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Research article

Determination of the physicochemical characteristics and bioactive compounds of the miracle fruit *(Synsepalum dulcificum)* considering different extraction and preservation methods

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ABSTRACT

The miracle fruit (*Synsepalum dulcificum*) is distinguished by its unique ability to alter taste perceptions, making it a low-calorie sugar substitute. This study aimed to determine its physicochemical characteristics and bioactive compounds by evaluating various extraction and preservation methods. Utilizing a Completely Randomized Block Design with a factorial arrangement (A*B), where factor A represented extraction methods (cold pressing, maceration, and pressurized liquids) and factor B represented preservation methods (fluid extract and ionic emulsion), the research found that bromatological analyses showed pH values ranging from 2.990 to 3.535, acidity between 0.13 and 0.14, soluble solids from 4.45 to 41.00, and protein content from 0.029 to 4.670. The maceration with fluid extract method achieved the most favorable results in terms of bioactive compounds, including flavonoids (20.14 mg Eq/g), anthocyanins (9.55 mg/g), total phenolic content (16.53 mg gallic acid/g), and antioxidant capacity (521.24 μg Trolox/g). Conversely, cold pressing combined with fluid extract was the most effective for carotenoid extraction (77.16 μg/g). Thus, maceration with fluid extract is the most efficient for obtaining bioactive compounds, while cold pressing combined with fluid extract excels in carotenoid extraction.

1. Introduction

Synsepalum dulcificum, commonly known as miracle fruit, is a shrub native to the tropical region of West Africa [1]. Currently, EFSA (European Food Safety Authority) has approved miracle berry as a novel food under Regulation (EU) 2015/2283 [2]. It has also gained

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significant attention among researchers and food manufacturers due to the plant's pharmacological properties, which include anti-diabetic, blood cholesterol-lowering, antihyperuricemic, antioxidant, anticonvulsant, and anticancer effects [3].

Berries are famous for their unique ability to temporarily alter taste perception [4]. This phenomenon is due to the presence of a glycoprotein called *miraculin*, which binds to taste receptors and transforms sour tastes into sweet ones [5]. In addition to its ability to modify taste, miracle fruit contains a variety of bioactive compounds including flavonoids, antioxidants, phenolic acids, and other substances with potential for pharmacological and food applications [6].

Previous research has shown that the miracle fruit is a rich source of antioxidant-rich phytochemicals, similar to blueberries, blackberries, cherries, and grapes [7]. From a gastronomic and nutritional perspective, this fruit is of notable interest due to its bioactive compounds. The pulp is rich in phenolic compounds (15.8 %) and flavonoids (11.9 %), while the skin contains even higher concentrations of these compounds: 36.7 % phenolics and 51.9 % flavonoids [8]. These compounds have been linked to antioxidant properties, enhancing their nutritional value and potential health benefits [9].

The most common routes used to extract compounds by traditional methods include hydrodistillation (HD), aqueous extraction, and Soxhlet extraction [10]. HD has been widely used to extract phenolic compounds from plants [11]. However, the time required for the process is quite long and the yield is lower and very expensive. These techniques can also lead to degradation of thermosensitive polyphenolic compounds and are often difficult to automate, making them unsuitable for larger scales [12].

Therefore, in recent years, many advanced extraction techniques have emerged, such as cold pressing, which involves extracting juice by crushing fruits without adding heat, in order to preserve enzymes and nutrients as close as possible to their original composition [13]. Among the different extraction methods used to obtain bioactive compounds from *Synsepalum dulcificum,* maceration, cold pressing, and extraction with pressurized liquids (supercritical CO_2) stand out [14]. Each of these methods has particular characteristics that make them suitable for different applications [15]. Maceration is a simple and economical process that, because it does not require heat, favors the preservation of compounds sensitive to high temperatures [16]. Cold pressing, on the other hand, is ideal for obtaining essential oils and liposoluble extracts, preserving the quality of nutrients and active principles. Finally, extraction with supercritical CO_2 is a highly selective method that allows obtaining pure extracts, without solvent residues, and is especially useful for the extraction of compounds with high biological value, such as flavonoids and terpenes [17].

The determination of the physicochemical characteristics and bioactive compounds of the fruit is crucial to understand its commercial and medicinal potential. In this context, the objective of this research was to determine the physicochemical characteristics and bioactive compounds of miracle fruit *(Synsepalum dulcificum)* by comparing different methods of extraction (cold pressing, maceration and pressurized liquid) and preservation (fluid extract and ionic gel). This approach allows to identify and optimize the most efficient methods for the extraction and preservation of bioactive compounds in order to develop products with improved functional properties.

2. Materials and methods

2.1. Materials

The miracle fruit was acquired from the company "Ecua Foresta" in the Quinindé canton, Esmeraldas province (Ecuador). The studies and experimental phase were conducted in the laboratories of the University of the Armed Forces "ESPE" in Santo Domingo, located at the geographic coordinates latitude: 00° 24′ 36″ and longitude: 79° 18′ 43″, at an altitude of 270 m above sea level.

2.2. Statistical analysis

A bifactorial A*B design was applied, where factor A = Extraction methods (Cold pressing; maceration; pressurized liquids) and factor B = Conservation methods (Fluid extraction; ionic emulsion), obtaining 6 treatments with 3 replicates. To determine statistical differences, the Tukey test was applied ($p < 0.05$) using the InfoStat program. The description of the treatments is presented in Table 1.

2.3. Experimental handling

2.3.1. Cold press extraction

The samples were first visually inspected to eliminate those in poor condition. They were then washed and disinfected. Once this phase was completed, the samples were stored for 24 h at −15 °C. The seeds were subjected to a cold pressing process using a hydraulic

Table 1
Treatments involved in the study.

Treatments	Description
T1	Cold pressing + fluid extract
T2	Cold pressing + ionic emulsion
T3	Maceration + fluid extract
T4	Maceration + ionic emulsion
T5	Pressurized liquids + fluid extract
T6	Pressurized liquids + emulsion

press applying 246–250 Bar of pressure and a temperature of 45 °C for 45 min. The seed extract was collected in stainless steel trays and finally filtered through a sieve [18].

2.3.2. Maceration extraction

The extraction involved placing 50 g of fruit samples in an oven (VWR Symphony, Colombia) for 24 h at 60 °C. Once dried, the samples were ground in a mortar and placed in a sterile amber glass container with 70 % ethyl alcohol added to fully cover the fruit. The jars were manually shaken three times a day for one week. After this period, the samples were filtered through a sterile sieve. The alcoholic content was evaporated using a rotary evaporator at 270 amber pressure at 40 °C with 100 rpm [19].

2.3.3. Pressurized liquid methods (LP) extraction

70 g of fresh material were added to blue-capped glass bottles with 100 ml of distilled water. These were then placed in an autoclave for 30 min at 100 °C and 60 bar pressure. After cooling, the samples were filtered through a sieve and sterile filter paper [20].

2.4. Preservation methods

2.4.1. Fluid extract

Each obtained extract was subjected to a second filtration to ensure the removal of impurities and was then placed in amber glass droppers (100 ml) for preservation [21].

2.4.2. Ionic emulsion

100 ml of an 11 % calcium chloride ($CaCl_2$) solution were prepared. This solution was poured into a beaker and placed on a stirring plate. Then, 50 ml of the miraculous fruit extract was mixed with 1 g of sodium alginate to obtain a 2 % solution. The tip of an automatic pipette was cut to increase the size of the spheres formed during the ionic emulsion process. 750 μL of the extract and alginate solution were quickly dropped into the 11 % calcium chloride solution while maintaining constant stirring. Once the spherifications were formed, they were washed with distilled water and stored in a glass container [22].

2.5. Bromatological characteristics of the extracts

Acidity, pH, soluble solids, moisture, protein, fiber, and fat were determined according to the procedures established in the Ecuadorian Technical Standard NTE INEN: acidity by titration using 25 ml in a 250 ml volumetric flask [23]; pH by direct reading with a potentiometer (Testo 206-PH2) [24]; soluble solids using a refractometer (POCKET PAL-3 from 0° - 30°Brix), by placing a drop on the glass and taking the reading [25]; moisture by oven drying (100 °C) [26]; protein by Micro-Kjeldahl [27]; fat by Soxtec System [28]; and fiber by muffle furnace (600 °C) [29].

2.6. Chemical characterization of the extracts

The characterization of bioactive compounds in different extracts was performed using gas chromatography-mass spectrometry (GC-MS) [30].

2.7. Determination of bioactive compounds

2.7.1. Flavonoid content

The total flavonoid content of the extracts was determined by the colorimetric method with aluminum chloride, following the procedure described by Popa et al. [31]. Quercetin was used as a standard to perform a calibration curve. For this purpose, from quercetin stock solutions (5 mg/ml), dilutions were prepared in methanol with concentrations of 0.1; 0.5; 1.0, 1.0; 2.5 and 5 mg/ml. To 100 μl of each quercetin dilution, 500 μl of distilled water, 100 μl of 5 % sodium nitrate, 150 μl of 10 % aluminum chloride and 200 μl of 1 M sodium hydroxide solution were added in sequence. The absorbance of the reaction mixture was measured at 510 nm using a UV spectrophotometer (Eppendorf UV–VIS). The total flavonoid content in the samples was calculated from the calibration curve obtained for the different quercetin concentrations using equation (1):

$$TFC \frac{C^{*}DF^{*}V}{M} \; Ecu\ 1 \tag{1}$$

TFC = total flavonoid content (mg EQ/g dry matter)
C = quercetin concentration (mg EQ/ml) determined from calibration curve.
DF = dilution factor.
V = extraction volume (ml)
M = mass of plant material of the extract (g).

2.7.2. Anthocyanin content

For anthocyanin quantification, extracts were filtered through a hydrophilic membrane (0.45 μm, Millipore, Bedford, MA, USA) and then transferred to a vial for injection into a Waters Alliance model 2690/5 high-performance liquid chromatograph (HPLC)

coupled to a photodiode array detector (Waters 2996). Chromatographic separation was carried out using a reverse column (C18, Thermo BDS Hypersil, 100 × 4.6 mm, 2.4 μm). The mobile phase consisted of an aqueous mixture of 5 % formic acid and acetonitrile, with a constant flow rate of 1.0 ml - min $^{-1}$ and an injection volume of 20 μl. The gradient for solvent B was as follows: 5 % at start (0 min), 7 % at 2 min, 10 % at 10 min, 13 % at 15 min, 15 % at 16 min, 17 % at 20 min, 20 % at 30 min, and returning to 5 % at 33 min, results were obtained at a wavelength of 520 nm [32].

2.7.3. Carotenoid content

Twenty grams of crushed sample was taken and homogeneously mixed with 50 ml of hexane and incubated at 60 °C for 30 min for carotenoid extraction. After 10 min of centrifugation, the hexane phase was transferred to a clean tube. The remaining residue was subjected to a second extraction with 50 ml of acetone, and the obtained acetone phase was combined with the previously extracted hexane phase. The final miracle berry extract was obtained by solvent evaporation using a centrifugal vacuum evaporator (Labconco, Kansas City, MO, USA) [33].

2.8. Total phenolic content and antioxidant capacity of Synsepalum dulcificum extracts considering different extraction and preservation methods

Total phenolic content was determined using the Folin-Ciocalteu method. A mixture was prepared by combining 200 μL of the extract with 1 ml of Folin-Ciocalteu reagent (0.2 N) and 800 μL of a 7.5 % Na2CO3 solution. The mixture was incubated for 2 h in darkness at room temperature. Absorbance was then measured at 760 nm using a spectrophotometer (Jenway 6405UV/VIS). Total phenolic compounds were expressed in mg/g, calculated as gallic acid equivalents based on a gallic acid standard curve and the dry weight of the extract [34]. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and expressed in terms of hydrogen donation or radical scavenging capacity using the stable radical [35].

3. Results

3.1. Bromatological results of the extract according to the extraction method (factor A)

Table 2 presents the results obtained for the content of various compounds, where it is evident that Factor A (Extraction Method) showed significant differences ($p < 0.05$) in each of the variables. The values ranged as follows: pH (3.07–3.40), acidity (0.09–0.011), and protein (0.89 %–2.35 %). Notably, the maceration extraction method resulted in a significant increase in the concentration of soluble solids compared to the other methods.

3.2. Bromatological results according to the conservation method (factor B)

Table 3 shows the results obtained for the immediate components, where it can be observed that Factor B (Conservation Method) presented significant differences ($p < 0.05$) in the variables of acidity (0.007–0.013), soluble solids (13.10 Bx - 17.70 Bx), and protein (0.076 %–3.06 %). However, the pH of the extracts was not significantly affected by the conservation method. Additionally, it was found that the ionic emulsion considerably reduced the values of the evaluated parameters.

3.3. Bromatological results of the factor combination (A*B)

Fig. 1 presents the bromatological characteristics of the extract from *Synsepalum* dulcificum considering 3 extraction methods and 2 conservation methods. Significant differences ($p < 0.05$) were observed in the variables pH, acidity, °Brix, and protein.

In the pH results, considerable variation ($p < 0.05$) was observed between the different treatments (Fig. 1A). The extraction method using pressurized liquids with fluid extract conservation resulted in the highest pH (3.535), while maceration significantly reduced this parameter (2.990). These results indicate that extraction methods can significantly influence the pH of the sample, which could affect its stability and functional properties.

Regarding acidity (Fig. 1B), the fluid extract conservation method showed higher values, ranging from 0.13g/100g (as citric acid) to 0.14g/100g (as citric acid). On the other hand, ionic emulsion reduced the values to a range of 0.006 %–0.007 % for extracts obtained using pressurized liquids and cold pressing, respectively.

As shown in Fig. 1C, the total soluble solids content (°Brix) was also influenced by the different treatments applied. The maceration method with fluid extract produced the highest Brix value, reaching 41.000, followed by ionic emulsion with a value of 30.000. In

Table 2
Values of the immediate components of the miracle fruit extract *(Synsepalum dulcificum)* and significance according to the extraction method.

Extraction Method	pH	*Acidity*	*Soluble Solids (Bx)*	Protein (%)
Cold Pressing	$3.37^{B} \pm 0.15$	$0.010^{B} \pm 0.02$	$4.80^{A} \pm 0.12$	$2.35^{C} \pm 0.11$
Maceration	$3.07^{A} \pm 0.10$	$0.011^{C} \pm 0.02$	$35.50^{B} \pm 1.20$	$0.89^{A} \pm 0.10$
Pressurized Liquids	$3.40^{B} \pm 0.03$	$0.009^{A} \pm 0.01$	$5.90^{A} \pm 0.16$	$1.37^{B} \pm 0.09$

$^{A, B,C:}$ statistically significant (using Tukey's test, $p < 0.05$).

Table 3
Values of the immediate components of the miracle fruit extract *(Synsepalum dulcificum)* and significance according to the conservation method.

Métodos de conservación	pH	*Acidity (%)*	*Soluble Solids (Bx)*	Protein (%)
Fluid Extract	$3.29^{A} \pm 0.11$	$0.013^{B} \pm 0.04$	$17.70^{B} \pm 1.16$	$3.06^{B} \pm 0.12$
Ionic Emulsion	$3.26^{A} \pm 0.10$	$0.007^{A} \pm 0.01$	$13.10^{A} \pm 0.61$	$0.076^{A} \pm 0.03$

$^{A, B:}$ statistically significant (using Tukey's test, $p < 0.05$).

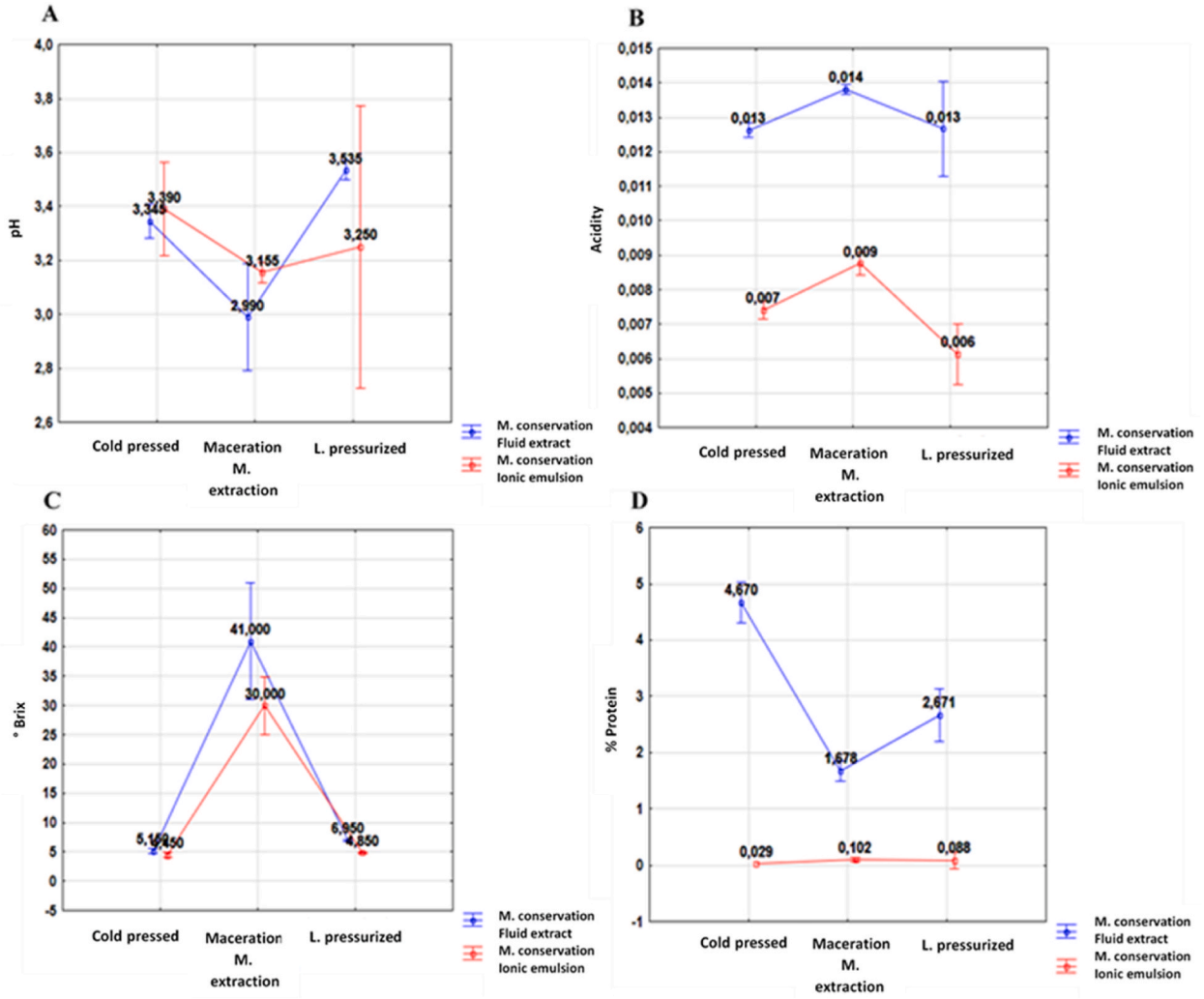

Fig. 1. Bromatological characteristics of the miracle fruit extract *(Synsepalum dulcificum)* based on different extraction and conservation methods: pH (A), acidity (B), °Brix (C) and protein content (D).

contrast, the combination of cold pressing and pressurized liquids with both conservation methods resulted in the lowest °Brix values, with concentrations of 4.450 and 4.850, respectively. These results suggest that maceration is particularly effective for extracting soluble solids from the sample.

Fig. 1D shows that extraction using cold pressing and fluid extract conservation better maintains the protein content, presenting higher values (4.670 %). In contrast, conservation through ionic emulsion significantly reduces ($p < 0.05$) the protein content, with the lowest values compared to the other two extraction methods (0.029 %, 0.102 %, and 0.088 %). These results indicate that cold pressing is the most efficient method for preserving proteins in the sample, while ionic emulsion and other extraction and pressurization methods could lead to significant protein losses.

3.4. Chemical characterization of the extracts

Fig. 2 shows significant variability in the chemical composition of six different samples, highlighting the presence of a high proportion of unidentified compounds in all of them. The results also indicate a complex mixture of substances, including pentacyclic triterpenes, steroids, monoglycerides, fatty acids, carbohydrates, organic acids, and alcohols. Notably, fatty acids and organic acids are the most abundant compounds, with higher concentrations in T1 (cold pressing + fluid extract) and lower concentrations in T6 (pressurized liquids + ionic emulsion). Pentacyclic triterpenes are also present at relatively high levels, while steroids and alcohols are found in low and fairly stable concentrations, except for a slight increase in alcohols in T3 (maceration + fluid extract), T5 (pressurized liquids + fluid extract), and T6 (pressurized liquids + ionic emulsion). Monoglycerides maintain a consistent presence across all samples, indicating stability in their concentration.

3.5. Bioactive compounds in extracts of Synsepalum dulcificum considering different extraction and preservation methods

The results obtained reveal significant variations in the concentration of flavonoids, anthocyanins and carotenoids (Fig. 3), with the cold pressing extraction method combined with extract fluid presenting the highest concentrations, with 19.75 mg Eq/g of flavonoids (Figs. 3A) and 77.16 μg/g of carotenoids (Fig. 3C). Maceration with fluid extract also showed elevated levels of flavonoids (20.14 mg Eq/g) and anthocyanins (9.55 mg/100 g of extract), although with a slight decrease in carotenoids (70.87 μg/g). Unlike the treatments with ionic emulsions, they generated lower concentrations of flavonoids and anthocyanins, with the lowest content of flavonoids found in the pressurized liquids with ionic emulsion (9.25 mg/100 g of extract) and the lowest content of anthocyanins in the same treatment (3.62 mg/100 g of extract), while carotenoids (Fig. 3B) also presented the lowest concentrations in these liquids, with 52.96 μg/g. These results show that both the extraction method and the type of emulsion used significantly influence the profile of bioactive compounds in the evaluated treatments.

3.6. Total phenolic content and antioxidant capacity of Synsepalum dulcificum extracts considering different extraction and preservation methods

Table 4 shows that the extraction method using maceration with fluid extract is the most efficient, yielding the highest total phenolic content (16.53 mg gallic acid/g) and the greatest antioxidant capacity (521.24 μg Trolox/g), indicating effective extraction and preservation of these compounds. In contrast, the pressurized liquid + ionic emulsion method resulted in the lowest total phenolic content (8.12 mg gallic acid/g) and the lowest antioxidant capacity (220.11 μg Trolox/g), reducing the composition of the extracts. On the other hand, although cold pressing methods also provide favorable results, particularly with fluid extract, they do not achieve the effectiveness of the maceration method.

4. Discussion

4.1. Bromatological characterization

In the pH variable, significant differences ($p < 0.05$) were observed between the extraction and conservation methods, with the pressurized liquid method with fluid extract presenting the highest pH content with 3.535 % (Fig. 1C). This result is comparable with the data reported by Valdez – López et al. [36], who determined a pH value of 4.40 in extracts obtained by the maceration method of

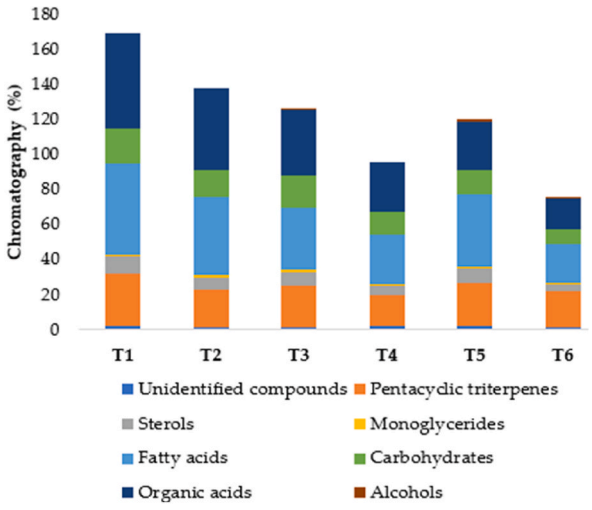

Fig. 2. Chromatographic (%) profile of each family characterized in the Synsepalum dulcificum extracts.

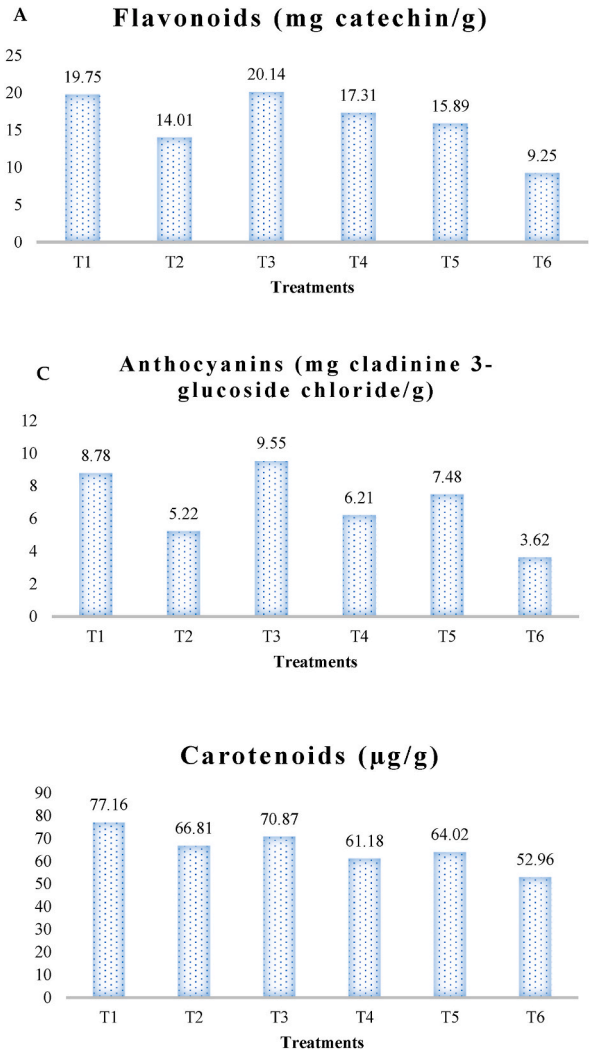

Fig. 3. Bioactive compounds in Synsepalum dulcificum extracts under different extraction and preservation methods. A: Flavonoids (mg of catechin/g), B: Anthocyanins (mg of cladinin-3-glucoside chloride/g) and C: Carotenoids (µg/g).

Table 4
Total phenolic content and in vitro antioxidant capacity of *Synsepalum dulcificum* extracts considering different extraction and preservation methods.

Tratamients	Total phenolic content (mg gallic acid/g)	Antioxidant capacity (µg Trolox/g)
Cold pressing + fluid extract	$14.23^{CD} \pm 0.58$	$438.89^{E} \pm 1.12$
Cold pressing + ionic emulsion	$13.60^{C} \pm 0.71$	$394.11^{C} \pm 0.98$
Maceration + fluid extract	$16.53^{D} \pm 0.12$	$521.24^{F} \pm 1.97$
Maceration + ionic emulsion	$12.21^{C} \pm 0.23$	$408.87^{D} \pm 1.01$
Pressurized liquids + fluid extract	$10.01^{B} \pm 0.45$	$320.01^{B} \pm 0.75$
Pressurized liquids + emulsion	$8.12^{A} \pm 0.57$	$220.11^{A} \pm 0.64$

$^{A,B,C,D,E,F:}$ statistically significant (using Tukey's test, $p < 0.05$).

Nephelium lappaceum L. The authors mention that the pH tends to decrease as the concentration of phenolic compounds increases, which is attributed to the more acidic characteristics that these compounds confer. Likewise, it has been confirmed that the pH in *Synsepalum dulcificum* pulp presents a pH of 3.20 [37] which compares well with other berries such as *Rubus subg. Rubus,* (3.4), *Fragaria* × *ananassa* (3.3–3.4) and *Rubus idaeus* (3.4) [14].

The titratable acidity content showed that the preservation method with fluid extracts presented the most relevant values (0.13 %–0.14 %), in contrast to the ionic preservation methods that significantly reduced the values (0.006 %–0.007 %). Studies on physicochemical properties of loquat (*Achras sapota* L.*)* extracts showed values ranging from 0.03 % to 0.06 % [38]. On the other hand, the determination of acidity is crucial to evaluate organoleptic characteristics, freshness and sour taste, so accurate methods for measuring

acidity are needed [39].

The analysis of soluble solids behavior revealed that the maceration method, using fluid extract as the preservation technique, produced the highest concentration reaching 41°Brix. This result demonstrates that maceration with fluid extract generates a significantly higher soluble solids content compared to other extraction methods and to previous studies. Authors such as Manzanarez-Tenorio et al. [40] reported a concentration of 23.8°Brix of soluble solids in their research on the antioxidant capacity of micro-encapsulated extracts of *Ficus carica*. Similarly, in studies on the properties of miracle fruit *(Synsepalum dulcificum),* both in its natural state and as an extract, a content of 10°Brix was observed [41].

The results obtained for protein content indicated that the cold pressing method with fluid extract was the most efficient, reaching 4.67 % protein. This result surpassed the extracts extracted by maceration and pressurized liquid, which indicated that cold pressing is the optimal method to preserve proteins. However, the values obtained were lower than those reported for *Synsepalum dulcificum* extracts, which presented a protein content of 8.42 % [42]. On the other hand, the results were similar to those found in *Chrysophyllum albidum*, which reported a protein content of 4.88 % [43].

4.2. Chemical composition

Previous research on chemical compounds in extracts of *Synsepalum dulcificum* (Schumach. & Thonn) Daniell has identified various bioactive compounds, such as glycoproteins, saponins, flavonoids, tannins, alkaloids, glucosides, phenols, resins, terpenoids, steroids, anthraquinones, cyanogenic glucosides, oleaginous substances, and phytosterols [44,45]. Additionally, a recent study isolated seventeen compounds from *S. dulcificum*, including (7S, 8R)-dihydrodeshydrodiconiferyl alcohol, (7S, 8R)-dihydrodeshydrodiconiferyl alcohol-9-β-D-glucopyranoside, quercetin, and quercetin-3-O-glucoside [46].

It is important to highlight that extraction and conservation methods affect the stability and efficacy of bioactive compounds. Cold extraction preserves heat-sensitive compounds such as flavonoids and antioxidants [47]. On the other hand, maceration with cold solvents extracts a broader range of compounds, depending on the solvent and time [48]. However, pressurized liquids may denature compounds due to the high temperatures involved in the process [49]. Regarding conservation, fluid extracts generally maintain compound stability better, minimizing oxidation [16], and ionic emulsions offer additional protection against degradation, though their effectiveness varies depending on formulation and specific compounds [17].

4.3. Bioactive composition

The bioactive composition of treatments showed variability in the results obtained. It was determined that the extraction method using pressurized liquids and conservation by ionic emulsion significantly reduces flavonoid content. Previous research indicated that microwave-assisted extraction yields higher amounts of total flavonoids (26.70 mg GAE/g) compared to ultrasound-assisted methanol extraction, which resulted in a lower amount of flavonoids (19.50 mg GAE/g PS) in *Vitis vinifera* waste [50]. On the other hand, extracts from the skin of the miracle fruit show higher free flavonoid content compared to the seed and pulp, providing approximately 15.00 mg of catechin per gram [51,52].

The amount of anthocyanins in the extracts showed significant variation depending on conservation conditions. Quantification of anthocyanins in the lyophilized sample revealed a total content of 11.4mg/100 g [6]. This value is comparable to the 14.3mg/100 g in dry weight reported by Cóndor-Soto (2019), suggesting that the main anthocyanin compound in miracle fruits is cyanidin-3-monogalactoside, which represents 70.70 % of the pigment [33]. According to one study, anthocyanin levels in *Synsepalum dulcificum* are relatively low compared to other anthocyanin-rich fruits, such as blueberries (*Vaccinium corymbosum*) and cherries (*Prunus avium*) [31].

Conservation methods significantly affect the total carotenoid content. Previous research has shown that freezing and canning increase phenolic and carotenoid content in processed samples, while drying causes a reduction compared to fresh samples. Additionally, storage time also has a notable impact on the bioactive compounds in processed samples of *Prunus armeniaca* [53]. On the other hand, carotenoid levels in extracts obtained using solvents exceeded those reported by Ladyslene et al. (2019), who extracted carotenoids from *Caryocar brasiliense* using acetone (30.0mg/100 g), hexane (19.9mg/100 g), and ethyl alcohol (29.60mg/100 g) [54].

4.4. Total phenolic content and antioxidant capacity of synsepalum dulcificum extracts considering different extraction and conservation methods

Table 4 shows the results of the total phenolic content and in vitro antioxidant capacity of extracts obtained from the miracle fruit. The phenolic compounds analyzed show variability in their concentration depending on the source and extraction method, where maceration combined with fluid extract presented the highest content with 16.53 mg gallic acid/g, while the pressurized liquid and ionic emulsion methods obtained the lowest content with 8.12. Research on *Physalis peruviana* extracts reported a value of 9.07gEAG/100g [55]. In the study by Wong-Paz et al. (2020), values ranged between 34.00 and 37.67 mg/g in citrus waste extracts [56]. Additionally, phenolic compounds play a crucial role in extending the shelf life of foods by offering antimicrobial properties, as well as preserving the color, flavor, and aroma of products [57].

Antioxidant content ranged between 220.11 and 521.24 μg Trolox/g, results that are higher than those obtained using pressurized liquid extraction (PLE) for bioactive compounds in *Moringa oleifera* leaves, with values between 39.46 and 124.29 μmol AAE/g [49]. In other studies where encapsulation extraction was determined, values ranged between 220.58 and 280.64 mg Trolox/g [50]. This demonstrates that the maceration + fluid extract method yields higher antioxidant content. On the other hand, bioactive compounds

found in fruit extracts are known for their antioxidant and antimicrobial capacity, making them increasingly relevant due to their safety for human use [58].

5. Conclusions

In conclusion, the study demonstrates that extraction and conservation methods significantly impact the bromatological and bioactive characteristics of *Synsepalum dulcificum* extracts. The combination of maceration and cold pressing with conservation in fluid extract resulted in higher pH, greater soluble solids concentration, and better protein preservation, indicating greater chemical and functional stability of the extract. Moreover, these methods achieved high levels of flavonoids, carotenoids, and antioxidant capacity, suggesting a more efficient extraction of bioactive compounds. In contrast, extraction methods using pressurized liquids combined with ionic emulsion produced significantly lower values in pH, soluble solids, and protein content, and reduced bioactive and phenolic compound concentrations, reflecting lower effectiveness in preserving nutritional and antioxidant properties. These results underscore the importance of carefully selecting extraction and conservation methods to optimize the quality and functionality of *Synsepalum dulcificum* extract, highlighting maceration with fluid extract as the most effective technique for applications requiring high bioactive activity and product stability.

CRediT authorship contribution statement

Juan Alejandro Neira Mosquera: Conceptualization. **Sungey Naynee Sánchez Llaguno:** Project administration, Methodology. **Karol Yannela Revilla Escobar:** Formal analysis, Data curation, Conceptualization. **Jhonnatan Placido Aldas Morejon:** Methodology, Investigation. **Angie Estefania Iguasnia Ureta:** Writing – original draft, Visualization, Validation. **Andy Sebastian Parrales Loor:** Software, Resources, Project administration. **Jonathan Alexander Arguello Cedeño:** Writing – original draft, Project administration, Formal analysis.

Data share statement

Data will be made available upon request. To request data, please write to the corresponding author.

Ethics statement

This study was reviewed and approved by all authors.

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.

References

[1] R. Buckmire, F. Francis, Anthocyanins and flavonols of miracle fruit, Synsepalum dulcificum, Schum, J. Food Sci. 41 (6) (2006) 1363–1365, https://doi.org/10.1111/j.1365-2621.1976.tb01172.x.
[2] D.C.E. Turck, A. Naska, C. Pelaez, K. Pentieva, A. Siani, F. Thies, S. Tsabouri, M. Vinceti, Safety of Dried Fruits of *Synsepalum Dulcificum* as a Novel Food Pursuant to Regulation (EU) 2015/2283, J.SFA, 2021, https://doi.org/10.2903/j.efsa.2021.6600.
[3] B. Van-Wyk, A review of commercially important African medicinal plants, J. Ethnopharmacology. 176 (2015) 118–134, https://doi.org/10.1016/j.jep.2015.10.031.
[4] K. Revilla-Escobar, R. Barre-Zambrano, J. Aldas-Morejón, C. Tubay-Bermúdez, D. Sánchez-Aguilera, M. Carrillo-Pisco, Determination of the impact of miracle fruit (*Synsepalum dulcificum*) on chocolate production and its effect on physical-chemical and sensory characteristics, Nutr. clín. diet. Hosp. 43 (2) (2023), https://doi.org/10.12873/432revilla.
[5] A. Afolabi-Clement, R.A. Aanuoluwapo, K.O. Kayode, Nutritional benefits, phytochemical constituents, ethnomedicinal uses and biological properties of miracle fruit plant (*Synsepalum dulcificum* Shumach. & Thonn. Daniell), Heliyon 6 (12) (2020) e05837, https://doi.org/10.1016/j.heliyon.2020.e05837.
[6] Z. He, J. Tan, O. Lai, A. Ariff, Optimization of conditions for the single step IMAC purification of miraculin from *Synsepalum dulcificum*, Food Chem. 181 (2015) 19–24, https://doi.org/10.1016/j.foodchem.2014.11.166.
[7] D. Liqing, S. Yixiao, Z. Xiumei, P. Witoon, X. Zhimin, Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts, Food. Chemi. (2013), https://doi.org/10.1016/j.foodchem.2013.03.077.
[8] L.A. Conde-Hernández, J.R. Espinosa-Victoria, A. Trejo, J.Á. Guerrero-Beltrán, CO2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*), J. Food Eng. 200 (2017) 81–86, https://doi.org/10.1016/j.jfoodeng.2016.12.022.
[9] S. Wagner, M. Gómez-De Cedrón, J. Navarro-Del Hierro, D. Martín-Hernández, M.D. Siles, S. Santoyo, L. Jaime, D. Martín, T. Fornari, A. Ramírez, Biological activities of miracle berry supercritical extracts as metabolic regulators in chronic diseases, Int. J. Mol. Sci. 24 (8) (2023) 6957, https://doi.org/10.3390/ijms24086957.
[10] A. Oreopoulou, E. Papavassilopoulou, H. Bardouki, M. Vamvakias, A. Bimpilas, V. Oreopoulou, Antioxidant recovery from hydrodistillation residues of selected Lamiaceae species by alkaline extraction, Appl. Res. Med. Aromat. Plants. 8 (2018) 83–89, https://doi.org/10.1016/j.jarmap.2017.12.004.
[11] N. Jeyaratnam, A.H. Nour, R. Kanthasamy, A.R. Yuvaraj, J.O. Akindoyo, Essential oil from *Cinnamomum cassia* bark through hydrodistillation and advanced microwave-assisted hydrodistillation, Ind. Crops Prod. 92 (2016) 57–66, https://doi.org/10.1016/j.indcrop.2016.07.049.
[12] E. Dobroslavić, I. Elez Garofulić, J. Šeparović, Z. Zorić, S. Pedisić, V. Dragović-Uzelac, Pressurized liquid extraction as a novel technique for the isolation of *Laurus nobilis* L. leaf polyphenols, Molecules 27 (16) (2022), https://doi.org/10.3390/molecules27165099.
[13] A. Prestes, M. Canella, C. Helm, A. Cruz, E. Prudencio, The use of cold pressing technique associated with emerging nonthermal technologies in the preservation of bioactive compounds in tropical fruit juices: an overview, Curr. Opin. Food Sci. 51 (2023), https://doi.org/10.1016/j.cofs.2023.101005.

[14] N. Azwanida, A review on the extraction methods used in medicinal plants, principle, Med. Aromat. Plants. 4 (196) (2015), https://doi.org/10.4172/2167-0412.1000196.

[15] E. Dobroslavić, M. Repajić, V. Dragović-Uzelac, G.I. Elez, Isolation of *Laurus nobilis* leaf polyphenols: a review on current techniques and future perspectives, Foods 11 (235) (2022), https://doi.org/10.3390/foods11020235.

[16] S. Ventura, F.A. Silva, M. Quental, D. Mondal, M.G. Freire, J.A. Coutinho, Ionic-liquid-mediated extraction and separation processes for bioactive compounds: past, present, and future trend, Chem. Reviews (2017), https://doi.org/10.1021/acs.chemrev.6b00550.

[17] N. Ortiz-Romero, L.A. Ochoa-Martínez, S.M. González-Herrera, O. Rutiaga-Quiñones, J.A. Gallegos-Infante, Advances in research on encapsulation through ionic gelation: a systematic review, TecnoL 24 (52) (2021), https://doi.org/10.22430/22565337.1962.

[18] T. Fonteles, D. Maia, B. Santos, F. Fernandes, S. Rodrigues, P. Campelo, Nonthermal processing as a tool to enhance fruit juice bioactive compounds' bioaccessibility, Processes 12 (8) (2024) 1640, https://doi.org/10.3390/pr12081640, 2024.

[19] C. Bitwell, S. Sen-Indra, C. Luke, M. Kenneth-Kakoma, A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants, Scie. Afr. 19 (2023), https://doi.org/10.1016/j.sciaf.2023.00223.

[20] H. Fraguela-Meissimilly, J.M. Bastías-Montes, J.A. Ortiz-Viedma, Y. Tamarit-Pino, M. Claret-Merino, J. Araneda-Flores, Valorization of extracts from maqui (*Aristotelia chilensis*) and calafate (*Berberis microphylla*) biowaste blends by supercritical fluid and pressurized liquid extraction, J. Agric. Res. 2024 15 (2024), https://doi.org/10.1016/j.jafr.2023.100950.

[21] J. Ahmad-Khan, S. Jabin, Chapter 8 - ionic liquids as valuable assets in extraction techniques, in: Adv. Appl. Ionic. Liquids, 2023, pp. 199–221, https://doi.org/10.1016/B978-0-323-99921-2.00014-8.

[22] E. Hernan-Lara, I. Rivera-Abascal, I. Gallegos-Marín, Encapsulation of hydroalcoholic extracts of *Moringa oleifera* seed through ionic gelation, LWT 203 (2024), https://doi.org/10.1016/j.lwt.2024.116368.

[23] Ecuadorian Institute of Standardization [INEN], Ecuadorian Technical Standard NTE INEN-ISO 750:2013. Plant and fruit products - determination of titratable acidity (IDT), Quito, Ecuador (2011). https://www.normalizacion.gob.ec/buzon/normas/381.pdf.

[24] Ecuadorian Institute of Standardization [INEN], Ecuadorian technical standard NTE INEN-ISO 1842:2013. Vegetable and fruit products - determination of pH. Quito. Ecuador. https://www.studocu.com/ph/document/university-of-st-la-salle/agriculture/inen-1842-productos-vegetales-y-de-frutas-determinacion-de-p-h-idt/65958580, 2013.

[25] Ecuadorian Institute of Standardization [INEN], Ecuadorian Technical Standard NTE INEN-ISO 2 337:2008. Juices, pulps, concentrates, nectars, fruit and vegetable drinks, Requisitos. Quito. Ecuador (2008). https://www.studocu.com/ec/document/universidad-de-guayaquil/analisis-de-alimentos/nte-inen-2337/10741008.

[26] Ecuadorian Institute of Standardization [INEN], Ecuadorian Technical Standard NTE INEN-ISO 1462:2013. Cereals and cereal products. Determination of moisture content, Basic reference method. Quito. Ecuador (2013). https://www.normalizacion.gob.ec/buzon/normas/381.pdf.

[27] Ecuadorian Institute of Standardization [INEN], Ecuadorian technical standard NTE INEN-ISO 16:1983. Determination of proteins, Quito. Ecuador (1983). https://www.normalizacion.gob.ec/buzon/normas/16.pdf.

[28] Ecuadorian Institute of Standardization [INEN], Ecuadorian Technical Standard NTE INEN-ISO 541:1980. Determination of fat content, Quito. Ecuador (1980). https://www.normalizacion.gob.ec/buzon/normas/541.pdf.

[29] Ecuadorian Institute of Standardization [INEN], Ecuadorian Technical Standard NTE INEN-ISO 522:2013. Plant-based flours. Determination of crude fiber, Quito. Ecuador (2013). https://www.normalizacion.gob.ec/buzon/normas/522-1R.pdf.

[30] L. Villarreal-Romero, J. Robles, G. Costa, State of the art in the standardization of plant extracts, Laboratory of Phytochem 52 (2) (2023) 842–867, https://doi.org/10.15446/rcciquifa.v52n2.110745.

[31] G. Popa, R. Toma, J. Francois-Boé, Experimental approaches targeting the biochemical and antioxidant potential of Synsepalum dulcificum dried fruits, Rom. Biotechnol. 26 (1) (2021) 2230–2235, https://doi.org/10.25083/rbl/26.1/2230.2235.

[32] G. Nunes-Mattos, M.C. Pessanga de Araujo-Santiago, A.C. Sampaio-Dotia, A. Rosenthal, R. Valeriano-Tonon, L.M. Correa-Cabral, Anthocyanin extraction from jaboticaba skin *(Myrciaria cauliflora Berg.)* using conventional and non-conventional methods, Foods 11 (6) (2022) 885, https://doi.org/10.3390/foods11060885.

[33] L. Du, Y. Shen, X. Zhang, W. Prinyawiwatkul, Z. Xu, Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts, Food Chem. 153 (2014) 279, https://doi.org/10.1016/j.foodchem.2013.12.072, 2.

[34] R. Huaraca-Aparco, F. Tapia-Tadeo, R. Cahuana-Lipa, F.G. Casas-Paz, Md Delgado-Laime, J.C. Machana-Mamani, Phenolic compounds and antioxidant activity in a functional beverage, J. Res. Agron. Vet. Sci. 7 (2023) 218–231, https://doi.org/10.33996/revistaalfa.v7i19.211.

[35] C. Proestos, K. Lytoudi, O. Konstantina-Mavromelanidou, P. Zoumpoulakis, V.J. Sinanoglou, Antioxidant capacity of selected plant extracts and their essential oils, Antioxidants 2 (1) (2013) 11–22, https://doi.org/10.3390/antiox2010011.

[36] L.L. Valdez-López, Y.I. Gutiérrez-Gaitén, V. García-Mir, O.M. Nieto-Acosta, Characterization of the extraction process by maceration using a fractional factorial design in *Nephelium lappaceum L. Cuban*, J. Pharm. (Lahore) 55 (3) (2022). https://revfarmacia.sld.cu/index.php/far/article/view/738.

[37] N.E. Njoku, C.N. Ubbaonu, S.O. Alagbaoso, I.M. Agunwa, C.N. Eluchie, Composición proximal, antinutricional y fitoquímica de la variedad amarilla de Synsepalum dulcificum (fruta milagrosa), J. Food Sci. Technol. 4 (4) (2016) 102–108.

[38] Y. Albornoz, B. Sulbarán, G. Ojeda-De Rodriguez, Caracterización fisicoquímica y contenido de minerales en pulpa de níspero *(Achras sapota L.)*, Boletín CIB 42 (2) (2008) 229–242. https://www.researchgate.net/publication/284551798_Caracterizacion_fisicoquimica_y_contenido_de_minerales_en_pulpa_de_nispero_Achras_sapota.

[39] L.D. Jimenez-Jumbo, S.P. Villacres-Parco, C.M. Cujilema-Tenezaca, G.M. Chuin-Vargas, Determination of titratable acidity in Amazonian citrus fruits: correlation with maturity and susceptibility to decay, Lat. Sci. Multidiscip. Sci. J. 8 (2) (2024), https://doi.org/10.37811/clrcm.v8i2.10681.

[40] L.E. Manzanarez-Tenorio, S. Ruiz-Cruz, E. Márquez-Ríos, Jd Ornelas-Paz, C.L. Del-Toro-Sánchez, F.J. Wong-Corral, G.M. Suárez-Jiménez, G.E. Dévora-Isiordia, R. Holguin-Soto, Microencapsulation of fig *(Ficus carica)* extracts by complex coacervation and evaluation of their antioxidant capacity, Botecnia 22 (2) (2020), https://doi.org/10.18633/biotecnia.v22i2.1247.

[41] G. Khakasar, K. Assatarakul, S. Sirikantaramas, Effect of cold-pressed and normal centrifugal juicing on quality attributes of fresh juices: do cold-pressed juices harbor a superior nutritional quality and antioxidant capacity, Heliyon 5 (6) (2019) e01917, https://doi.org/10.1016/j.heliyon.2019.e01917.

[42] O. Lanre-Aeotedu, P. Oluwatimilehin-Ogunbamowo, Nutritional, anti-nutritional and phytochemical profile of the leaves and fruits of *Synsepalum dulcificum (Schumach. & Thonn*, Daniell. Am. J. Biol. Chem. (2019) 53–59. https://miraburst.com/wp-content/uploads/2020/01/Nutritionals-Anti-Nutritional-and-Phytochemical-Profile-of-the-Leaves-and-Fruit-of-the-Miracle-Fruit.pdf.

[43] Gibson Lucky Arueya, Grace Febechi Ugwu, Development and evaluation of African star apple (*Chrysophyllum albidum*) based food supplement and its potential in combating oxidative stress, J. Funct.Foods 33 (2017) 376–385, https://doi.org/10.1016/j.jff.2017.04.004.

[44] H. Ibrahim, N. Kassim, N. Che-Soh, Z. Othman, T. Tuan-Ismail, Antibacterial property of Synsepalum dulcificum leaves aqueous extract against oral pathogens and its chemical compounds, Arch. Orofac. Sci. 15 (1) (2020) 55–64. https://aos.usm.my/docs/Vol_15/aos-article-427.pdf.

[45] S. Afzal, A. Manap, C. Zelynn, A comprehensive review on chemical and pharmacological potential of Synsepalum dulcificum (Schumach. & Thonn.) Daniell. Bol. Latinoam. Caribe. Plant, Med. Aromat. 23 (5) (2024) 706–731, https://doi.org/10.37360/blacpma.24.23.5.44.

[46] J. Wang, S. Shao, R. Huang, Shaohua Wu, Chemical constituents and their antioxidant activities from the leaves of *Synsepalum dulcificum*, Nat. Prod. Res. 34 (23) (2020) 6141–6146, https://doi.org/10.1080/14786419.2020.1830394.

[47] M.F. Ramadan-Hassanien, Cold pressed oils: green technology, bioactive compounds, functionality, and applications, Food. Sci. Technol. Nutr. (2020), https://doi.org/10.1016/C2018-0-03151-5.

[48] H. Andishmand, B. Masoumi, M. Torbati, A. Homayouni-Rad, S. Azadmard-Damirchi, H. Hamishehkar, Ultrasonication/dynamic maceration-assisted extraction method as a novel combined approach for recovery of phenolic compounds from pomegranate peel, Food Sci. Nutr. (2023), https://doi.org/10.1002/fsn3.3642.

[49] T. Chatzimitakos, V. Athanasiadis, K. Kotsou, M. Mantiniotou, D. Kalompatsios, I. Makrygiannis, E. Bozinou, S. Lalas, Optimization of pressurized liquid extraction (PLE) parameters for extraction of bioactive compounds from Moringa oleifera leaves and bioactivity assessment, Int. J. Mol. Sci. 25 (9) (2024) 4628, https://doi.org/10.3390/ijms25094628.
[50] R. Casquete, M. Benito, A. Martín, A. Martínez, M. Rivas, M. Córdoba, Influence of different extraction methods on the compound profiles and functional properties of extracts from solid by-products of the wine industry, LWT 170 (2022) 114097, https://doi.org/10.1016/j.lwt.2022.114097.
[51] G.E. Inglett, D. Chen, Contents of phenolics and flavonoids and antioxidant activities in skin, pulp, and seeds of miracle fruit, J. Food Sci. 76 (3) (2011) 79–82, https://doi.org/10.1111/j.1750-3841.2011.02106.x.
[52] R. Abdulaziz, B. Dhiya-Dalila, Y. Alkasim-Kabiru, M. Jahan, In vitro anti-oxidant potential, total phenolic content and total flavonoid content of methanolic flower and seed extract of miracle tree (Moringa oleifera Lam.), Aust. J. Basic. Appl. Sci. 9 (20) (2015) 27–31. https://d1wqtxts1xzle7.cloudfront.net/38360828/australian_journal-libre.pdf?1438538134=&response-content-disposition=inline%3B+filename%3DIn_Vitro_Anti_Oxidant_Potential_Total_Ph.pdf&Expires=1725055648&Signature=Zs5cFu7uaeBC8chqJo2RridTÑG~V5u8DMHXKVRa1bf.
[53] S.M. Wani, F. Masoodi, H. Ehtishamul, A. Mukhtar, S. Ganai, Influence of processing methods and storage on phenolic compounds and carotenoids of apricots, LWT 132 (2020), https://doi.org/10.1016/j.lwt.2020.109846.
[54] C. Ladyslene, F. Silva, E. Silva, E. Asquieri, C. Damiani, Influence of preservation methods on the bioactivity of mangaba *(Hancornia speciosa Gomes)* from the Brazilian savannah, Food. Sci. Technol. 39 (2) (2019) 403–409, https://doi.org/10.1590/fst.31917.
[55] E.S. Ordoñez, A. Leon-Arevalo, H. Rivera-Rojas, Quantification of total polyphenols and antioxidant capacity in cacao *(Theobroma cacao L.)* husks and seeds, prickly pear *(Opuntia ficus indica Mill),* grape *(Vitis vinifera),* and nanches, Sci. Agropecu. 12 (2) (2019), https://doi.org/10.17268/sci.agropecu.2019.02.02.
[56] J.E. Wong-Paz, P. Aguilar-Zárate, F. Veana, D.B. Muñiz-Márquez, Impact of green extraction technologies for obtaining bioactive compounds from citrus fruit waste, J. Spec. Chem-Biol. Sci. 23 (2020). https://www.redalyc.org/journal/432/43266574023/43266574023.pdf.
[57] A. Mueed, S. Shibli, D. Al-Quaie, M.F. Ashkan, M. Alharbi, H. Alanazi, N. Binothman, M. Aljadani, K. Ali-Majrashi, M. Huwaikem, M.A. Abourehab, S. Korma, M. El-Saadony, Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition, Front. Nutr. 10 (2023), https://doi.org/10.3389/fnut.2023.1125106.
[58] S. Suriyaprom, P. Mosoni, S. Leroy, T. Kaewkod, M. Desvaux, Y. Tragoolpau, Antioxidants of fruit extracts as antimicrobial agents against pathogenic bacteria, Antioxidants 11 (3) (2022) 602, https://doi.org/10.3390/antiox11030602.