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Extraction of polyphenols and antioxidant compounds from SCOBY, as a by-product of Kombucha, using different types of extraction

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Abstract
Kombucha is obtained through a symbiosis fermentation of bacteria and yeast, generating a floating film called SCOBY (Symbiotic Culture of Bacteria and Yeast). After a period of use, SCOBY becomes a production waste. Thus, alternative uses of SCOBY can add value to Kombucha production. In this way, this work aimed to produce and evaluate antioxidant activity and total phenolic contents of SCOBY ethanolic extracts, which were obtained by two different solid–liquid extraction with three different waste weight/extractor solvent volume ratios. The SCOBY evaluated had a high concentration of cellulose (9.42 ± 1.00%) and low concentrations of proteins (0.84 ± 0.04%) and lipids (0.29 ± 0.05%). All evaluated extracts showed a high concentration of total phenolics (40.7–64.3 mg GAE/100 g of SCOBY) and high antioxidant activity ($ABTS^{\bullet+}$, FRAP and ORAC assays). The most efficient extraction of bioactive compounds was obtained by decoction using the highest solvent volume (ratio 1:20). The PCA showed clusters of the samples both by the type of extraction and by the different ratios of the weight of SCOBY to the solvent volume. Of our knowledge, our work is the first in the literature that describes such data. Therefore, the present work showed the possibility of using this industrial waste mainly to obtain active compounds and antioxidants.

Keywords Fermented drinks · Antioxidant potential · Phenolics · Bioactive compounds · Lactic acid bacteria

1 Introduction

Currently, the search for healthy foods and beverages has stimulated research and development of products with high probiotic potential. Kombucha is one of the health drinks that originated in northeast China [1]. This drink has gained prominence for having therapeutic effects on several diseases [2]. Previous research has shown that Kombucha's medicinal properties are mainly due to its composition rich in organic acids, vitamins, antioxidants and a probiotic culture rich in bacteria and yeasts [3].

Kombucha fermentation is carried out with a symbiotic culture of bacteria and yeast [4]. Thus, using this symbiosis, fermentation produces an important floating layer mainly composed of a cellulosic network, among other compounds [5]. This floating biofilm is called SCOBY (Symbiotic Culture of Bacteria and Yeast) and consists of various microbial populations of bacteria and yeasts, such as the genera *Gluconobacter*, *Acetobacter*, *Zygosaccharomyces*, *Saccharomyces* and *Schizosaccharomyces*, among others [6].

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SCOBY has important functions such as fermenting the sugar-rich medium, fixing and protecting cells against conditions such as ultraviolet radiation and high hydrostatic pressure, as well as keeping bacteria exposed to surface oxygen, which is essential for fermentation [7]. On the other hand, the formation of increasingly thick layers of this biofilm can prevent oxygen from reaching the liquid medium, making the bacteria inactive [8].

Therefore, the development of the SCOBY continues in the presence of sucrose, so the biofilm continues to grow, forming several separate layers, thus needing to be removed from the tank [9]. In this way, the production process of Kombucha generates a high amount of by-product, composed of SCOBY and other organic compounds, which can lead to environmental problems if discarded without proper management [10].

Thus, the reuse of SCOBY as a Kombucha by-product is necessary and relevant. On the other hand, according to Laavanya, Shirkole and Balasubramanian [1] studies on the application of SCOBY in new products and/or raw materials for industry are still limited and incipient. Most studies are carried out on a small-scale [1]. They are mainly focused on the physical characteristics of the cellulosic matrix that composed SCOBY, which can be used in textiles [11], food packaging [12], use in the adsorption of heavy metals [5] and animal feed [13]. In addition, investigations aimed at evaluating the bioactive compounds from SCOBY are incipient, and no studies have been found in the literature that demonstrate the potential of the SCOBY to be used as a benefit to human health, making this an innovative field of research.

Such studies would be of interest since some works report the presence of bioactive compounds in both Kombucha and SCOBY [14]. In this way, the extraction of bioactive compounds from SCOBY would add value to industrial waste and reduce possible environmental damage resulting from the production of Kombucha.

There are numerous methods for extracting active compounds and antioxidants from industrial waste, with decoction and maceration being the simplest, most cost-effective and highly applicable [15]. In addition, the solvents commonly used in industry must be low-cost, low-toxicity solvents with high interaction with the compounds of interest [16]. Ethanol, which is considered a green solvent, is therefore the most suitable because, in addition to being non-toxic and low-cost, it has a high intermolecular interaction with phenolic compounds [17].

Therefore, besides the several industrial applications of SCOBY, utilizing its bioactive compounds is also an important field of research, which can add more value to the Kombucha production process. Thus, this work aimed to evaluate the total phenolic content and antioxidant potential of ethanolic extracts of SCOBY obtained by different solid–liquid extractions to give evidence to propose an alternative use for this industrial waste.

2 Material and methods

2.1 Materials

The SCOBY (processing by-product) was donated by Tribos Kombucha® (located at Campinas/SP, Brazil, latitude: 22º 54′ 23" south, longitude: 47º 03′ 42" west). Reagents used were 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Folin–Ciocalteau reagent, gallic acid, fluorescein, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2′-azobis(2-methyl-propanimidamide) dihydrochloride (AAPH): all were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Hydrochloric acid, sodium carbonate, glacial acetic acid, ethanol, sodium phosphate, and ferric chloride were purchased from Synth (São Paulo, Brazil).

2.2 SCOBY preparation

The SCOBY was prepared according to the methodology proposed by Amarasekara, Wang e Grady [18], with modification. SCOBY was washed in running water for 2 min to remove the excess sugars from the fermentation process. After washing, it was chopped into small pieces (approximately 0.5 cm) to increase the contact surface and improve the interaction with the solvent (ethanol). Subsequently, the pieces were stored under refrigeration (≈ 4 ± 2 °C) until they were analyzed and submitted to extractions.

2.3 SCOBY characterization

2.3.1 Moisture content

The moisture content of the SCOBY was determined by drying its pieces in the air oven at 105 °C [19] until constant weight.

2.3.2 Chemical composition

The chemical composition of the SCOBY was determined using the methodologies by AOAC [19] and Silva and Queiroz [20] for protein, crude fiber, ash, lipid, and carbohydrate analysis. In this way, the ash content was determined by muffle incineration (500 °C to 600 °C, Marconi, MA 385/3, Brazil). The determination of fiber content was performed using the Van Soest method. Lipids were determined by extraction in a Soxhlet apparatus, using petroleum ether as solvent. Protein analysis was performed using the Kjedahl method. Finally, carbohydrates were determined by exclusion (total weight less protein, fiber, ash, and lipid).

2.3.3 Cell viability by counting lactic acid bacteria and yeasts

In order to determine the efficiency of SCOBY for new fermentations or identify it as an industrial by-product from the production of Kombucha, an analysis of cell counts of lactic acid bacteria and molds and yeasts was carried out to identify if such microorganisms were alive or not.

2.3.3.1 Viable lactic acid bacteria The methodology proposed by Silva et al. [21] was used to determine the total count of viable lactic acid bacteria. For this, serial dilutions (from 10^{-1} to 10^{-5}) were plated using the pour plate technique on plates containing De Man, Rogosa, and Sharpe agar (MRS) and incubated at 37 °C for 48 h. For sample preparation, sterile 0.1% peptone water was used. Thus, the number of viable lactic acid cells was obtained by directly counting in plates, expressed in colony forming unit (CFU) per g, as shown in Eq. 1.

$$\text{Lactic acid bacteria}\left(\frac{\text{CFU}}{\text{g}}\right) = \frac{\text{n x}\left(\frac{1}{\text{D}}\right)}{\text{A}} \tag{1}$$

where: *n* is the number of colonies obtained by direct plate counting, *D* is the sample dilution and *A* is the aliquot of the plated sample, in mL.

2.3.3.2 Viable molds and yeasts In turn, molds and yeasts were determined according to the methodology proposed by Downes and Ito [22]. Thus, total mold and yeast counts were obtained from serial dilutions (from 10^{-1} to 10^{-5}) in sterile 0.1% peptone water, and spread plate plating on plates containing Potato Dextrose Agar (PDA) acidified with 10% tartaric acid (to pH = 3.5). After preparing the plates, they were incubated in a bacteriological oven at 25 °C for 5 days. Then, a direct plate count was performed to determine the number of colonies forming units (CFU) of molds and yeasts per g, as described in Eq. 2.

$$\text{Molds and yeasts}\left(\frac{\text{CFU}}{\text{g}}\right) = \frac{\text{n x}\left(\frac{1}{\text{D}}\right)}{\text{A}} \tag{2}$$

where: *n* is the number of colonies obtained by direct plate counting, D is the sample dilution and *A* is the aliquot of the plated sample in mL.

2.4 Preparation of ethanolic extracts from SCOBY

In order to obtain the ethanolic extracts from industrial SCOBY, two solid–liquid techniques were used: maceration and decoction. For both extractions, samples (5 g) were added in different volumes of ethanol (25, 50, and 100 mL) in order to

obtain different sample:solvent ratios (1:5; 1:10; 1:20, respectively). The extraction procedure methodology was proposed by our research group, which has been studying different solid–liquid extraction methods of bioactive compounds from plants for several years. One of these works is reported in Boffo et al. [23].

For the maceration technique, the samples and ethanol were added to Erlenmeyer. Then, they were submitted to shaking for 1 h at room temperature (25 ± 2 °C) (300 rpm; shaker of Marconi, MA 420, Piracicaba, Brazil). After this time, the samples were vacuum filtered (Tecnal, TE058, Brazil) using filter paper (Whatchman n°1). In turn, for the decoction extraction, the samples and the solvent, in the previously mentioned proportions, were added to a round-bottomed flask with glass shards to avoid the solvent's turbulent heating. Then, the flask was placed in a heating mantle (Quimis, Q-321A, Brazil), and a ball type condenser was attached. After the solvent started boiling (≈ 78 °C), the time was set (1 h), and after that the samples were vacuum filtered (Tecnal, TE058, Brazil) using filter paper (Whatchman n°1). All extracts were prepared in triplicate (nine analyses for each sample) and stored at -22 °C until total phenolics content and antioxidant activity were performed.

2.5 Characterization of SCOBY ethanolic extracts

2.5.1 Total phenolics content

The SCOBY alcoholic extracts were evaluated for the concentration of total phenolics using the methodology proposed by Singleton, Orthofer and Lamuela-raventós [24]. SCOBY alcoholic extracts samples (0.5 mL) was added to 2.5 mL of Folin–Ciocalteau reagent and was homogenized. The samples were then incubated for 2 h (25 ± 2 °C; absence of light). Using a spectrophotometer at 740 nm (PerkinElmer, Lambda 35 UV–Vis, USA), the samples' absorbances were obtained. Using gallic acid (22.4 to 64.0 µg/mL) as external standard, the results were expressed as mg of gallic acid equivalents/ 100 g of SCOBY.

2.5.2 Ferric reducing antioxidant power (FRAP)

For the determination of the antioxidant activity by the FRAP method, the methodology proposed by Benzie and Strain [25] was used. For this, each extract (0.1 mL) was dispersed in the FRAP solution (2.9 mL), homogenized and incubated in a heated bath at 37 °C (Marconi, MA 159, Piracicaba, Brazil). After 30 min, the sample's absorbance was measured at 593 nm (PerkinElmer, Lambda 35 UV–Vis, USA). Results were expressed as µmol Trolox equivalent/ 100 g of SCOBY, using Trolox as an external standard (2.5–22.5 µmol/L).

2.5.3 ABTS radical scavenging assay

The methodology described by Re et al. [26] for determination of the antioxidant activity by the $ABTS^{\bullet+}$ method was used. Dilutions of the extracts (33 µL) were dispersed in 3 mL of the ABTS radical, being then kept at rest for 6 min. Using a spectrophotometer at 593 nm (PerkinElmer, Lambda 35 UV–Vis, USA) the samples' absorbance was obtained after this time. For external standard, Trolox was used and the results were expressed in µmol of Trolox equivalent / 100 g of SCOBY.

2.5.4 Oxygen radical absorbance capacity (ORAC)

The methodology described by Ou, Hampsch-Woodill and Prior [27] was used to determine the antioxidant potential by ORAC method. Using a microplate (96-cell), the fluorescein solution (81 mM, 150 µL) and 25 µL of dilution of each ethanolic extract were added to each cell, and then incubated (37 °C) in a spectrofluorometer (BMG Labtech, FLUOstar OPTIMA, German) for 10 min. After incubation, AAPH (152 mM, 25 µL) was added to each cell. For determine the fluorescein decay in each cell, the fluorescence was measured every minute for a total period of 120 min at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Trolox (8–96 µM) was used as an external standard, and the results were expressed as µmol of Trolox equivalent / 100 g of SCOBY.

2.6 Chemometric analysis of total phenolic and antioxidant activity data

Chemometric analysis of total phenolic and antioxidant activity data was performed from a data matrix containing 54 rows (total of extracts: 2 extraction methods × 3 different concentrations x extraction triplicate × assay triplicate) and 4 columns (number of assays: total phenolics and antioxidants – FRAP, ORAC, $ABTS^{\bullet+}$).

Chemometric treatment was performed using the software Pirouette® version 4.5 (InfoMetrix Inc., Bothell, Washington, USA). Data were mean centered and analyzed by PCA (Principal Component Analysis).

2.7 Statistical analysis

The SCOBY characterization analyzes were performed in triplicates (3 points per analysis). Results were expressed as mean value ± standard deviation. The analyzed data were statistically evaluated through the difference between the means, using Duncan's test with a 95% confidence interval by the SAS software (Version 9.2, SAS, Inc.).

3 Results and discussion

3.1 SCOBY industrial by-product characterization

3.1.1 Moisture content

The SCOBY as an industrial by-product was evaluated for water content. It was found that the water content in the SCOBY is high (94.02 ± 0.08 g/100 g), as expected, since its composition is made up of water-rich microorganisms (bacteria and yeasts). These results corroborated with that obtained by Jayabalan et al. [28], which investigated the composition of SCOBY in different fermentation periods (96.39–97.38 g/100 g).

3.1.2 Chemical composition

In addition to the water content, the fiber and carbohydrate content (Table 1) are also high in the SCOBY. On the other hand, the protein, lipid and ash content determined (Table 1) are reduced. This behavior is also directly linked to the composition of the microorganisms present in the SCOBY, mainly due to the high cellulosic mass and carbohydrates presence. Some authors in the literature describe SCOBY mainly as a cellulosic network [28], corroborating with the results obtained in this work.

The results found in the literature are diverse for all the compounds evaluated. As an example, Jayabalan et al. [28] evaluated the chemical composition of SCOBY excluding the concentration of carbohydrates and determined proteins (23.1%), crude lipids (5.4%), crude fibers (14.79%) and ash (3.9%) in different fermentation periods. In turn, Ahmed and Dirar [29], when evaluating the centesimal composition of SCOBY, also found slightly different values (crude protein = 4.8%; crude fiber = 49.5%; crude lipid = 1%; ash = 1.5%; carbohydrates = 43.2%). Possibly the differences between such values are associated to: the substrates used in fermentation; the types of microorganisms; and, the period between the development of the cellulose matrix and its analysis.

According to Jayabalan et al. [28], the longer the fermentation period of tea, the higher its chemical composition. Possibly, this behavior can be understood by the growth of microorganisms in this material throughout time. In this way, the highest levels of proteins, lipids, fibers, ash and carbohydrates can be obtained at the end of the SCOBY's useful life.

Table 1 shows that SCOBY has high concentration of fibers and reduced levels of proteins and lipids. Therefore, the use of SCOBY for animal feeding is favored. However, it is necessary to highlight that the protein found in SCOBY is of the microbial type, which can be an interesting source of unconventional proteins. The fiber content is high,

Table 1 Chemical composition of SCOBY as an industrial by-product from Kombucha production

Chemical composition	Value ± SD
Proteins (g/100 g)	0.84 ± 0.04
Lipids (g/100 g)	0.29 ± 0.05
Ash (g/100 g)	0.58 ± 0.09
Fibers (g/100 g)	10.49 ± 0.29
Cellulose (g/100 g)	9.42 ± 1.00
Hemicellulose (g/100 g)	0.80 ± 0.24
Carbohydrates (g/100 g)	86.51 ± 0.55

All values are expressed on a dry basis

due to the high concentration of bacterial cellulose, which is not digested by the human tract, being inadequate for human consumption. However, this cellulosic mass can be widely used as raw material for other products. Paximada et al. [30], for example, evaluated the use of bacterial cellulose as a stabilizer in oil and water emulsions, and concluded that it can be widely used in the food industry. According to Paximada et al. [30], despite of bacterial and plant cellulose have the same chemical composition, the first has numerous advantages, since it has lower density, higher crystallinity, higher water retention, better mechanical property, due to the structural form of the cellulosic network, its purity and for not being associated with lignin and hemicelluloses.

Also, SCOBY have a high carbohydrate content (Table 1), which can provide important characteristics for any industry sectors, due to its high energy content. However, studies regarding the energy content of SCOBY are incipient in the literature, which indicates a promising research area.

With this exposed, it is possible to verify the high applicability of SCOBY in several industrial sectors mainly due to its structural properties. However, the bioactive compounds' contribution in this waste must also be considered.

3.1.3 Cell viability by counting lactic acid bacteria and yeasts

SCOBY is composed of bacteria and yeasts, which carry out the fermentation of teas to produce Kombucha. However, these microorganisms have a useful life in this process, since their functions become less effective after some times of use and unfeasible for the continuation of such process. To continue its use (or not) in the fermentation of teas, this material should be microbiologically evaluated. If they are not, SCOBY can be considered a production waste and should become an environmental problem.

In this work, SCOBY was evaluated in relation to the viability of the largest class of bacteria in it, lactic acid bacteria, molds, and yeasts. Figure 1 shows that, regardless of the degree of dilution of the sample, there are not lactic acid bacteria, molds, and yeasts in the SCOBY used in this work. Therefore, it can be considered an industrial by-product of the Kombucha production.

According to Esa et al. [8], several factors are responsible for inactivating bacteria and yeasts in the SCOBY, mainly the difficult oxygenation of such microorganism in thicker layers of this material.

3.2 Characterization of SCOBY ethanolic extracts

Ethanol extracts from SCOBY were obtained using pure ethanol (99.7%) and two solid–liquid extraction techniques: a cold technique, maceration; and, a hot technique, decoction. Regardless of the technique, the extracts were obtained in the sample:solvent proportions of 1:5, 1:10 and 1:20, respectively. Different proportions were used to verify the relationship between bioactive compounds concentration and antioxidant activity.

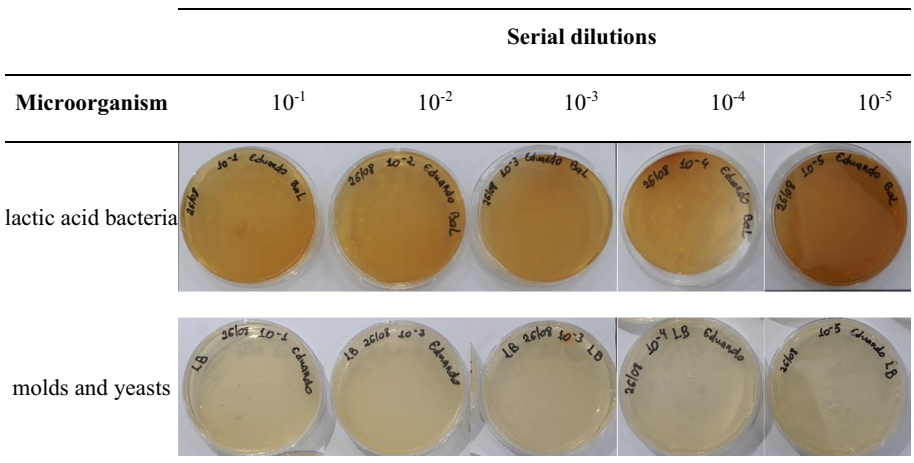

Fig. 1 Visual inspection of microbiological viability assays (lactic acid bacteria, molds and yeasts) of SCOBY as industrial by-product from Kombucha production

3.2.1 Total phenolics content

Regarding the total phenolic content of the extracts obtained by maceration and decoction from the SCOBY, a significant difference ($p > 0.05$) was observed (Fig. 2). The decoction process extracted high quantities of phenolic compounds, which are known by their antioxidant properties. In Fig. 2, we can also observe that different proportions (1:5, 1:10 and 1:20) of sample:solvent afforded extracts with significative ($p > 0.05$) difference in their phenolic content only in the decoction process: the higher the solvent volume, the higher the total phenolic content.

So, the increase in the sample:solvent ratio in decoction process caused an increase in the final concentration of total phenolics. This behavior may be associated with the better interaction between sample and solvent, when there is an increasing in the volume of solvent. This result is expected, as a higher volume of solvent provides higher solubility of the solute and prevents solvent saturation.

Thus, it can be seen from Fig. 2 that the most efficient way of extracting phenolic compounds from SCOBY was by the decoction method, using larger volumes of solvent for a smaller sample mass, as is the case of the 1:20 ratio (sample:solvent). Figure 2 also shows that the decoction was always more efficient in extracting phenolic compounds from the SCOBY than the maceration, in the different SCOBY/solvent proportions used to prepare extracts. Possibly, this behavior is also related to the higher solubility of most compounds at higher temperatures.

In the literature, studies regarding the concentrations of bioactive compounds in SCOBY are still incipient. However, we believe that the phenolic compounds present in SCOBY are similar to those found in Kombucha, with differences only in their concentrations. Based on this hypothesis, SCOBY may contain the same phenolic compounds that Kombucha, such as, flavanols (e.g. gallocatechin, catechin gallate and epicatechin), which represent around 70% of the total phenolics present in green tea [2].

Many studies show the high concentration of total phenolics in Kombucha. Kallel et al. [31], when evaluating the compounds present in Kombucha, in different fermentation periods, found values between 0.78 and 1.01 mg gallic acid equivalent (GAE) per mL of Kombucha. Also, Jayabalan et al. [32], when evaluating the influence of the fermentation period of Kombucha on its antioxidant properties, determined high concentrations of phenolic compounds (≈ 0.1 mg GAE/mL of Kombucha). According to Jayabalan et al. [28], the properties of the liquid (Kombucha) can also be verified in SCOBY. Thus, a high concentration of bioactive compounds can be found in both Kombucha and SCOBY.

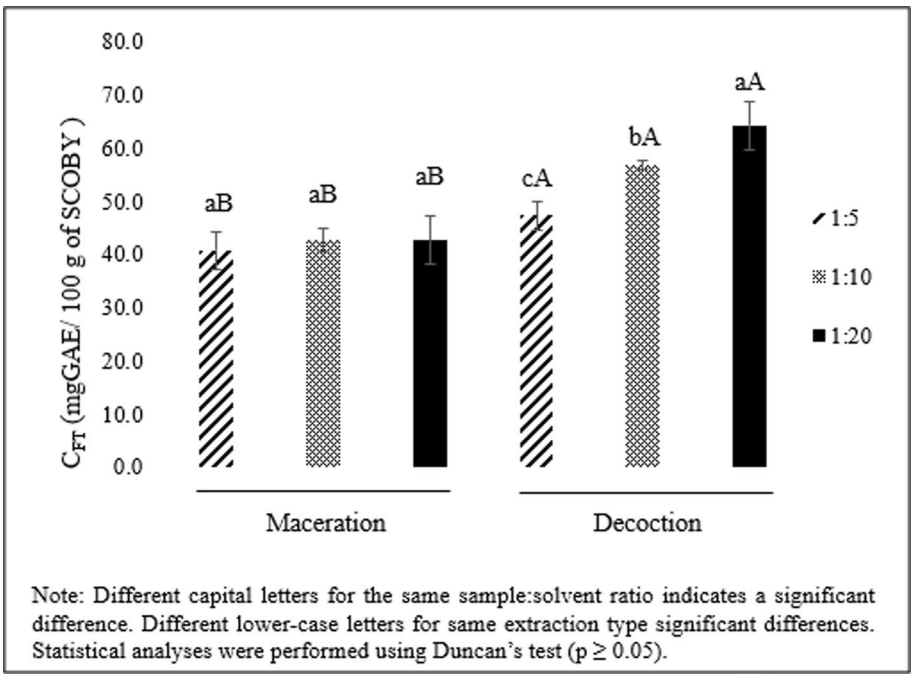

Fig. 2 Concentration of total phenolics (C_{FT}) in SCOBY ethanolic extracts by two different extraction techniques: maceration and decoction, and different sample:solvent ratios (1:5, 1:10 and 1:20)

3.2.2 Antioxidant potential of SCOBY ethanolic extracts

The antioxidant activity of SCOBY ethanolic extracts was evaluated by three methods: FRAP (ferric reduction antioxidant potential), $ABTS^{\bullet+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical decay) and ORAC (oxygen radical antioxidant capacity). The results are shown in Table 2.

Table 2 shows that the three antioxidant assays indicated the same behavior of antioxidant activity, that is, all decoction processes generated extracts with higher antioxidant potential than those obtained by maceration ($p < 0,05$). The higher antioxidant activity of the extracts obtained by the hot process is expected, as, in general, the solubility of bioactive compounds increases with increasing temperature. However, this effect just does not happen when the compounds are thermolabile. In addition, as observed for total phenolics, the higher the volume of solvent for the preparation of extracts, the higher the antioxidant activity, probably, as explained above, by better extraction of bioactive compounds (phenolics, for example).

Thus, the ethanolic extracts from SCOBY produced by the decoction technique and using a fraction of 1:20 (sample:solvent) was the one that showed the highest antioxidant action.

According to Genovese et al. [33], the antioxidant potential is directly proportional to the concentration of total phenolics. This result was also observed in the present work as it can be seen that the extract with the highest concentration of total phenolics (Fig. 2) also has the highest antioxidant activity (Table 2).

As previously mentioned, studies on the antioxidant potential of SCOBY are still incipient. However, as the composition of SCOBY can be similar to that of Kombucha, including the secondary metabolites produced during fermentation [8], antioxidant activity of both may also be related. In the literature, some studies [34, 35] point out the high antioxidant activity of Kombucha. Detailly, the study by Jakubczyk et al. [35] evaluated the chemical profile and antioxidant activity of Kombucha fermented in different types of teas and determined the antioxidant activity measured by the FRAP method from 2.29–5.37 µmol Trolox equivalent / mL of kombucha for the different types of tea. In turn, Cardoso et al. [34] evaluated Kombucha fermented in green and black tea concerning the phenolic profile, and its impacts on antioxidant, bactericidal and cytotoxic activity and they found an antioxidant activity of 8.22 and 13.59 µmol Trolox equivalent / mL of Kombucha made with green and black tea, respectively, for the ABTS method. So, the similar chemical composition of Kombucha and SCOBY and the antioxidant activity of the first one reported in the literature could explain the high antioxidant potential of SCOBY extracts of the present study.

3.3 Chemometrics from biological data of SCOBY ethanolic extracts

A data matrix was created from the data of total phenolics and antioxidant activity of the ethanolic extracts of SCOBY. After the data were mean centered, they were submitted to PCA analysis. Chemometrics applied to such data allowed the discrimination between samples. The PC1 *vs* PC2 scores plot (Fig. 3) shows that the samples were grouped into practically six groups according to the extraction method and mainly the sample/solvent relationship. From negative to positive values of PC1 (from left to right) in Fig. 3, six vertical ellipses refer to the extracts M_1:5 (extract obtained by maceration in the proportion of 1 g of SCOBY to 5 mL of ethanol), D_1:5 (extract obtained by decoction in the proportion of 1 g of

Table 2 Antioxidant activities determined by the FRAP, $ABTS^{\bullet+}$ and ORAC methods of SCOBY ethanolic extracts obtained by two different techniques: maceration and decoction and using different extraction fractions for each of the techniques (sample:solvent; 1:5, 1:10 and 1:20)

Extraction method	Fraction (sample:solvent)	Antioxidant potential (µmol of Trolox equivalent / 100 g of SCOBY)		
		FRAP	$ABTS^{\bullet+}$	ORAC
Maceration	1:5	11.2 ± 0.2^{cB}	198 ± 12^{cB}	575 ± 67^{bB}
	1:10	12.5 ± 0.7^{bB}	284 ± 7^{bB}	540 ± 107^{bA}
	1:20	13.3 ± 1.3^{aB}	461 ± 16^{aB}	851 ± 113^{aA}
Decoction	1:5	14.0 ± 1.0^{cA}	242 ± 24^{cA}	706 ± 90^{bA}
	1:10	16.0 ± 0.9^{bA}	379 ± 11^{bA}	687 ± 107^{bA}
	1:20	18.2 ± 1.3^{aA}	617 ± 43^{aA}	878 ± 72^{aA}

Different capital letters in the same column for the same sample:solvent ratio indicates a significant difference. Different lower-case letters in the same column for same extraction type indicate significant difference. Statistical analyses were performed using Duncan's test ($p \geq 0.05$)

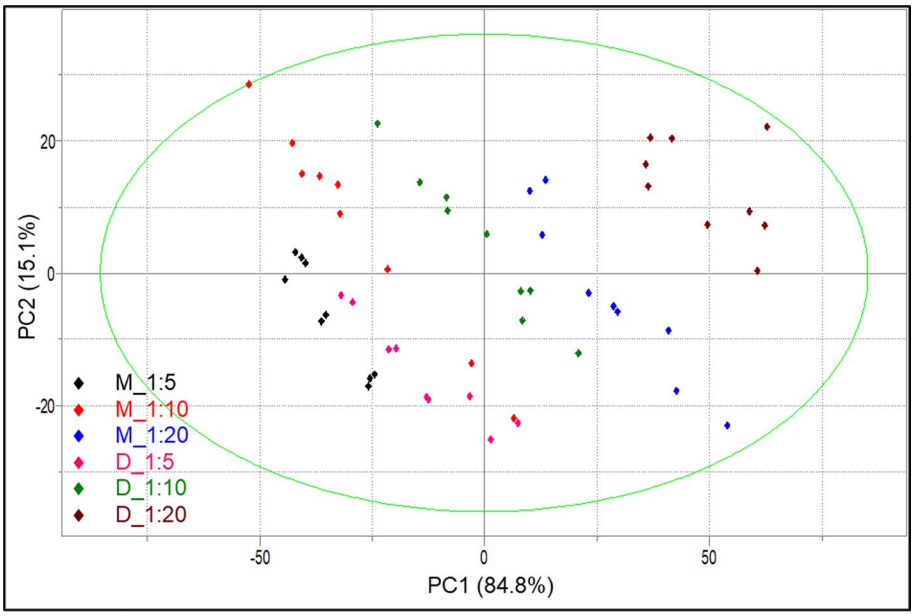

Fig. 3 PC1 vs PC2 scores plot of total phenolic data and antioxidant activity of SCOBY ethanolic extracts obtained by different extraction methods, in different mass/solvent ratios

SCOBY to 5 mL of ethanol), M_1:10, D_1:10, M_1:20 and D_1:20, respectively. This plot (Fig. 3) presents 99.9% of the variability of original information, with PC1 representing 84.8% of the variance of the data set.

Samples that are close in Fig. 3 are chemically similar and, consequently, biologically similar. The proximity of each type of sample in the PCA scores plot shows that the extraction processes were quite reproducible and that both the type of extraction (maceration and decoction) and the relationship between SCOBY mass and solvent volume (1:5, 1:10, and 1:20) are essential for differentiating the chemical composition of the samples and, consequently, for the different antioxidant and total phenolic activities observed.

During the PCA analysis, the loading plot was also obtained (Fig. 4), which allows for verifying the influence of different factors on the separation of the samples. Loading values of the variables associated with the PCA scores plot (Fig. 3) were used to explain the discrimination among the extracts. By analyzing Fig. 4, FRAP and total phenolics values have the same importance in the discrimination of samples and influence the result by taking them to lower values of PC1.

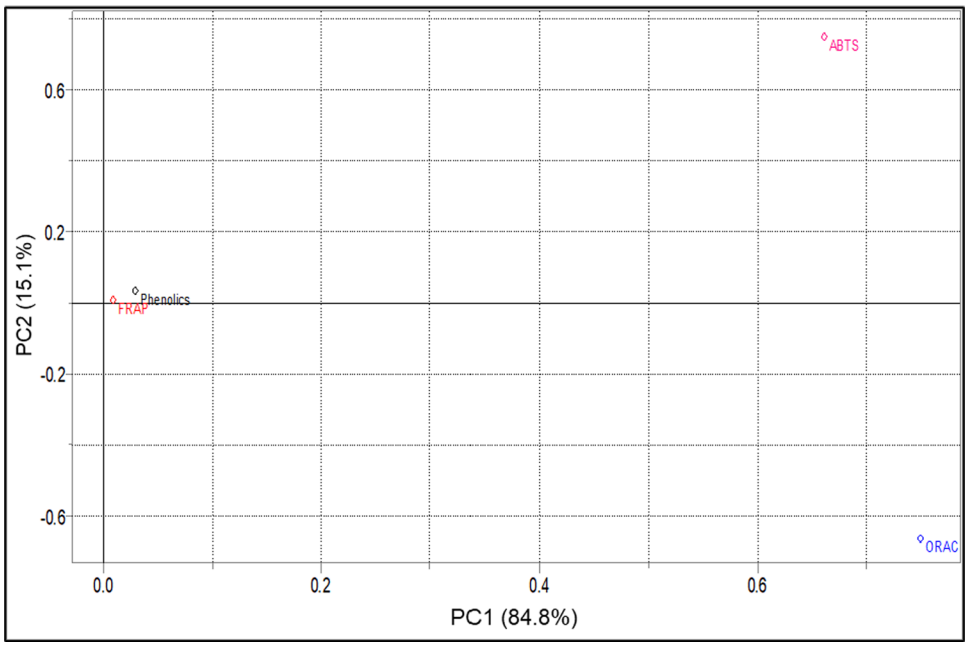

Fig. 4 Loading plot of chemometric analysis of data on phenolic compounds concentration and antioxidant potential of SCOBY ethanolic extracts

On the other hand, ORAC took the samples to positive values of PC1 and negative values of PC2 and $ABTS^{\bullet+}$ to positive values of PC1 and PC2. However, both ($ABTS^{\bullet+}$ and ORAC) have similar magnitude values of PC2.

Figures 3 and 4 show that elevated values of $ABTS^{\bullet+}$ mainly influenced the discrimination of samples D_1:20 (decoction, 1:20). The grouping of M_1:20 extracts (maceration, 1:20) were influenced by ORAC and $ABTS^{\bullet+}$ values (that were smaller than D_1:20). On the other hand, the lowest FRAP and total phenolics values led the samples M_1:5 (maceration, 1:5) and D_1:5 (decoction, 1:5) to negative PC1. Finally, samples M_1:10 (maceration, 1:10) and D_1:10 (decoction, 1:10) have intermediate total phenolics and antioxidant activity, which did not significantly influence the discrimination.

4 Conclusion

This investigation showed that extracting active compounds from SCOBY is a promising alternative to using this industrial waste. Extraction by decoction with high amount of solvent (1:20) afforded ethanolic extracts with higher total phenolic content and antioxidant activity than those obtained by maceration and/or in other sample/solvent proportions. The chemometric analysis showed that the extracts' similarity depends on the solid–liquid extraction and the residue mass: solvent volume ratio. Therefore, the extraction of active compounds from SCOBY is interesting and can be applied in different industries, such as, pharmaceutical, cosmetic, and food, but new investigations must be carried out.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate This article does not contain any studies with human or animal subjects.

Competing interests The authors declare that they have no conflict of interest.

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