Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 17(6): 2564-2574, October 2023

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Effect of heat treatment on physico-chemical parameters and extractability of free radical scavengers from *Hibiscus sabdarifa* juice

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Received: 21-06-2023 Accepted: 07-10-2023 Published: 31-10-2023

ABSTRACT

Hibiscus sabdariffa also known as Roselle is a shrub generally measuring between one and two meters in height. After flowering, white or red calyxes appear, depending on the variety. These calyxes are highly prized by African populations for the production of drinking juice. Thus, this work was initiated to study the thermal effects on physico-chemical parameters of the juice obtained. The pH was determined using a pH meter, the conductivity using a conductivity meter. Polyphenols were quantified by the ferric chloride reaction and Folin-Ciocalteu's reagent. The antiradical activity was determined by the ABTS method and the mineral content was determined using an atomic absorption spectrometer. According to the results, the production of juice at 100°C allowed the extraction of more phytochemical compounds among which: flavonoids (66.6 mg EQ/100 g dry extract) and polyphenols (49.3 mg EAG/100 g dry extract). The results also showed a better anti-free radical capacity for the juice obtained at 100°C compared to the other juices in this study. Indeed, the highest percentage of inhibition was obtained with the lowest concentration of juice extracted at 100°C (0.446 mg/mL). Hence, this study allowed the determination of the optimal temperature for preparing hibiscus sabdarifa juice.

Keywords: Temperature, pH, polyphenols, minerals content, conductivity

INTRODUCTION

Hibiscus sabdariffa, or Guinea sorrel, is a shrub usually between one and two meters in height. It originated in Southeast Asia and was brought to Africa in the 19th century. It is a tropical plant that needs sun and heat. After flowering, the plant produces white or red calyxes, depending on the variety (Maldonado-Astudillo et al., 2019). These calyxes are highly prized for the production of juice by African populations in Guinea Conakry, Senegal, Mali, Burkina Faso, Benin, Egypt, Sudan, etc. (Cisse et al., 2009). In Senegal, this juice is better known as bissap juice, which derives from the plant's vernacular name in Wolof, the national language. Studies had

DOI: https://dx.doi.org/10.4314/ijbcs.v17i6.33

shown that this plant is rich in bioactive compounds with benefits for both humans and animals (Heim et al., 2002).

A number of antiradical and pharmacological properties associated with the use of fruits, herbal teas and fruit juices have also been described in the literature (Leong and Shui, 2002; Obouayeba et al., 2014; Maghsoudlou et al., 2019; Naco et al., 2023). However, the extraction of bioactive phytochemicals depends, among other factors, on the extraction temperature which could influence extraction capacity and the nature of the compounds. For this reason, it is necessary to work under optimal temperature conditions to avoid degradation of heat-sensitive compounds. In additional, many conventional techniques for producing and preserving natural beverage juices essentially use heating processes at different temperatures. It was with this in mind that this study was initiated, with the aim of defining optimal extraction temperature conditions while preserving the nutritional qualities and properties of the juice constituents obtained.

MATERIALS AND METHODS

Reagents and chemicals

All solvents and reagents used in this study were of analytical grade. Ethanol was purchased from Valdafrique laboratory Canonne (Senegal). 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and potassium persulfate were purchased from Sigma Aldrich (St-Louis, USA). Aluminum chloride ($AlCl_3$) was purchased from Panreac Applichem (Darmstadt, Germany). Sodium carbonate (Na_2CO_3) was purchased from Prolabo (France). Folin ciocalteu, Ascorbic acid, quercetin and gallic acid were purchased from Sigma Aldrich (Steinheim, Germany).

Plant material

It consisted of dried calyxes of the white variety of *Hibiscus sabdarifa*. The samples were purchased at the Fass Delorme market in Dakar in February 2023. The identification was carried out by the Laboratory of Pharmacognosy and Botany of the Faculty of Medicine, Pharmacy and Odontology of the Cheikh Anta Diop University of Dakar. The samples were ground using a mortar and pestle in the Physical Chemistry, Organic Chemistry, Inorganic Chemistry and Therapeutic Chemistry laboratories. A powder was then obtained and packaged in a clean bag pending physico-chemical analysis.

Juice extraction procedure

A mass of 15 g of fine powder of the white variety of *Hibiscus sabdarifa* was introduced into 500 ml erlenmeyer flasks, then 150 ml of distilled water was added and the whole was boiled for two hours. To recover as many compounds as possible, extraction was carried out in triplicates at different temperatures: 25°C, 50°C, 100°C and 150°C. The resulting solutions were filtered and concentrated using a rotary evaporator (Buchi B-480, Switzerland). The evaporation residues were then dried in a ventilated oven (Memmert^{TM} UN110) at 37°C.

pH determination

Acid-base balances play an essential role in the functioning of the human organism. Hence, slight variations in pH can lead to adverse health effects such as alkalosis or acidosis, which can be fatal. For these reasons, the pH of juices has been determined to assess their potential impact on people's health. Three measurements of each juice decocted was taken for two hours at different temperatures from 10% *Hibiscus sabdarifa* powders of the white variety. The pH of the filtrate was read directly using a pH meter (HANNA HI 9813-5 Portable pH/EC/TDS/Temperature Meter). A preliminary calibration was carried out using buffers of pH = 4 and 7. Before and between two measurements of different solutions, the probe should be immersed in a beaker of distilled water, then wiped lightly with absorbent paper.

Phytochemical compound content

Determination of flavonoid content by the aluminum chloride ($AlCl_3$) method

Flavonoid content was determined using a colorimetric method. The principle consists in adding aluminum chloride ($AlCl_3$), which reacts with flavonoids to form a stable acid complex (Figure 1) (Blaise et al., 2021; Makuasa and Ningsih, 2020).

For the quantification of the flavonoids, quercetin was used as reference molecule and the results were expressed in milligrams of quercetin equivalent per 100g of dry extract (mg EQ/100g of dry extract). Experimentally, 2 ml of extract was mixed with 2 ml of a 2% (w/v) aluminum chloride solution, then after homogenization, the mixture was incubated at room temperature for 10 minutes in the dark to allow the reaction to proceed. After this time, an absorbance reading was taken at a wavelength of 430 nm against a blank.

Determination of total phenolic content

Total phenolic content was assessed using the Folin colorimetric method (Figure 2) (Ainsworth and Gillespie, 2007; Mensah and Thompson, 2023; Ouedraogo et al., 2015).

According to the protocol used, to 500 µl of extract, 2.5 ml of Folin-Ciocalteu's reagent (0.1N) was added. After 5 minutes, 2 ml of sodium carbonate (75 g/l) was added, followed by incubation at 50°C for 5 minutes. After cooling, absorbance measurements were taken using a spectrophotometer (Thermo Scientific, Evolution 300 UV-Vis Spectrophotometer) at a wavelength of 760 nm against a blank. The quantification of total phenols was carried out using gallic acid as a reference. The total phenol content was calculated using the gallic acid calibration curve equation. Results were expressed in milligrams of gallic acid equivalent per 100 g of dry extract (mg GAE/100 g dry extract).

Determination of total polyphenolic content

The same method used to determine total phenol content was used with a few modifications. 0.1 ml of aqueous extract was mixed with 2 ml of a freshly prepared solution of 2% sodium carbonate (Na_2CO_3), then vortexed (Heidolph REAX 2000 Vortex Mixer). After five minutes, 100 µl of Folin-Ciocalteu reagent (1N) was added to the mixture, followed by 30 minutes of incubation at ambient temperature before reading with a spectrophotometer at 700 nm. The results were expressed in milligrams of gallic acid equivalent per 100 g of dry extract (mg GAE/100 g of dry extract).

Determination of free radical scavenging properties

The method used was that described by Leong and al (2002), which was adapted to evaluate free radical scavenging properties according to the basic principle of redox reactions (Balde et al., 2018; Sarr et al., 2015). This reaction principle allowed the reduction of the radical cation of 2,2'-Azino-bis(3-Ethylbenzothiazoline-6-sulfonic) acid (ABTS) ($ABTS^{•+}$) resulting from its oxidation by potassium persulfate (Figure 3).

A quantity of 38.40 mg of ABTS was first dissolved in 10 ml of distilled water. To obtain the radical cation, 6.75 mg of potassium persulfate was added to the solution. To ensure a smooth reaction, the resulting mixture was kept in the dark at room temperature for 12 hours before use. The absorbance was adjusted using ethanol to obtain a solution with an absorbance value of 0.7 for reliable quantification according to the Beer-Lambert law. To carry out the tests, 2 ml of the extract was mixed with 2 ml of the solution of the radical cation ABTS ($ABTS^{•+}$). Absorbance was then read after two minutes using a spectrophotometer at 734 nm, using ethanol as a blank.

Determination of mineral content

The mineral elements were dosed directly from juices obtained at different temperatures: 25°C, 50°C, 100°C, 150°C. After filtration (0.45µm filter), atomic absorption spectrometry (Thermo Scientific iCE 3300 AA Spectrometer) was used to quantify the mineral composition of the juices.

Conductivity determination

Measurements were taken with a conductivity meter (HANNA HI 9813-5 Portable pH/EC/TDS/Temperature Meter) previously calibrated with a standard conductivity solution, 1,413 µS/cm. Three measurements of each juice decocted from 10% *Hibiscus sabdarifa* powders for two hours at different temperatures were taken from the white variety. Before and between two measurements of different solutions, the probe is immersed in a beaker of distilled water, then lightly wiped with absorbent paper.

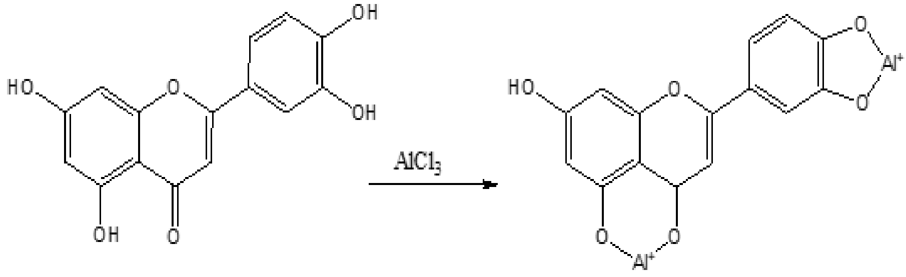

Figure 1: Principle of the reaction between quercetin and aluminium chloride

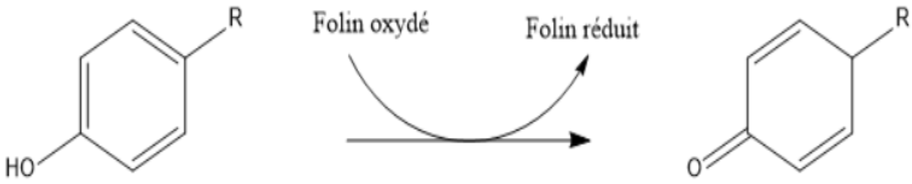

Figure 2: Principle of the reaction between phenolic compounds and Folin

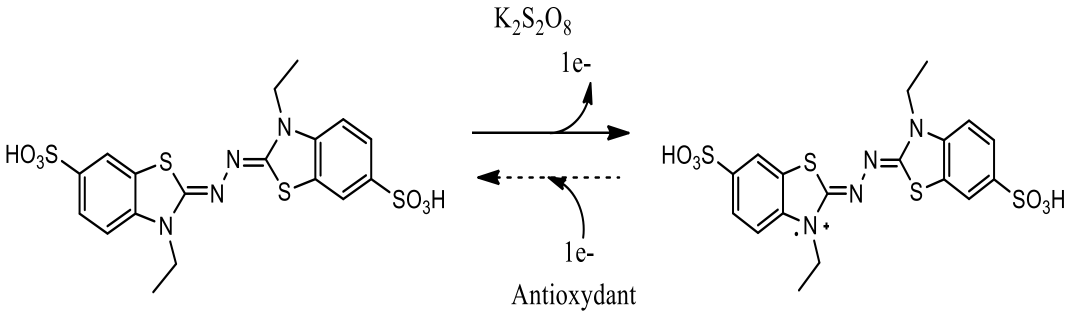

Figure 3: Mechanism of formation of ABTS radical cation ($ABTS^{\bullet +}$)

RESULTS

Juice extraction procedure

The juice obtained is a slightly reddish yellow solution with a more viscous appearance than the solvents used for the extraction.

pH determination

At the start of the experiment, pH dropped rapidly to a temperature of 100°C, before reaching a plateau between 100 and 150°C. A temperature-dependent decrease in pH was observed, from 2.4 to 2.1 between 25 to 100°C (Figure 4). This drop in pH could be explained by the presence of phenolic compounds in the juices obtained. Indeed, these compounds have acidic properties due to the presence of H^+ protons available at the level of the hydroxyl groups of the polyphenols. The low pH variation between 100 and 150°C could be explained by the progressive depletion of polyphenolic compounds in the matrix.

Phytochemical compound determination

The phytochemical contents of the juices are as presented in the Figures 5; 6 and 7. The content of polyphenolic compounds obtained during extraction increased progressively until reaching a maximum at 100°C. Indeed, the maximum contents of flavonoids (66.6 mg EQ/100 g dry extract) polyphenols (49.3 mg EAG/100 g dry extract) and phenols (13.8 mg EAG/100 g dry extract) were obtained at a juice extraction temperature of 100°C. However, the results showed a drop in these contents above 100°C, which could be explained by their thermosensitivity.

Determination of free radical scavenging properties

The graph (Figure 8) showed that the free radical scavenging capacity was a function of the juice extraction temperature. The lower the median inhibitory concentration (IC_{50}), the better the anti-free radical scavenging capacity. Thus, in this study, the lowest IC_{50} value (0.446 mg/mL) was obtained at a juice preparation temperature of 100°C. This means that juices obtained at this temperature contained more polyphenolic compounds and therefore had a better anti-free radical scavenging capacity. Thus, to obtain the best activity from *Hibiscus sabdarifa* juices, they need to be prepared at an optimum temperature of 100°C.

Determination of mineral content

The mineral content of juice as a function of temperature was presented in Figure 9. The search for mineral elements showed a higher calcium (Ca^{2+}) content for all juices, followed by potassium (K^{+}). The presence of iron (Fe^{2+}) and magnesium (Mg^{2+}) was also noted. The best mineral element contents were obtained at a temperature of 150°C.

Conductivity determination

The curve showing the variation of conductivity as a function of temperature was shown in Figure 10. The results showed an increase in the electrical conductivity of the juices as a function of temperature. It went from 6.53 to 6.71 µS/cm between 25 and 50°C. It then remained constant between 50 and 100°C, before increasing to reach a maximum value of 6.94 µS/cm at 150°C. This maximum conductivity value also corresponded to higher mineral content.

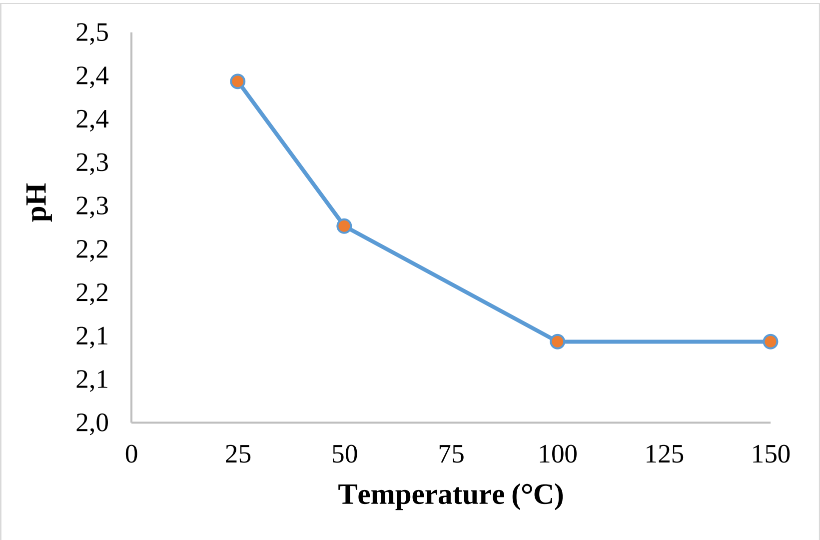

Figure 4: pH control.

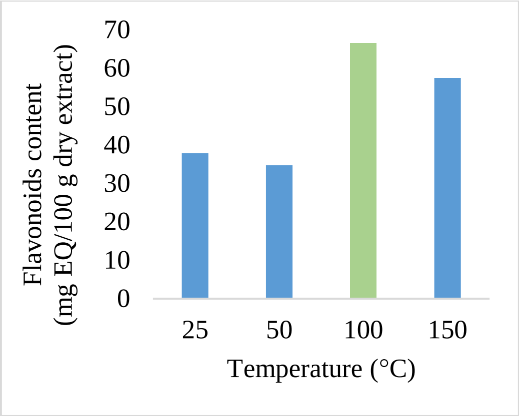

Figure 5: Flavonoids content.

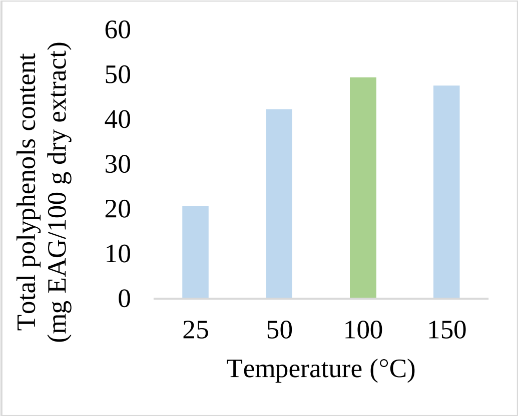

Figure 6: Total polyphenols content.

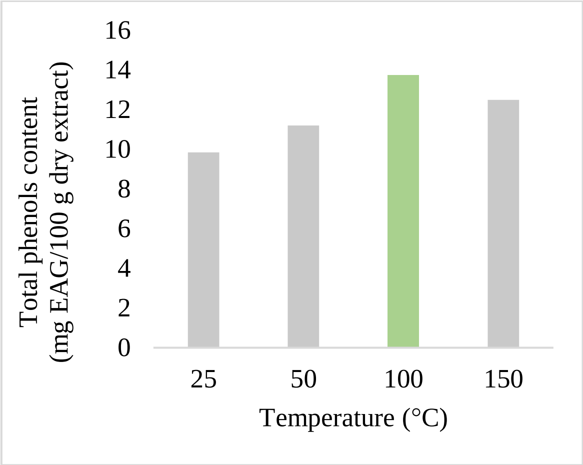

Figure 7: Total phenols content.

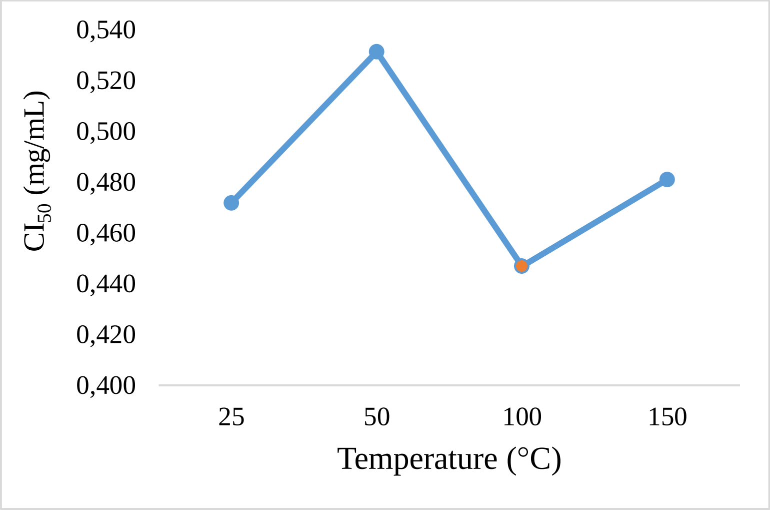

Figure 8: Anti-free radical scavenging capacity - $ABTS^{\bullet +}$.

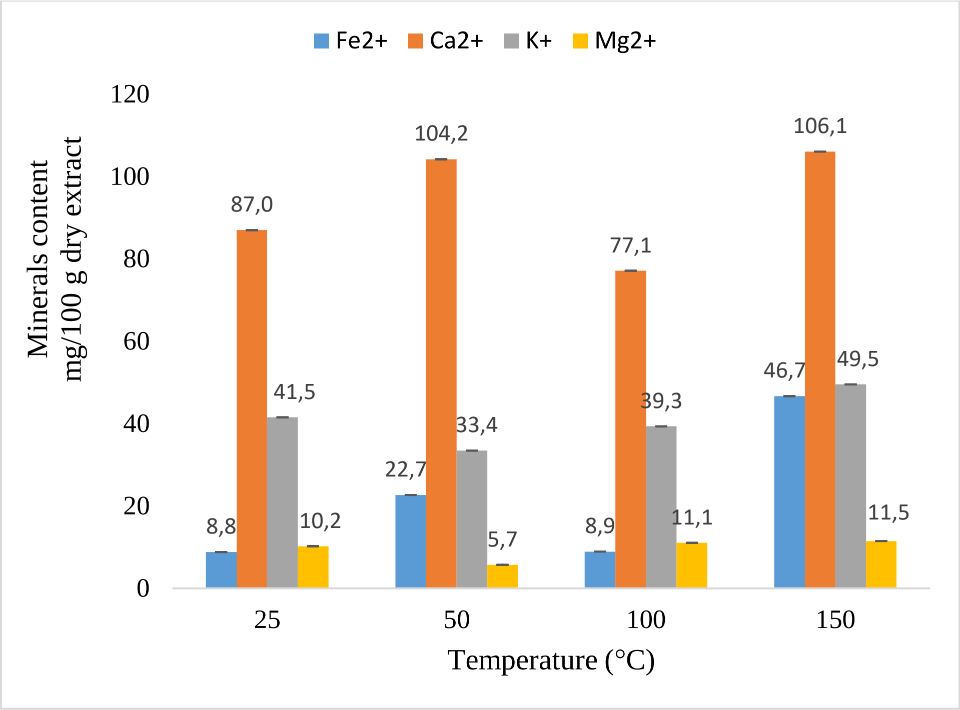

Figure 9: Minerals content.

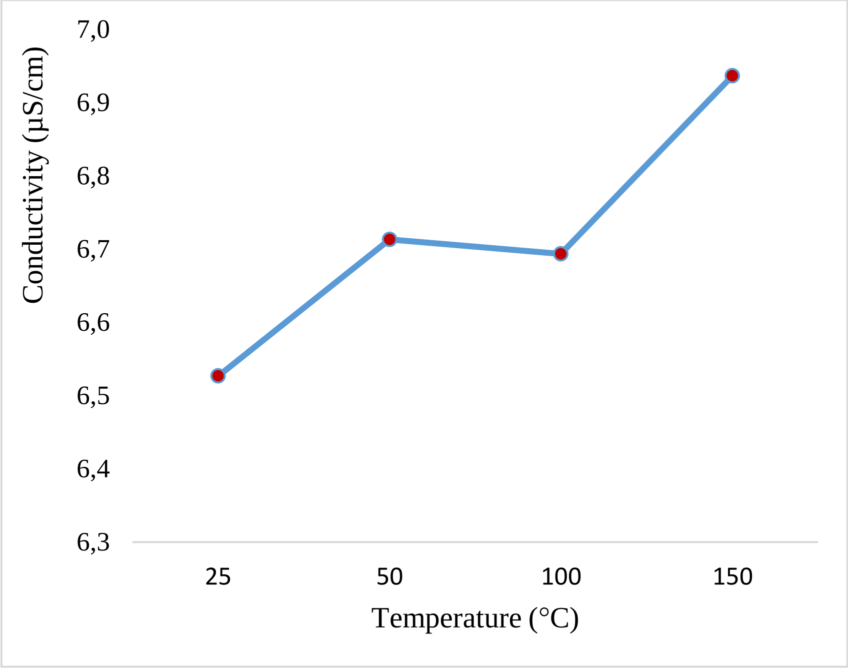

Figure 10: Conductivity measurement.

DISCUSSION

Hibiscus sabdarifa is a shrub whose calyxes were commonly used in many countries to make fruit juices. In Senegal, this juice is better known as bissap juice, which derives from the plant's vernacular name in Wolof, the national language. Numerous studies have shown that bissap juice is rich in bioactive compounds with beneficial effects on the human organism, preventing the onset of complications in several pathologies such as diabetes, hypertension and cancer etc. (Mahadevan et al., 2009). Thus, in this study, compounds such as flavonoids, phenols and polyphenols were revealed in the juice of *Hibiscus sabdarifa* and could play a role in the acid-base balance, which is the balance between the acidity and alkalinity in the body, generally influenced by diet. In addition, plasma pH (or blood pH) is finely regulated around a slightly alkaline value of 7.4. When the pH deviates from this normal value, acidosis (pH < 7.38) or alkalosis (pH > 7.42) may occur. To prevent these possible variations in pH, the body uses phosphate, carbonate and protein buffer systems (Hamm et al., 2015) to restore the acid-base balance. A good acid-base balance in the blood also helps prevent osteoporosis, osteotendinous disorders, inflammation and chronic fatigue. Nutrition therefore has an important role to play in achieving this acid-base balance, in connection with health prevention (Remer, 2001). In this study, raising the juice preparation temperature led to a slight decrease in the pH of the solutions. This could be explained by an increase in the entropy of the system, that is to say a disorder at the molecular level, due to the rise in temperature, which favors the release of protons H^+ by the hydroxyl groups of the flavonoids and polyphenols, reinforcing the acid character of the solutions and consequently a slight decrease in the pH of the juices. Additionally, flavonoids, phenols, and polyphenols are known to be highly soluble in hot water (Cuevas-Valenzuela et al., 2014). The same trends were observed in this study, with a maximum extraction rate of these chemical families obtained at a temperature of 100°C. This could support the way local people prepare *Hibiscus Sabdarifa* juices, to better benefit from the benefits of these chemical compounds. Indeed, these compounds were also known for their anti-free radical scavenging properties, helping to fight against oxidative stress (Ali et al., 2005; Djamilatou et al., 2021). The latter is responsible for many adverse health effects, as it has been linked to the development of complications in several diseases such as diabetes, hypertension, cancer and neurodegenerative diseases (Giacco et al., 2010; Jay et al., 2006). Polyphenolic compounds could also reduce inflammation, improve blood circulation, lower bad cholesterol, and boost the immune system (Ali et al., 2008).

In this study, the anti-free radical scavenging capacity was evaluated by determining the median inhibitory concentration (IC_{50}) of the radical cation ABTS ($ABTS^{\bullet+}$), thanks to the reducing properties of the phytochemicals contained in the juices of *Hibiscus Sabdarifa*. For quantification, ascorbic acid was used as a reference, with an estimated IC_{50} of 0.001 mg/ml. Regarding the juice samples, the one obtained at a temperature of 100°C showed better anti-free radical capacity, with a lower IC_{50} estimated at 0.446 mg/ml. The other juices obtained at temperatures of 25; 50°C and 150°C had estimated IC_{50s} of 0.471, 0.531 and 0.481 mg/ml, respectively. These results could be supported by the fact that the highest contents of polyphenolic compounds were obtained at the same temperature of 100°C and which are known for their anti-free radical capacity. However, it would be important to take into account their thermosensitivity above 100°C, otherwise their physico-chemical properties will be lost (Antony and Farid, 2022; Chaaban et al., 2017; Zapata et al., 2021; Zeng et al., 2017). Indeed, according to some studies, polyphenols such as gallic acid have very low thermal stability at temperatures above 190°C (Elhamirad and Zamanipoor, 2012; Krungkri and Areekul, 2019).

In addition, the maximum content of mineral elements present in the juices was obtained at a temperature of 150°C. This content was correlated with the maximum conductivity value obtained at the same temperature in this study. This would seem logical, given that the mineral elements would be able to conduct electric current in a solution.

In addition, the increase in electrical conductivity has been described in some works and has generally been correlated with the presence of mineral elements such as calcium (Ca^{2+}), iron (Fe^{2+}), sodium (Na^{+}), potassium (K^{+}) and chlorine (Cl^{-}) ions (Charriau et al., 2013). Some of these ions, such as sodium (Na^{+}) and potassium (K^{+}), are also very important in maintaining osmotic pressure and water movement in the body (hydration/dehydration), as well as in the acid-base balance. Indeed, the Na^{+}, K^{+}/ATPase pump or sodium pump is a cellular component essential to the functioning of almost all animal cells. It is the transport system that maintains an electrochemical gradient for the two main cations of physiological fluids: sodium, which has a high extracellular concentration and a low intracellular concentration, and potassium, with an inverse concentration gradient (Horisberger, 2006; Sherwood, 2015).

Conclusion

The results obtained in this study enabled the determination of the optimal temperature of extraction and the nature of the phytochemical compounds contained in the juice of *Hibiscus sabdarifa*. According to the results, the juices should be prepared by heating them to a temperature of 100°C, in order to reap the maximum benefit from the phytochemicals.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Plant material sampling, the extraction, the tests and the drafting of the article were carried out by MB and HT. The manuscript was reviewed by RSG, ID, YT, AD, ADF, DF, MS and AD. Supervision was provided by SOS and AW.

ACKNOWLEDGMENTS

The co-authors would like to thank all those who have contributed in any way to obtaining these results and writing this manuscript.

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