedor-Calvo and Morales, 2023) reinforced the current knowledge about the fermentation process and alleviated some disadvantages of produced kombuchas, such as too high or low pH values and high ethanol levels. To achieve this objective, eight kombuchas were prepared using individual fruits (cherry, plum, apricot, strawberry, persimmon, grape, orange, and pomegranate) and without tea leaves and a specific SC. The phenolic, carbohydrate, and ethanol contents, as well as the pH and antioxidant activity of these fruit-based kombuchas were analyzed during the whole fermentation process from 0 to 21 days. The effects of SCs on the elaboration of fruit-derived kombuchas were also investigated in kombuchas elaborated using four selected fruits and two additional SCs.

2. Materials & methods

2.1. Biological material

Eight fruit species were selected because of their well-described phenolic composition and antioxidant capacity (Ayub et al., 2023; Ben Hsouna et al., 2023; Gonçalves et al., 2023; Koraqi et al., 2023; Silva et al., 2023; Vo et al., 2022; Zargar et al., 2023), and were purchased from local markets: cherry (*Prunus avium*), plum (*Prunus domestica*), apricot (*Prunus armeniaca*), strawberry (*Fragaria* x *ananassa*), persimmon (*Diospyros kaki*), grape (*Vitis vinifera*), orange (*Citrus* x *sinensis*), and pomegranate (*Punica granatum*). Pedicels were manually removed, and whole fruits, including skins, seeds, and pulp were mechanically mixed and freeze-dried (LyoAlfa 15–85, Telstar, Terrasa, Spain) until a fine powder was obtained, which was stored in darkness at −20 °C until kombucha elaboration. Cherry, plum, apricot, and persimmon seeds, as well as orange and pomegranate peels, were removed before trituration. Powdered dried green tea leaves (Hornimans, London, United Kingdom) were acquired from a local market. Three different SCs were utilized: SC1, kindly provided by a local producer; SC2, purchased in "Oh My Kefir!" and SC3, prepared in the laboratory following the procedure described by Tejedor-Calvo and Morales (2023). Briefly, tap water (3 L) was heated (80 °C) before the addition of powdered tea leaves (8 g/L) and white cane sugar (80 g/L) (Acor, Valladolid, Spain). The mixture was vigorously stirred, homogenized, and cooled to room temperature (RT = 24.5 ± 0.5 °C) prior to the addition of non-pasteurized commercial kombucha (275 mL) (Kombutxa, Mataró, Spain). Then, the resultant mixture was transferred to a sterile glass receptacle that was kept open but covered with filter paper and stored in the dark at RT for 21 days.

2.2. Reagents

Methanol (HPLC grade) was obtained from Lab-SCAN (Gliwice, Poland). Sodium carbonate (Na_2CO_3) and sulfuric acid (H_2SO_4) were purchased from Panreac (Barcelona, Spain). Phenol, HCl (37%), Folin-Cioucalteu's phenol reagent, D-glucose, gallic acid, 2,2-diphenyl-1-pircrylhydrazyl (DPPH●), and Trolox were acquired from Sigma-Aldrich Química (Madrid, Spain).

2.3. Kombucha elaboration

The fruit kombuchas were prepared (in duplicate) according to the protocol described by Tejedor-Calvo and Morales (2023), with some modifications. Briefly, lyophilized fruit powder (12 g/L) was added to hot tap water (80 °C), together with white cane sugar (70 g/L). The mixture was vigorously stirred to obtain a homogenized suspension that was cooled down to RT (24.5 ± 0.5 °C) to include SC1, SC2, or SC3 (40 g/L) and 10% v/v of the medium where the SCOBY was previously maintained ('*old kombucha*') to ensure an initial pH value (<4.2) that avoided the growth of undesired microorganisms. The recipients with kombuchas were kept open, cover with filter paper, and stored in darkness at RT for 21 days in the case of SC1 and 14 days with SC2 and SC3. The first experiments were carried out with SC1 in all fruits, and dried tea leaves were subjected to the same process to elaborate traditional kombucha, which was used as a control. After analysing the results, four fruits (plum, strawberry, grape, and pomegranate) were selected (mainly targeting TPC counts and antioxidant capacity but also considering pH, total carbohydrate, and ethanol levels) for further assays using SC2 and SC3. The microbial composition of the SCs was described by Tejedor-Calvo and Morales (2023).

2.4. Characterization of kombuchas

Kombuchas were characterized at different fermentation times (0, 2, 4, 7, 11, 14, 17, and 21 days). pH was measured using a MicropH 2000 pH meter (Crison Instruments, Barcelona, Spain). The total carbohydrate content (TCC) was quantified using the phenol-sulfuric acid method described by Ribeiro Smiderle et al. (2017). The kombucha samples were thoroughly diluted in water and added (25 μL) in triplicate to a 96-well plate, followed by a 5 % phenol solution (25 μL) and concentrated H_2SO_4 (125 μL). The plate was sealed and incubated at 80 °C for 30 min, and absorbance was measured at 490 nm. D-glucose was used as the standard for quantification. To determine the total alcohol content, a hand-held refractometer (Alla France, Anjou, France) was used. The results were expressed as % (v/v). Finally, the total phenol concentration (TPC) of the kombuchas was determined using the Folin-Ciocalteu method following the procedure described by Ramírez-Anguiano et al. (2007) and adapted by Tejedor-Calvo and Morales (2023). Kombucha samples (50 μL) were mixed with 1.48% HCl (300 μL) and methanol (150 μL) in triplicates and centrifuged for 2 min at 13000 rpm. Supernatants (50 μL) were added to 1 mL of 2% Na_2CO_3 (w/v), and the mixtures were vigorously stirred and incubated at RT for 3 min prior to the addition of Folin-Ciocalteu reagent (25 μL). After incubating the samples (RT, 30 min), the absorbance was measured at 750 nm. Gallic acid was used as a standard for quantification.

2.5. *Determination of free radical scavenging activity*

The DPPH$^{\bullet}$ scavenging activity of kombucha samples was determined adapting the protocol described by Morales et al. (2018). Aliquots collected on different fermentation days (0, 2, 4, 7, 11, 14, 17, and 21) were mixed at different concentrations (1.5–100 μL/mL) with methanolic DPPH$^{\bullet}$ solution (76 μM). Absorbance at 517 nm was recorded after 15 min of incubation at RT in the dark. Results were calculated and expressed as TEAC (Trolox equivalent antioxidant capacity) values.

2.6. *Statistical analysis*

Differences were evaluated at a 95% confidence level ($p \leq 0.05$) using one-way analysis of variance (ANOVA), followed by Tukey's Multiple Comparison test. Statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, USA). Outliers were identified using the same program and removed before the statistical analysis.

3. Results & discussion

3.1. *Evolution of pH along SC1 fermentation*

The initial pH of fruit solutions (before SC1 fermentation) oscillated between 3.30 and 3.44, with apricot and cherry solutions showing the highest pH values (3.44 and 3.43, respectively), while orange solution presented the lowest starting pH value (Fig. 1). All showed lower pH than that of the tea solution (3.66). These differences ($p < 0.0001$) could be due to the dissimilar composition of the raw materials, particularly because of the organic acid content (Walker & Famiani, 2018). This fact was also observed in a recent study that investigated the addition of Indian gooseberry (*Phyllantus emblica*) fruits and juices to traditional tea kombucha, with an initial pH close to 4.0 in the non-treated tea beverage and close to 3.0, in the case of gooseberry-treated ones (Klawpiyapamornkun et al., 2023). Despite these slight initial variations, most of the fruit-derived kombuchas showed a very similar pattern in decreasing pH due to SC1 fermentation: slight and slow reduction during the first days followed by a pronounced fall (particularly with pomegranate after day 7, similar to that in tea, and with plum and persimmon after days 2 and 4, respectively) and finally reaching pH values from 2.03 to 2.75 after 21 days, with the lowest values observed in persimmon-derived kombucha and the highest in grape-derived kombucha (Fig. 1). These results are in concordance with previous studies that utilized the same SC to ferment strawberry tree fruits (Tejedor-Calvo and Morales, 2023) or other microbial consortia to ferment green, black, and oolong teas (Kaewkod, Bovonsombut, & Tragoolpua, 2019; X. Wang et al., 2022) and also fruits such as grape juice (Ayed et al., 2017), papaya (Sharifudin et al., 2021), apple (Zubaidah et al., 2018), black grape, black mulberry and rosehip fruits (Tomar, 2023). This acidity increase is due to the activity of AAB because i) these bacteria produce not only acetic acid, but also gluconic and glucuronic acids, and ii) the release of fruit acids from the fruit matrix to the kombucha media (De Filippis et al., 2018; Li et al., 2022).

The pH range considered safe for human consumption is between 2.5 and 4.2, as lower values indicate excessive acetic acid levels, and higher values are correlated with a significant risk of undesired microorganism growth (Cardoso et al., 2020). Therefore, the aim of kombucha producers is to bottle kombuchas at a pH range of 2.5–4.2. The upper limit was not exceeded by any kombucha analog during the whole process in the present study; however, values below 2.5 were reached after 7 days for cherry- and persimmon-derived kombuchas, 11 days for pomegranate kombuchas, and 21 days for plum-derived kombuchas (Fig. 1). These results suggest that fermentation must be stopped before acetic acid production surpasses the safe range. Normally, kombucha fermentation is carried out for 7–10 days, so this 3 week-experiment can be easily shortened since the last days are not required for the final product development (Kapp & Sumner, 2019).

3.2. *Evolution of total carbohydrate content along SC1 fermentation*

Because a high amount of sugar was added to allow SC1 activity, its consumption by kombucha microorganisms must be monitored to

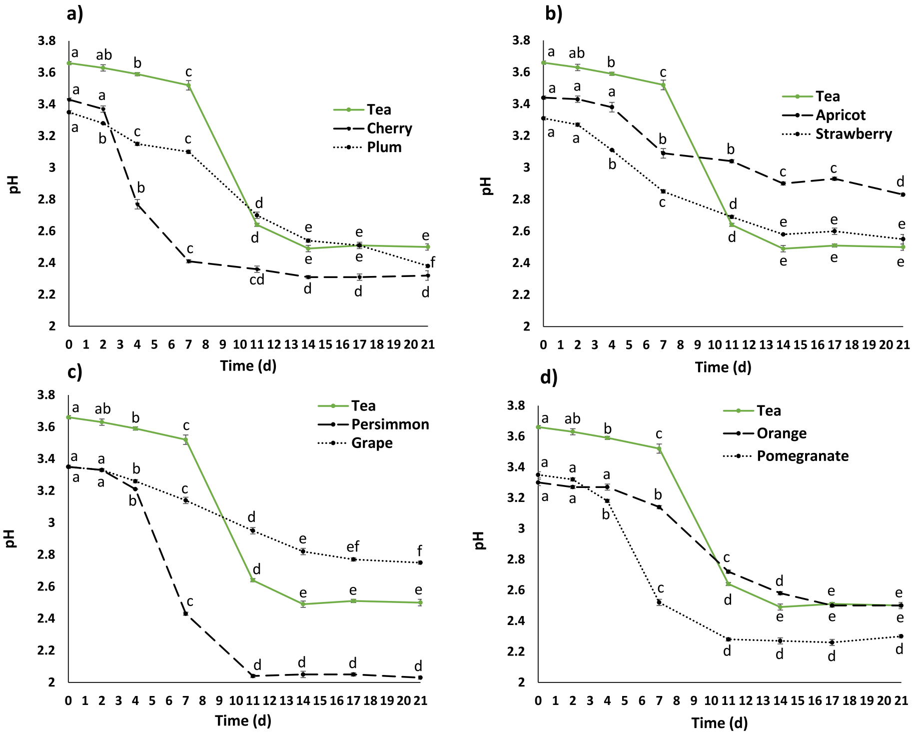

Fig. 1. Evolution of pH values along fermentation (21 days) of fruit-derived kombuchas: cherry, plum (a), apricot, strawberry (b), persimmon, grape (c), orange and pomegranate (d) with SCOBY (SC) 1. Tea kombucha was included as a reference of traditional kombucha. Different letters (a–f) denote significant differences (One-way ANOVA, Tukey's test, $p \leq 0.05$) for the same kombucha at different fermentation times. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

obtain a final product with reduced carbohydrate content that can be consumed as an interesting drink from a nutritional perspective. When kombuchas were prepared, the total carbohydrate content (TCC) was measured, and slight differences were observed, ranging from 7.0% in pomegranate-derived kombucha to 11.1% in cherry kombucha ($p < 0.001$ comparing the lowest and highest values) (Fig. 2). These variations are caused by the dissimilar carbohydrate composition of the tea and fruits, as the TCC provided by the sugar cane and SC1 is the same in all cases. Despite the different starting contents, all kombuchas experienced an evident decrease in TCC due to the sugar utilization by SC1 microorganisms (Fig. 2). During the first week, the reduction was particularly rapid in the case of kombuchas from strawberry and persimmon, with consumption rates of 47 (from 9.5 to 5.0%) and 43 % (from 8.6 to 4.9%), respectively. On the contrary, tea and pomegranate recorded the lowest drops, consuming the 13 (from 10.9 to 9.5%) and 11 % (from 7.0 to 6.2%) of the TCC, respectively. The second week followed a linearly diminishing tendency for most of the kombuchas, with two exceptions: i) persimmon beverage, which reached a TCC of 3.6% on day 11, and no significant decreases were observed below this value, suggesting that the remaining carbohydrates could not be utilized by SC1 bacteria or yeasts, and ii) pomegranate-derived kombucha, which showed very stable values from day 2 to day 17, and the only significant decrease was registered from day 17 to day 21. The reason for this delayed consumption might be linked to the structure of pomegranate carbohydrates, since there is a relevant presence of pectins and/or high molecular weight polysaccharides, particularly in the fruit peels and membranes that were included to prepare the powder used to prepare the kombuchas (Shakhmatov et al., 2019). Therefore, these molecules may require further activity from microorganisms and their enzymes to process the complex structures and then release available and simpler sugar forms that can be accessible for consumption by SC1 (Laavanya, Shirkole, & Balasubramanian, 2021).

After 3 weeks of fermentation, some remnant carbohydrate levels were found in the samples, ranging from 1.5 (grape) to 4.0% (cherry), corresponding to non-utilizable carbohydrates and possibly to those structures directly linked to SC1 (Laavanya, Shirkole, & Balasubramanian, 2021). These behaviours were previously observed in studies on traditional kombuchas; for instance, initial sucrose content (5%) in green and black tea kombucha was reduced after 10 days of fermentation to a remaining concentration of 2% and 3.5%, respectively (Cardoso et al., 2020). Similar results were reported for fruit kombuchas: strawberry tree kombuchas reduced its TCC from 8.8% to 2.4% after 21 days (Tejedor-Calvo and Morales, 2023); papaya kombuchas °Brix halved from 14° to 7° after 4 days (Sharifudin et al., 2021); and total sugar content in apple kombuchas was reduced from 14-16% to 4–6% depending on the apple variety after 14 days (Zubaidah et al., 2018). Other substrates such as cashew nuts beverages noticed these reductions along kombucha fermentation since the utilized SC seemed to consume almost 60% of the available sucrose in 72 h (from 0.25 to 0.10 mg/100 mL) (Araujo Filho et al., 2023).

From a nutritional and functional perspective and considering that conventional kombuchas are bottled stopping fermentation 7–10 days after the initial preparation, the TCCs of fruit kombuchas that were obtained in this study were remarkable. The observed values were lower than those shown by tea kombucha used as a traditional reference after 7 days (9.5%), particularly in the case of strawberry, persimmon, and orange (4.9–5%). Moreover, these levels were significantly lower than those described for other beverages such as carbonated drinks or fruit juices (9–10%) (Lin et al., 2018), being these fruit-based kombuchas a promising candidate to replace them as a nutritional and healthy alternative.

3.3. *Evolution of alcohol content along SC1 fermentation*

One of the major concerns in kombucha production is the control of ethanol production during the process, as its functional and potentially healthy properties must be within the context of a non-alcoholic beverage. Drinks must be labelled as alcoholic beverages when their alcohol volume exceeds 1.2%, and they are obviously excluded from any nutritional or health claims (European Parliament & Council of the European Union, 2011). In the present study, the 1.2% limit was surpassed by only a few fruit-derived kombuchas on specific days (Table 1). All of them showed non-detectable ethanol levels before the fermentation process started. For instance, in the case of tea kombucha, used as a

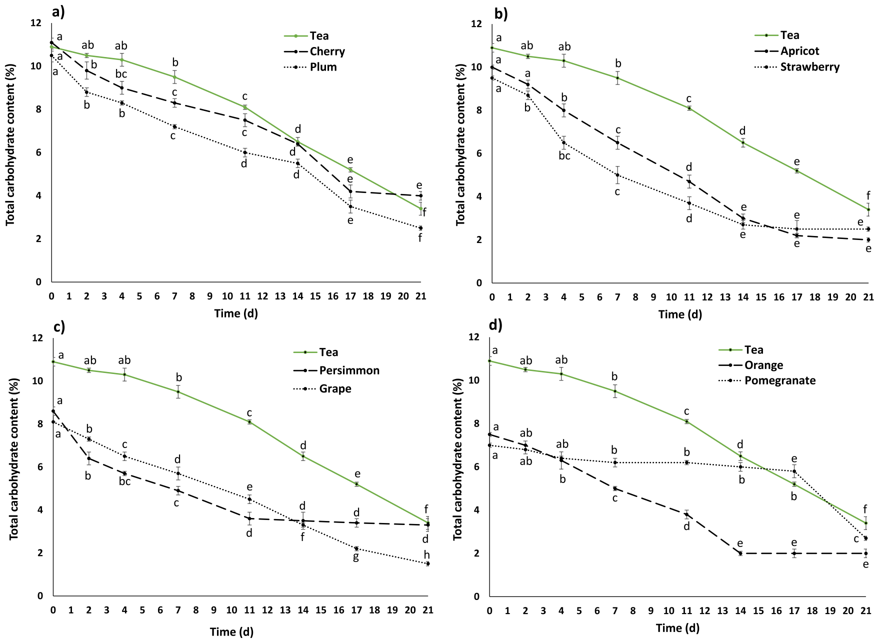

Fig. 2. Evolution of total carbohydrate content along fermentation (21 days) of fruit-derived kombuchas: cherry, plum (a), apricot, strawberry (b), persimmon, grape (c), orange and pomegranate (d) with SCOBY (SC) 1. Tea kombucha was included as a reference of traditional kombucha. Different letters (a–h) denote significant differences (One-way ANOVA, Tukey's test, $p \leq 0.05$) for the same kombucha at different fermentation times. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reference, ethanol levels were over 1.2% from day 2 to day 11 (ranging from 1.7 to 2.6%). However, these values decreased during the last week owing to the action of AAB. Moreover, cherry-derived kombucha also showed high levels from day 2 to day 4 (2.6%), as did apricot and orange kombuchas on day 4 (1.6 and 3.1%, respectively), plum on day 7, and grape on day 14 (1.6% in both cases) (Table 1). It can be noticed that each fruit led to different ethanol production rates by yeasts and bacteria and to different ethanol consumption kinetics by the action of AAB. The obtained tendency was in concordance with previous studies that determined the ethanol content in tea and fruit kombuchas during fermentation of various lengths; however, the particular values significantly differed depending on the sample. For instance, kombucha obtained from green and black tea reached values below 0.6% after 10 days (Cardoso et al., 2020); however, a previous study on black tea kombucha showed a higher alcoholic degree, registering levels up to 4.11% (after 11 days), which decreased only to 1.4% after 21 days (exceeding the 1.2% limit) (Villarreal-Soto et al., 2019). Moreover, kombucha prepared with papaya juices showed ethanol curves that were similar to those obtained in the present study, although the process seemed to be faster: ethanol levels were practically null before fermentation started, reached maximum percentages on day 2 (0.95–1.18%), and promptly decreased again close to 0% after 4 days (Sharifudin et al., 2021). Furthermore, when Indian gooseberry fruits or juices were added to tea kombucha, ethanol production was higher than that in our fruit-based kombuchas, with the highest levels observed after 12 days (2% for beverages that included gooseberry juices and less than 0.5% for tea kombucha) (Klawpiyapamornkun et al., 2023). The ethanol generated in gooseberry drinks was rapidly consumed and reduced to values close to 0.5%, without significant differences when compared with tea kombucha (Klawpiyapamornkun et al., 2023).

Returning to the results of the present study (Table 1), the prepared fruit-derived kombuchas should be bottled before or after the ethanol peaks, preferably when the levels are below 1.2%. Furthermore, dealcoholisation procedures may be applied, as long as these methods do not significantly increase the process expenses or negatively affect the concentration of biologically active molecules (Müller et al., 2016).

3.4. Evolution of total phenolic content along SC1 fermentation

Moreover, TPC was analyzed in the prepared kombuchas (Table 2). Initially, the TPC of fruit solutions (before the fermentation) ranged between 9.7 and 2.9 mg/100 mL, with the highest values being for strawberry (9.7 mg/100 mL), followed by plum (6.6 mg/100 mL) and the lowest for orange and persimmon (2.9 and 3.0 mg/100 mL, respectively). In all cases, the TPC was significantly lower than that of the tea preparation (18.4 mg/100 mL), all these differences were caused by the dissimilar phenolic content and profile of tea and the studied fruits (Fu et al., 2011). When SC1 was added to the fruit or tea solutions, TPC increased from day 2 of fermentation to day 7 in most of the fruit-derived kombuchas, except for plum-derived kombucha (which, as well as tea, showed this peak at day 2) and for orange and persimmon-derived kombuchas, which exerted a delayed increase and maximum points on days 11 and 14, respectively. This increase was followed by a subsequent reduction in TPC in all cases (Table 2). The earlier TPC increase observed in tea kombucha was also detected in other reported kombuchas elaborated using black, oolong, and green tea, which showed a rapid increase in TPC in only 3 days (Kaewkod, Bovonsombut, & Tragoolpua, 2019). Although research on black grape, black mulberry, and rosehip fruit kombuchas noticed a direct reduction in TPCs, it seems to be because the first quantification was carried out after 7 days and the TPC increase could occur before this time (Tomar, 2023). In addition, a rising-declining movement was also observed in red grape juice and strawberry tree kombuchas (Ayed et al., 2017; Tejedor-Calvo and Morales, 2023). This phenomenon may be explained by different factors such as low pH and microbial action, which contribute to the enzymatic release of phenolic species (acid hydrolysis, depolymerization, etc.), particularly small monomers that augment TPC counts. In this regard, these factors (pH and microbial enzymatic activity, which are also related to the utilized SC) contribute to the degradation of the vegetal cell wall components (lignin, cellulose, hemicellulose, etc.), which are in varied proportions depending on the fruit, resulting in different TPC release kinetics for each kombucha (Szymanska-Chargot, Chylinska, Gdula, Koziol, & Zdunek, 2017). Later, the successive decrease may have been caused by acidic degradation at extremely low pH, microbial activity, and polymerization of some of the small phenols into high-molecular-weight polyphenolic structures, resulting in lower TPC values (Ayed et al., 2017).

Although fruit kombuchas did not reach the highest TPC noticed in tea one (27.1 mg/100 mL), interesting values were recorded for some fruit-derived kombuchas such as those obtained from cherry, strawberry, grape and plum (16.5, 13.7, 12.3 and 11.4 mg/100 mL, respectively), placing these kombucha analogues as relevant tea kombucha alternatives.

3.5. Evolution of $DPPH^{\bullet}$ scavenging activity along SC1 fermentation

Similar to the TPC, the antioxidant capacity of the prepared fruit kombuchas increased because of the fermentation activity of SC1 (Table 3). In most cases, the highest TEAC values were obtained between days 4 and 11 and seemed to be linked to the observed TPC pattern. However, this correlation is not always strict, indicating that not only is the amount of total phenols relevant for antioxidant power, but also their structure and availability (Li et al., 2023; Moazzen, Öztinen, Ak-Sakalli, & Koşar, 2022). In this sense, tea kombucha showed the highest TPC values and it was also the most active in terms of $DPPH^{\bullet}$ scavenging capacity, showing a TEAC = 4.16 μmol Trolox eq/mL at day 11, when phenolic degradation was already started. Even superior TEAC values (8.2 μmol Trolox eq/mL) have been reported for green tea kombucha (Cardoso et al., 2020). In the case of fruit kombuchas, as occurred with TPC, they did not reach the TEAC value shown by tea

Table 1
Evolution of alcohol content (%) along SCOBY 1 (SC1) fermentation of tea and fruit kombuchas. Different letters denote statistical significance (One-way ANOVA, Tukey's test, $p \leq 0.05$) between different fermentation days for the same kombucha (A-D) and between different raw materials for the same fermentation day (a-c). n.d. = non detected.

	Alcohol content (% v/v)							
	Day 0	Day 2	Day 4	Day 7	Day 11	Day 14	Day 17	Day 21
Tea	n.d.^{D,a}	$1.8 \pm 0.1^{B,b}$	$2.6 \pm 0.2^{A,b}$	$1.8 \pm 0.2^{B,a}$	$1.7 \pm 0.2^{B,a}$	$0.6 \pm 0.3^{CD,b}$	$1.0 \pm 0.3^{C,a}$	n.d.^{D,a}
Cherry	n.d.^{C,a}	$2.6 \pm 0.4^{A,a}$	$2.6 \pm 0.3^{A,b}$	$1.2 \pm 0.2^{B,ab}$	$0.9 \pm 0.3^{BC,b}$	$0.4 \pm 0.1^{BC,bc}$	n.d.^{C,b}	n.d.^{C,a}
Plum	n.d.^{C,a}	$0.9 \pm 0.1^{B,c}$	$0.8 \pm 0.0^{B,cd}$	$1.6 \pm 0.1^{A,ab}$	$0.6 \pm 0.2^{BC,bc}$	$0.3 \pm 0.1^{C,bc}$	$0.3 \pm 0.1^{C,b}$	n.d.^{C,a}
Apricot	n.d.^{B,a}	$1.0 \pm 0.1^{A,c}$	$1.6 \pm 0.1^{A,c}$	$1.0 \pm 0.3^{A,b}$	n.d.B,^{c}	n.d.^{B,c}	n.d.^{B,b}	n.d.^{B,a}
Strawberry	n.d.^{C,a}	n.d.^{C,d}	$0.3 \pm 0.1^{B,d}$	$0.5 \pm 0.1^{A,b}$	$0.3 \pm 0.0^{B,bc}$	n.d.^{C,c}	n.d.^{C,b}	n.d.^{C,a}
Persimmon	n.d.^{C,a}	n.d.^{C,d}	n.d.C,^{d}	n.d.^{C,b}	n.d.C,^{c}	$0.4 \pm 0.1^{B,bc}$	$1.3 \pm 0.2^{A,a}$	n.d.^{C,a}
Grape	n.d.^{C,a}	n.d.^{C,d}	$0.4 \pm 0.0^{C,d}$	$1.1 \pm 0.1^{B,ab}$	$1.2 \pm 0.3^{AB,ab}$	$1.6 \pm 0.1^{A,a}$	$0.4 \pm 0.0^{C,b}$	n.d.^{C,a}
Orange	n.d.^{C,a}	n.d.^{C,d}	$3.1 \pm 0.4^{A,a}$	$0.2 \pm 0.1^{C,b}$	$1.3 \pm 0.2^{B,ab}$	n.d.^{C,c}	$0.1 \pm 0.0^{C,b}$	$0.1 \pm 0.0^{C,a}$
Pomegranate	n.d.^{C,a}	n.d.^{C,d}	$0.2 \pm 0.0^{B,d}$	$0.6 \pm 0.1^{A,b}$	n.d.C,^{c}	n.d.^{C,c}	n.d.^{C,b}	n.d.^{C,a}

Table 2
Evolution of total phenolic content (mg/100 mL) along SCOBY 1 (SC1) fermentation of tea and fruit-derived kombuchas. Different letters denote statistical significance (One-way ANOVA, Tukey's test, $p \leq 0.05$) between different fermentation days for the same kombucha (A-G) and between different raw materials for the same fermentation day (a-h).

	Total phenolic compounds (mg/100 mL)							
	Day 0	Day 2	Day 4	Day 7	Day 11	Day 14	Day 17	Day 21
Tea	$18.41 \pm 0.23^{C,a}$	$27.07 \pm 0.17^{A,a}$	$17.61 \pm 0.50^{C,a}$	$23.19 \pm 0.57^{B,a}$	$14.62 \pm 1.14^{D,a}$	$13.30 \pm 0.83^{D,a}$	$7.73 \pm 0.17^{E,b}$	$8.61 \pm 0.26^{E,a}$
Cherry	$3.72 \pm 0.09^{E,f}$	$11.45 \pm 0.00^{C,c}$	$13.39 \pm 0.38^{BC,b}$	$16.47 \pm 0.41^{A,b}$	$13.79 \pm 1.05^{B,a}$	$12.30 \pm 0.84^{C,a}$	$8.90 \pm 0.06^{D,a}$	$7.86 \pm 0.03^{D,b}$
Plum	$6.59 \pm 0.17^{D,c}$	$11.40 \pm 0.10^{A,c}$	$8.08 \pm 0.19^{C,d}$	$9.38 \pm 0.09^{B,e}$	$7.70 \pm 0.18^{C,cd}$	$5.72 \pm 0.21^{E,c}$	$3.24 \pm 0.06^{F,f}$	$5.38 \pm 0.06^{E,e}$
Apricot	$3.52 \pm 0.00^{C,f}$	$4.05 \pm 0.06^{B,f}$	$4.29 \pm 0.09^{B,e}$	$5.27 \pm 0.09^{A,g}$	$2.36 \pm 0.12^{E,f}$	$2.95 \pm 0.12^{D,e}$	$1.28 \pm 0.10^{G,g}$	$1.73 \pm 0.06^{F,i}$
Strawberry	$9.70 \pm 0.09^{D,b}$	$12.29 \pm 0.29^{B,b}$	$12.31 \pm 0.25^{B,c}$	$13.66 \pm 0.43^{A,c}$	$10.29 \pm 0.18^{C,b}$	$6.69 \pm 0.12^{F,bc}$	$6.70 \pm 0.11^{F,c}$	$7.06 \pm 0.13^{E,c}$
Persimmon	$2.97 \pm 0.09^{D,g}$	$4.96 \pm 0.26^{BC,e}$	$4.67 \pm 0.75^{BC,e}$	$4.56 \pm 0.00^{BC,g}$	$5.97 \pm 0.16^{A,de}$	$6.62 \pm 0.06^{A,bc}$	$4.13 \pm 0.15^{C,de}$	$4.97 \pm 0.11^{B,f}$
Grape	$5.35 \pm 0.09^{D,d}$	$8.77 \pm 0.17^{B,d}$	$11.49 \pm 0.09^{A,c}$	$12.31 \pm 0.68^{A,d}$	$8.63 \pm 0.10^{B,c}$	$7.49 \pm 0.21^{C,b}$	$4.47 \pm 0.39^{E,d}$	$3.65 \pm 0.09^{E,g}$
Orange	$2.92 \pm 0.00^{CD,g}$	$5.35 \pm 0.64^{A,e}$	$4.35 \pm 0.09^{B,e}$	$4.42 \pm 0.03^{B,g}$	$4.96 \pm 0.12^{AB,e}$	$4.55 \pm 0.22^{B,cd}$	$3.05 \pm 0.06^{C,f}$	$2.25 \pm 0.03^{D,h}$
Pomegranate	$4.06 \pm 0.09^{E,e}$	$5.41 \pm 0.17^{CD,e}$	$5.00 \pm 0.19^{D,e}$	$7.38 \pm 0.09^{A,f}$	$6.55 \pm 0.27^{B,d}$	$4.21 \pm 0.41^{E,d}$	$3.85 \pm 0.06^{E,e}$	$5.77 \pm 0.03^{C,d}$

Table 3
Evolution of DPPH● scavenging activity (expressed as TEAC value, μmol Trolox eq/mL) along SCOBY 1 (SC1) fermentation of tea and fruit-derived kombuchas. Different letters denote statistical significance (One-way ANOVA, Tukey's test, $p \leq 0.05$) between different fermentation days for the same kombucha (A-D) and between different raw materials for the same fermentation day (a-c).

	TEAC value (μmol Trolox equivalents/mL)							
	Day 0	Day 2	Day 4	Day 7	Day 11	Day 14	Day 17	Day 21
Tea	$2.19 \pm 0.22^{BC,a}$	$2.44 \pm 0.33^{B,a}$	$2.55 \pm 0.04^{B,a}$	$3.59 \pm 0.28^{A,a}$	$4.16 \pm 0.14^{A,a}$	$3.97 \pm 0.40^{A,a}$	$2.17 \pm 0.21^{BC,a}$	$1.60 \pm 0.12^{C,a}$
Cherry	$0.07 \pm 0.01^{D,c}$	$0.25 \pm 0.02^{B,c}$	$0.20 \pm 0.01^{BC,c}$	$0.41 \pm 0.04^{A,bc}$	$0.16 \pm 0.02^{C,c}$	$0.21 \pm 0.03^{BC,b}$	$0.16 \pm 0.01^{C,c}$	$0.12 \pm 0.00^{CD,c}$
Plum	$0.26 \pm 0.03^{B,c}$	$0.25 \pm 0.05^{B,c}$	$0.23 \pm 0.02^{B,c}$	$0.56 \pm 0.01^{A,b}$	$0.20 \pm 0.02^{B,c}$	$0.15 \pm 0.01^{C,b}$	$0.15 \pm 0.02^{C,c}$	$0.14 \pm 0.02^{C,c}$
Apricot	$0.13 \pm 0.03^{B,c}$	$0.20 \pm 0.02^{A,c}$	$0.19 \pm 0.02^{A,c}$	$0.18 \pm 0.01^{A,c}$	$0.13 \pm 0.01^{B,c}$	$0.06 \pm 0.01^{C,b}$	$0.05 \pm 00^{C,c}$	$0.04 \pm 0.00^{C,c}$
Strawberry	$0.86 \pm 0.09^{B,b}$	$2.05 \pm 0.04^{A,b}$	$0.54 \pm 0.06^{C,bc}$	$0.49 \pm 0.04^{C,bc}$	$0.34 \pm 0.04^{D,bc}$	$0.35 \pm 0.07^{D,b}$	$0.26 \pm 0.01^{D,bc}$	$0.27 \pm 0.00^{D,b}$
Persimmon	$0.19 \pm 0.02^{B,c}$	$0.10 \pm 0.01^{CD,c}$	$0.09 \pm 0.02^{D,c}$	$0.09 \pm 0.02^{D,c}$	$0.29 \pm 0.02^{A,bc}$	$0.15 \pm 0.02^{BC,b}$	$0.13 \pm 0.01^{CD,c}$	$0.14 \pm 0.01^{C,c}$
Grape	$0.27 \pm 0.08^{B,c}$	$0.38 \pm 0.02^{AB,c}$	$0.34 \pm 0.04^{AB,c}$	$0.47 \pm 0.03^{A,bc}$	$0.38 \pm 0.08^{AB,bc}$	$0.39 \pm 0.04^{AB,b}$	$0.38 \pm 0.07^{AB,b}$	$0.35 \pm 0.03^{AB,b}$
Orange	$0.16 \pm 0.02^{BC,c}$	$0.15 \pm 0.03^{BC,c}$	$0.19 \pm 0.05^{BC,c}$	$0.23 \pm 0.05^{B,c}$	$0.43 \pm 0.04^{A,b}$	$0.13 \pm 0.02^{C,b}$	$0.08 \pm 00^{C,c}$	$0.11 \pm 0.03^{C,c}$
Pomegranate	$0.31 \pm 0.05^{B,c}$	$0.20 \pm 0.01^{BC,c}$	$0.65 \pm 0.19^{A,b}$	$0.50 \pm 0.02^{AB,bc}$	$0.21 \pm 0.04^{BC,c}$	$0.17 \pm 0.02^{BC,b}$	$0.09 \pm 0.01^{C,c}$	$0.10 \pm 0.00^{C,c}$

kombucha (Table 3), but some of these analogues could be considered promising antioxidant beverages, highlighting strawberry, pomegranate, plum, and grape kombuchas, which registered TEAC values of 2.05, 0.65, 0.56 and 0.47 μmol Trolox eq/mL, respectively, obtained on days 2, 4, 7, and 7. Furthermore, the utilized fermentation conditions seemed to be more favourable for the stability of their antioxidant power when compared with other studies, since kombuchas prepared with different fruits such as black grape, black mulberry, and rosehip fruits showed a rapid and continuous decrease in antioxidant capacity over 21 days of fermentation (Tomar, 2023).

Moreover, the results are particularly interesting when compared with other beverages traditionally considered antioxidant products, such as fruit juices. For instance, those elaborated from strawberry and pomegranate, which have much higher fruit content (10–30 times) than our fruit-based kombuchas, showed TEAC values between 3 and 4 μmol Trolox eq/mL (Piljac-Žegarac et al., 2009). Thus, the elaborated alternative kombuchas with a lower fruit amount (12 g/L) than fruit juices might act as an alternative antioxidant drink.

3.6. *Effect of the use of different SCs on selected fruit kombuchas*

The promising insights obtained from the fruit strawberries, pomegranates, plums, and grapes encouraged further research to evaluate the effects of different SCs on the antioxidant capacity, TPC, and other kombucha quality parameters, as the dissimilar microbial content of SCs may lead to modified phenol release and biological activities. As reported by Tejedor-Calvo and Morales (2023), SC1 contained higher counts of yeasts than SC3, whereas SC2 contained smaller populations of aerobic mesophilic microorganisms and lactic acid bacteria. Moreover, the total fermentation time was shortened to 14 days after evaluating the pH and alcohol levels.

3.6.1. *Effect of the use of different SCs on selected fruit kombuchas pH, total carbohydrate content and alcohol levels*

When different SCs were added to the sugared fruit solution, different initial pH values were observed (Fig. 3). This phenomenon was expected since this starting preparation included a relevant volume (10% v/v) of '*old kombucha*', the medium where each SCOBY was kept. This was previously observed when the same SCs were applied to other materials such as strawberry tree fruits (Tejedor-Calvo and Morales, 2023). In all cases, the highest and lowest pH values were recorded for SC3 and SC1, respectively, but the progression along the fermentation time was quite similar, regardless of SC or fruit. None of the SCs had pH levels above the 4.2-limit in any fruit, with the highest records registered for pomegranate-derived kombucha using SC2 and SC3 (pH 4.1). Moreover, this kombucha also showed a decrease below the 2.5-limit, reaching a pH of 2.47 for SC2 and SC3 on day 14, together with the already commented values for SC1 (Fig. 3), suggesting that fermentation must be stopped before this pH decrease. The observed variations between SCs might be explained, together with the differences in '*old kombucha*' pH, by the dissimilar microbial composition of the three SCs (Tejedor-Calvo and Morales, 2023). Differential proportions of microbial species have different impacts on pH evolution in the prepared kombuchas, and vice versa, which is in agreement with the related scientific literature. These studies confirmed that these variations also affected the sensory attributes of beverages (Tejedor-Calvo and Morales, 2023; Wang et al., 2020).

Regarding TCC, before fermentation started, fruit solutions containing SC1 showed a higher total carbohydrate percentage than ones containing SC2 and SC3, and no significant differences were observed between the other two SCs (Fig. 4). These variations were provoked by the carbohydrate composition of the SCs and were previously observed in other products elaborated with these consortia, with SC1 having the highest content (Tejedor-Calvo and Morales, 2023). Once the action of fermentative microorganisms began, SC2 and SC3 consumption rates and tendencies were very close to those exerted by SC1, but the lowest carbohydrate content was registered for SC2 and SC3 kombuchas at 14 days (0.8–3.0% for SC2/SC3; 2.7–6.0% for SC1) because the initial amount was also lower (6.4–9.3 % for SC2/SC3; 7.0–10.5 for SC1) (Fig. 4). This reduced content was also observed at day 7 (2.4–5.8% for SC2/SC3; 5.0–7.2% for SC1) (Fig. 4), being this

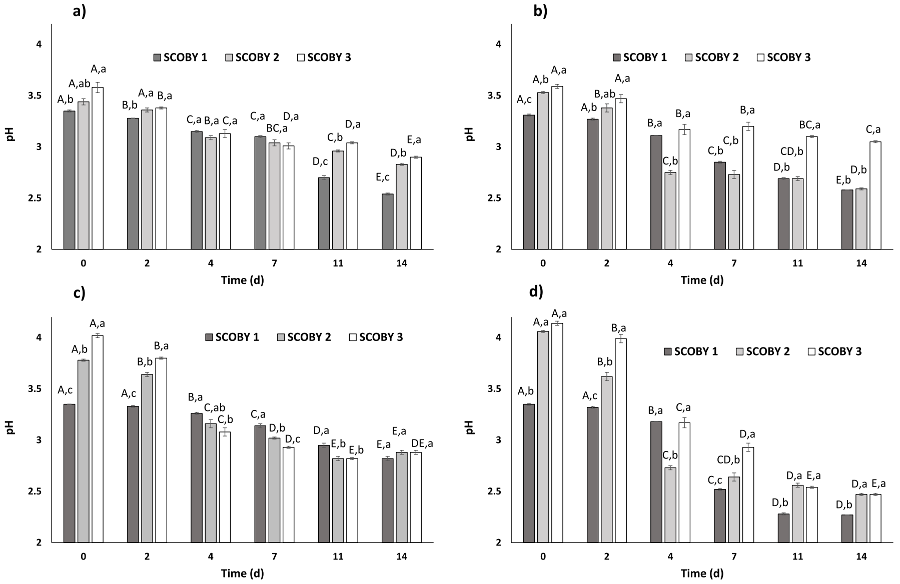

Fig. 3. Evolution of pH values along fermentation (14 days) of selected fruit kombuchas: plum (a), strawberry (b), grape (c) and pomegranate (d) with SCOBYs (SCs) 1, 2 and 3. Different letters denote significant differences for the same SC at different fermentation times (A–E) and for different SCs at the same fermentation time (a–c) (One-way ANOVA, Tukey's test, $p \leq 0.05$).

moment very common as final point in traditional kombucha fermentation so, from a nutritional and health perspective, SC2 and SC3 might be more adequate to design low-carbohydrate kombuchas, particularly when compared to the previously mentioned drinks (carbonated drinks, fruit juices, etc.) (Lin et al., 2018).

Furthermore, the replacement of SC1 by SC2 and SC3 was useful to mitigate ethanol production, avoiding increases that exceeded the 1.2% limit that was significantly surpassed by SC1 for many fruits (Tables 1 and 4). Thus, plum kombucha alcohol was below this limit and was only close after 11 days in SC3, with a 1.0% value that was rapidly neutralized by the action of AAB. A very similar situation was noticed for pomegranate kombucha with this SC, with 0.8% on day 11, which reduced again to 0.2% three days later. Grape kombucha also reached a close value, 0.9% at day 4 with SC2 and it was reduced even earlier, at day 7. Moreover, the alcohol content of strawberry kombucha was low in all SCs (Table 4). Compared to the alternative consortia, SC1 showed higher and faster ethanol production. This fact might be due to the initially higher load of yeast and carbohydrate content (not high enough to induce osmotic stress in microbial cells), leading to a more intense fermentative action during the first days (Da Silva Fernandes et al., 2022; Kostas et al., 2016). Later, low pH stimulated the growth of AAB, which reduced the alcoholic degree, in agreement with previous studies (Sharifudin et al., 2021; Villarreal-Soto et al., 2019).

3.6.2. Effect of the use of different SCs on selected fruit kombuchas total phenolic content and DPPH$^{\bullet}$ scavenging activity

As previously mentioned, the initial TPC of fruit solutions was lower than that observed in traditional tea kombuchas (Table 5), both in the

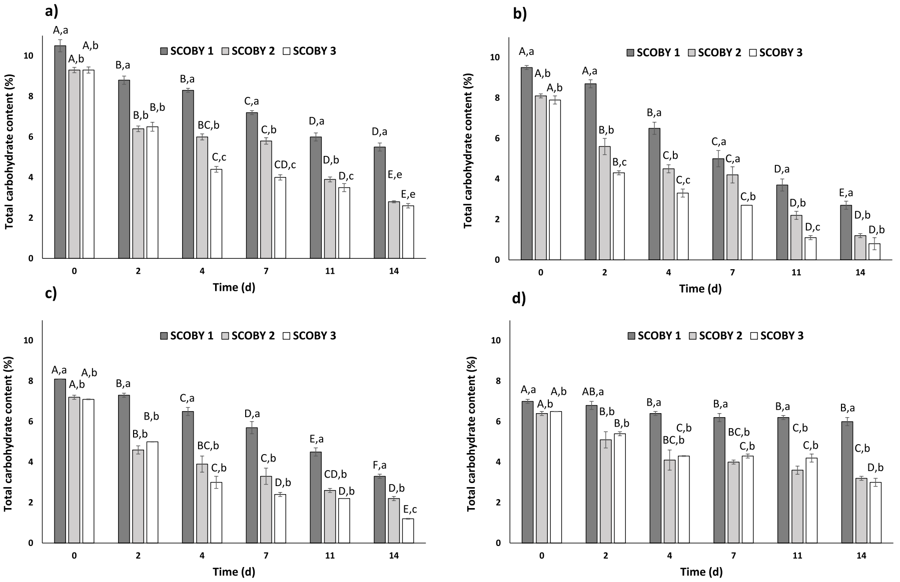

Fig. 4. Evolution of total carbohydrate content (%) along fermentation (14 days) of selected fruit-derived kombuchas: plum (a), strawberry (b), grape (c) and pomegranate (d) with SCOBYs (SCs) 1, 2 and 3. Different letters denote significant differences for the same SC at different fermentation times (A–F) and for different SCs at the same fermentation time (a–c) (One-way ANOVA, Tukey's test, $p \leq 0.05$).

Table 4

Evolution of alcohol content (% v/v) along fermentation (14 days) of selected fruit-derived kombuchas: plum, strawberry, grape and pomegranate with SCOBYs (SCs) 1, 2 and 3. Different letters denote significant differences for the same SC at different fermentation times (A-C) and for different SCs at the same fermentation time (a-c) (One-way ANOVA, Tukey's test, $p \leq 0.05$).

		Alcohol content (% v/v)			
		Plum	Strawberry	Grape	Pomegranate
Day 0	SC1	n.d.^{C,a}	n.d.^{B,a}	n.d.^{B,a}	n.d.^{C,a}
	SC2	n.d.^{B,a}	n.d.^{C,a}	n.d.^{B,a}	n.d.^{C,a}
	SC3	n.d.^{B,a}	n.d.^{B,a}	n.d.^{A,a}	n.d.^{C,a}
Day 2	SC1	$0.9 \pm 0.1^{B,a}$	n.d.^{B,b}	n.d.^{B,b}	n.d.^{C,b}
	SC2	n.d.^{B,b}	$0.4 \pm 0.0^{AB,a}$	$0.7 \pm 0.1^{A,a}$	$0.2 \pm 0.0^{BC,a}$
	SC3	n.d.^{B,b}	n.d.^{B,b}	n.d.^{A,b}	n.d.^{C,b}
Day 4	SC1	$0.8 \pm 0.0^{B,a}$	$0.3 \pm 0.1^{A,a}$	$0.4 \pm 0.0^{B,b}$	$0.2 \pm 0.0^{B,b}$
	SC2	$0.1 \pm 0.0^{B,b}$	$0.4 \pm 0.0^{AB,a}$	$0.9 \pm 0.1^{A,a}$	$0.6 \pm 0.1^{AB,a}$
	SC3	n.d.^{B,b}	n.d.^{B,b}	n.d.^{A,c}	$0.2 \pm 0.0^{B,b}$
Day 7	SC1	$1.6 \pm 0.1^{A,a}$	$0.5 \pm 0.1^{A,a}$	$1.1 \pm 0.1^{A,a}$	$0.6 \pm 0.1^{A,a}$
	SC2	n.d.^{B,b}	$0.5 \pm 0.1^{A,a}$	n.d.^{B,b}	$0.7 \pm 0.1^{A,a}$
	SC3	n.d.^{B,b}	n.d.^{B,b}	n.d.^{A,b}	$0.2 \pm 0.0^{B,b}$
Day 11	SC1	$0.6 \pm 0.2^{BC,a}$	$0.3 \pm 0.0^{A,a}$	$1.2 \pm 0.3^{A,a}$	n.d.^{C,c}
	SC2	$0.5 \pm 0.1^{A,a}$	$0.3 \pm 0.0^{B,a}$	n.d.^{B,b}	$0.4 \pm 0.1^{B,b}$
	SC3	$1.0 \pm 0.1^{A,a}$	$0.4 \pm 0.1^{A,a}$	n.d.^{A,b}	$0.8 \pm 0.1^{A,a}$
Day 14	SC1	$0.3 \pm 0.1^{C,a}$	n.d.^{B,a}	$1.6 \pm 0.1^{A,a}$	n.d.^{C,b}
	SC2	n.d.^{B,b}	$0.1 \pm 0.0^{C,a}$	n.d.^{B,b}	$0.1 \pm 0.0^{C,ab}$
	SC3	n.d.^{B,b}	n.d.^{B,a}	n.d.^{A,b}	$0.2 \pm 0.0^{B,a}$

present study and in published studies (Kaewkod, Bovonsombut, & Tragoolpua, 2019; Wang et al., 2022). However, these levels were significantly increased by the microbial action. Prior to starting this activity, differences in TPC content were observed depending on the SC utilized; while SC2 and SC3 showed similar TPC, SC1 showed significantly higher values (Table 5). This additional phenolic supply came from the '*old kombucha*', which contained the tea phenolic species that were released during SC development, indicating an upper production and long-term stability in the case of SC1. This consortium led to a clear trend for all fruits, as mentioned in Section 3.4., with an initial increase until approximately days 7–11 and a posterior degradation. However, SC2 and SC3 showed the highest TPCs after 2 weeks (Table 5), suggesting that phenolic losses are delayed with these SCs, probably because of the lower acidity that was measured in their liquid media. Moreover, although TPC was lower when compared to SC1 kombuchas during the first days, SC2 achieved the highest TPC among strawberry kombuchas (17.7 mg/100 mL) and SC2 among pomegranate ones (10.3 mg/100 mL), both after 11 days. In addition, SC1 led to the highest values in plum and grape kombuchas (11.4 and 12.3 mg/100 mL, respectively), but SC3 obtained very close values in plum (10.8 mg/100 mL) and SC2 in grape (11.3 mg/mL) (Table 5).

One of the conclusions drawn from the obtained results was the absence of a strict correlation between TPC and TEAC values (Tables 5 and 6). This fact was clearly illustrated in the case of strawberry kombuchas: at day 0, SC2 and SC3 showed lower TPC than SC1 but higher TEACs (1.25 and 1.24 μmol Trolox eq/mL) (Table 6). Moreover, TEAC was even increased until day 4 (1.46 and 1.21 μmol Trolox eq/mL) and drastically reduced from day 7, while phenolic degradation was not noticed for SC2 and SC3 kombuchas during the whole studied period (14 days). Therefore, as suggested in Section 3.5., TPC is not the only relevant factor that may affect the antioxidant activity of kombuchas; the structural features of the phenolic species, their proportions and abundance, and also their availability are crucial points for the scavenging capacity of the samples, as well as the presence of other antioxidants such as lipids or vitamins (Piljac-Žegarac et al., 2009).

Interestingly, in terms of TEAC, SC3 succeeded improving the scavenging capacity of plum and pomegranate kombuchas, showing the highest values at day 7: 0.70 and 0.79 μmol Trolox eq/mL, respectively.

Table 5

Evolution of total phenolic compounds content (mg/100 mL) along fermentation (14 days) of selected fruit kombuchas: plum, strawberry, grape and pomegranate with SCOBYs (SCs) 1, 2 and 3. Different letters denote significant differences for the same SC at different fermentation times (A-E) and for different SCs at the same fermentation time (a-c) (One-way ANOVA, Tukey's test, $p \leq 0.05$).

		Total phenolic compounds (mg/100 mL)			
		Plum	Strawberry	Grape	Pomegranate
Day 0	**SC1**	$6.59 \pm 0.17^{D,a}$	$9.70 \pm 0.09^{C,a}$	$5.35 \pm 0.09^{D,a}$	$4.06 \pm 0.09^{D,a}$
	SC2	$4.68 \pm 0.00^{E,b}$	$6.69 \pm 0.08^{D,c}$	$2.36 \pm 0.08^{E,b}$	$2.27 \pm 0.35^{F,b}$
	SC3	$4.64 \pm 0.00^{D,b}$	$7.57 \pm 0.08^{D,b}$	$2.49 \pm 0.38^{F,b}$	$2.14 \pm 0.00^{E,b}$
Day 2	**SC1**	$11.40 \pm 0.10^{A,a}$	$12.29 \pm 0.29^{B,a}$	$8.77 \pm 0.17^{B,a}$	$5.41 \pm 0.17^{C,a}$
	SC2	$6.47 \pm 0.00^{D,b}$	$10.81 \pm 0.16^{C,b}$	$6.75 \pm 0.14^{D,b}$	$3.02 \pm 0.08^{E,b}$
	SC3	$6.42 \pm 0.78^{C,b}$	$10.19 \pm 0.29^{C,b}$	$5.67 \pm 0.08^{E,c}$	$3.21 \pm 0.15^{D,b}$
Day 4	**SC1**	$8.08 \pm 0.19^{C,a}$	$12.31 \pm 0.25^{B,a}$	$11.49 \pm 0.09^{A,a}$	$5.00 \pm 0.19^{C,a}$
	SC2	$7.09 \pm 0.08^{C,b}$	$12.88 \pm 0.00^{B,a}$	$9.26 \pm 0.17^{B,b}$	$4.09 \pm 0.08^{D,b}$
	SC3	$8.20 \pm 0.25^{B,b}$	$12.45 \pm 0.08^{A,a}$	$8.63 \pm 0.14^{B,c}$	$4.58 \pm 0.38^{C,ab}$
Day 7	**SC1**	$9.38 \pm 0.09^{B,a}$	$13.66 \pm 0.43^{A,b}$	$12.31 \pm 0.68^{A,a}$	$7.38 \pm 0.09^{A,a}$
	SC2	$8.64 \pm 0.30^{B,b}$	$17.41 \pm 0.32^{A,a}$	$8.49 \pm 0.40^{BC,b}$	$5.15 \pm 0.00^{C,c}$
	SC3	$7.99 \pm 0.31^{B,c}$	$10.97 \pm 0.23^{B,c}$	$7.53 \pm 0.18^{C,b}$	$5.81 \pm 0.09^{B,b}$
Day 11	**SC1**	$7.70 \pm 0.18^{C,a}$	$10.29 \pm 0.18^{C,c}$	$8.63 \pm 0.10^{B,a}$	$6.55 \pm 0.27^{B,b}$
	SC2	$6.21 \pm 0.14^{D,b}$	$17.68 \pm 0.37^{A,a}$	$8.36 \pm 0.45^{C,a}$	$5.71 \pm 0.16^{B,c}$
	SC3	$6.36 \pm 0.14^{C,b}$	$11.99 \pm 0.56^{A,b}$	$6.45 \pm 0.29^{D,b}$	$10.27 \pm 0.24^{A,a}$
Day 14	**SC1**	$5.72 \pm 0.21^{E,b}$	$6.69 \pm 0.12^{D,c}$	$7.49 \pm 0.21^{C,c}$	$4.21 \pm 0.41^{D,c}$
	SC2	$10.55 \pm 0.00^{A,a}$	$17.58 \pm 0.56^{A,a}$	$11.34 \pm 0.43^{A,a}$	$7.10 \pm 0.21^{A,b}$
	SC3	$10.83 \pm 0.14^{A,a}$	$11.71 \pm 0.08^{AB,b}$	$9.38 \pm 0.08^{A,b}$	$10.17 \pm 0.08^{A,a}$

Table 6
Evolution of DPPH● scavenging activity (expressed as TEAC value, μmol Trolox eq/mL) along fermentation (14 days) of selected fruit kombuchas: plum, strawberry, grape and pomegranate with SCOBYs (SCs) 1, 2 and 3. Different letters denote significant differences for the same SC at different fermentation times (A-D) and for different SCs at the same fermentation time (a-c) (One-way ANOVA, Tukey's test, $p \leq 0.05$).

		TEAC value (μmol Trolox equivalents/mL)			
		Plum	Strawberry	Grape	Pomegranate
Day 0	**SC1**	0.26 ± 0.03^{B,a}	0.86 ± 0.09^{B,b}	0.27 ± 0.08^{B,ab}	0.31 ± 0.05^{B,a}
	SC2	0.26 ± 0.04^{B,a}	1.25 ± 0.11^{B,a}	0.35 ± 0.01^{B,a}	0.27 ± 0.02^{C,ab}
	SC3	0.30 ± 0.01^{BC,a}	1.24 ± 0.11^{A,a}	0.22 ± 0.00^{C,b}	0.20 ± 0.01^{C,b}
Day 2	**SC1**	0.25 ± 0.05^{B,a}	2.05 ± 0.04^{A,a}	0.38 ± 0.02^{AB,a}	0.20 ± 0.01^{B,b}
	SC2	0.30 ± 0.08^{B,a}	1.46 ± 0.20^{B,b}	0.50 ± 0.09^{AB,a}	0.45 ± 0.08^{B,a}
	SC3	0.36 ± 0.01^{B,a}	1.21 ± 0.19^{A,b}	0.43 ± 0.03^{B,a}	0.26 ± 0.01^{C,b}
Day 4	**SC1**	0.23 ± 0.02^{B,a}	0.54 ± 0.06^{C,c}	0.34 ± 0.04^{AB,a}	0.65 ± 0.19^{A,a}
	SC2	0.24 ± 0.01^{B,a}	1.94 ± 0.17^{A,a}	0.44 ± 0.09^{AB,a}	0.51 ± 0.04^{B,a}
	SC3	0.32 ± 0.07^{BC,a}	1.42 ± 0.12^{A,b}	0.43 ± 0.04^{B,a}	0.60 ± 0.06^{B,a}
Day 7	**SC1**	0.56 ± 0.01^{A,b}	0.49 ± 0.04^{CD,a}	0.47 ± 0.03^{A,a}	0.50 ± 0.02^{AB,b}
	SC2	0.47 ± 0.05^{A,b}	0.53 ± 0.04^{D,a}	0.55 ± 0.05^{A,a}	0.66 ± 0.01^{A,a}
	SC3	0.70 ± 0.02^{A,a}	0.55 ± 0.08^{B,a}	0.55 ± 0.05^{A,a}	0.79 ± 0.10^{A,a}
Day 11	**SC1**	0.20 ± 0.02^{BC,a}	0.34 ± 0.04^{D,b}	0.38 ± 0.08^{AB,a}	0.21 ± 0.04^{B,a}
	SC2	0.07 ± 0.03^{C,b}	0.47 ± 0.04^{D,a}	0.18 ± 0.01^{C,b}	0.22 ± 0.06^{C,a}
	SC3	0.25 ± 0.03^{C,a}	0.18 ± 0.02^{C,c}	0.18 ± 0.01^{C,b}	0.12 ± 0.04^{CD,a}
Day 14	**SC1**	0.15 ± 0.01^{C,a}	0.39 ± 0.04^{D,a}	0.39 ± 0.04^{AB,a}	0.17 ± 0.02^{B,a}
	SC2	0.07 ± 0.03^{C,b}	0.40 ± 0.06^{D,a}	0.09 ± 0.00^{C,b}	0.09 ± 0.01^{D,b}
	SC3	0.07 ± 0.03^{C,b}	0.40 ± 0.06^{D,a}	0.09 ± 0.00^{C,b}	0.09 ± 0.01^{D,b}

Both SC2 and SC3 also registered the greatest TEAC for grape kombucha (0.55 μmol Trolox eq/mL) but it was not statistically higher than SC1 record (0.47 μmol/mL). Finally, strawberry kombucha TEAC obtained on day 2 (2.05 μmol/mL) with SC1 was not reached by either SC2 or SC3 (Table 6).

4. Conclusions

Alternative kombuchas produced using fruits (without tea leaves) have shown results that make them as interesting candidates because of their composition and functionality. The obtained products maintained a safe pH range and lower carbohydrate content than other related drinks (tea kombucha, fruit juices, and carbonated drinks), particularly that using strawberries. The alcohol content of fruit-based kombuchas prepared using SC2 and SC3 at conventional bottling times did not exceed the alcohol limit for being considered a non-alcoholic product. Moreover, SC fermentative activity increased TPC in kombuchas, followed by subsequent losses over long fermentation times. However, SC2 and SC3 displayed higher stability in the phenolic content of kombuchas over time than SC1 did. *In vitro* antioxidant activity also increased after microbial action on fruits, but was later reduced in all cases. The highest TEAC value was recorded for the strawberry kombucha (2.05 μmol Trolox eq/mL). No strict correlation was observed between the TPC and TEAC values.

Therefore, the results showed that fruits, mainly strawberries, could be used to prepare alternative kombuchas with *in vitro* antioxidant activity without the addition of tea leaves, although future research focusing on *in vivo* evaluation of antioxidant activity is needed. As phenolic compounds exert a wide range of bioactivities, these fruit-based kombuchas might also exhibit other bioactivities. Additional studies focusing on the assessment of other bioactivities, characterization of the phenolic profile, and bioavailability of these bioactive compounds could be of interest to evaluate the potential of these alternative kombuchas and to identify the compounds responsible for their bioactivity.

Funding

This work was supported by grant number PID2020-113739RB-I00 funded by MCIN/AEI/10.13039/501100011033. D.M. is recipient of the grant FJC2020-044585-I funded by MCIN/AEI/10.13039/501100011033 and cofounded by European Union NextGenerationEU/PRTR. F.I.B is a Serra Húnter Fellow.

CRediT authorship contribution statement

Diego Morales: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Roger Gutiérrez-Pensado:** Formal analysis, Investigation, Methodology. **Francisca Isabel Bravo:** Funding acquisition, Supervision, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Begoña Muguerza:** Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Tea House (Tarragona, Spain) is acknowledged by its information support for SC3 preparation.

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