African Journal of Food Science and Technology (ISSN: 2141-5455) Vol. 14(6) pp. 01-06, July, 2023
DOI: http:/dx.doi.org/10.14303//ajfst.2023.026
Available online @https://www.interesjournals.org/food-science-technology.html

Research Article

Impacts of Extraction Procedure and Freeze-Drying on Anthocyanins and Volatile Compositions of Hibiscus Extracts and Freeze-Dried Hibiscus Extracts

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Abstract

There has been a great deal of interest in Roselle (*Hibiscus sabdariffa* L.), called bissap in Senegal, recently because of consumer demand for nutraceutical products. However, beverages made from hibiscus have short self-lives due to anthocyanin and flavor degradation. The purpose of our study was to examine the impacts of freeze-drying on the anthocyanins and the volatiles compositions of hibiscus extracts. Senegalese hibiscus was extracted with hot and cold water and one part of each extract was freeze-dried. A ratio of 1:15 w/v was used; temperature was 98°C for 30 min for hot extraction and 22°C for 4 hours for cold extraction. The anthocyanins were determined using reversed phase High Performance Liquid Chromatography (HPLC) and the volatiles were measured using headspace-Solid Phase Micro-Extraction and Gas Chromatography-Mass Spectrometry (SPME-GCMS) Freeze-drying had no effect on the anthocyanins in cold extracts. However, a significant difference between the hot extract and its freeze-dried product was observed. Volatile profiles were different between cold and hot extracts and their freeze-dried powders. The results of this study show freeze-dried hibiscus has volatiles and anthocyanins similar to non-dried, suggesting that freeze drying is an option for stabilizing hibiscus.

Keywords: Hibiscus, Volatiles, Anthocyanins, Hibiscin, Lyophilization

INTRODUCTION

In developing countries, research has often focused mainly on agricultural production (Camelo-Mendez et al., 2013).

Because of this, product development and food processing are often not well studied (Khumbah Foote, 2014); this has negative impacts, particularly on the economy. People who live in large cities rely heavily on imported, processed food. According to Van Wyk in recent years research has been conducted on many African medicinal plants in order to find the best ways to promote growth in their use

Received: 06-Sep-2019, Manuscript No. AJFST-23-2176; **Editor assigned:** 11-Sep-2019, Pre QC No. AJFST-23-2176 (PQ); **Reviewed:** 25-Sep-2019, QC No. AJFST-23-2176; **Revised:** 12-Jun-2023, Manuscript No. AJFST-23-2176 (R); **Published:** 10-Jul-2023

Citation: O'Keefe SF, et al (2023). Impacts of Extraction Procedure and Freeze-Drying on Anthocyanins and Volatile Compositions of Hibiscus Extracts and Freeze-Dried Hibiscus Extracts AJFST: 026.

and sales. *Hibiscus sabdariffa* is among the targeted plants and is considered a functional food. Hibiscus has reported health benefits, such as lowering blood pressure, anticancer activity and bactericidal properties; consequently, consumer demand for this nutraceutical product has increased worldwide (Chen et al.,1998). Furthermore, some scientific studies found hibiscus to be a valuable ingredient for pharmaceutical, cosmetic and food industries.

Some researchers consider hibiscus to be one of the most important botanical products on the global market. Research has shown that hibiscus contributes to the development of the rural economy and strengthens food security and others point out that hibiscus can be an alternative crop in Senegal. However, some studies have suggested that beverages made from hibiscus calyces, the main edible of the plant, have a short self-life due to degradation of flavors and anthocyanins. This makes it difficult to ship, transport, and commercialize processed hibiscus products abroad. Processed hibiscus products add value and have greater economic benefits (Chiou et al., 2007). Therefore, finding ways to obtain ready-to-use and easy-to-export products from hibiscus that are safe, have good qualities, high consumer acceptability and good shelf-life can improve hibiscus accessibility and availability throughout the world. A number of studies have been conducted on *Hibiscus sabdariffa* to develop products that are commercially successful.

For instance, Duangmal et al. have investigated the stability of anthocyanins in freeze-dried hibiscus using maltodextrin or trehalose as stabilizer. They found that the addition of these products retarded color degradation and that 3 g/100 ml maltodextrin provided the best stability. The effect of the temperature on spray drying of hibiscus extracts (using ethanol as solvent) has also been investigated. They reported a significant effect of spray drying temperature on the volatiles. However, further investigations are needed in order to improve hibiscus calyces' transformation, commercialization and consumption worldwide. The purpose of our study was to investigate the effects of origin, water, and temperature on the anthocyanin contents of hibiscus extracts and also to determine the effect of freeze-drying on anthocyanin contents and aroma compositions of powder obtained from hibiscus extracts. The results of this current work will help determine factors relevant to processing hibiscus freeze dried powder (Cisse et al., 2009).

MATERIALS AND METHODS

This work was conducted in the Human and Agricultural Biosciences Building 1 (HABB1) laboratory in the department of food science and technology of Virginia polytechnic institute and state university (Virginia Tech), Virginia, U.S.A (Cisse et al., 2009).

Plant material used for this study

The raw materials used for this study are dried calyces of *Hibiscus sabdariffa* L, Vimto ariety, purchased in Senegal, West Africa. The calyces were produced in villages located in the department of Nioro, region of Kaolack. The packaging material used was large plastic bags designed so that moisture, light and insects could not penetrate and alter the calices. The samples were stored at room temperature before use. Samples were manually cleaned twice to remove seeds and contaminants (stones, etc) (Cisse et al., 2012).

Aqueous extract preparation and freeze-drying

Distilled water was used for juice extraction. As the calices were quite large, before extraction they were ground to increase contact area with the water and facilitate juice extraction. For this purpose, a Ninja brand grinder was used (model GFP-200) (Da-Costa-Rocha et al., 2014). A mass ratio of 1:15 (one kilogram of calyces per 15 liters of water) was used. Cold extractions were conducted at room temperature (around 22°C) and the extraction time was 4 hours. A time temperature of 30 minutes at 98°C was used for hot extractions the hibiscus calyces were always added to boiling water. Temperature was monitored, and cold extraction was done in two replicates while hot was repeated three times. All macerates were filtered using gravity filtration with a stainless-steel sieve with 1 mm openings (No. 22 mesh) to remove the larger residues before performing a fine filtration with a cloth filter with 25 µm pore size (No. 500 mesh) (Duangmal et al., 2008). After filtration, pH, Brix, anthocyanin content and aroma compounds of the solutions were immediately measured. Then, the extracted solutions were frozen at -80°C (model 88600A Thermo Fisher Scientific Ashville, NC USA). The frozen extracts were then freeze-dried for 72 hours in a Freeze-dryer. At the end of freezing-drying, the dried hibiscus powder was immediately collected in plastic bags and completely covered with aluminum foil to avoid the effect of moisture and light. Anthocyanins and aroma compounds of this freeze-dried hibiscus were later determined (Ebert, 2014).

Anthocyanins and volatiles were determined on powders obtained from freeze-drying the cold and hot extracts. To evaluate the effect of freeze-drying on the dried products, comparison of the results from each fresh extract to that of its powder (or freeze-drying product) was made (Gonzalez-Palomares et al., 2009).

Total anthocyanins and hibiscin measurement

Hibiscin (delphinidin 3-sambubioside) standard was obtained from Indofine Chemicals (Hillsborough NJ). A high-performance liquid chromatograph (Agilent Technologies model 1260, Santa Clara, CA) was fitted with a 25 cm × 0.45 cm i.d. 5µ C18(C2) Luna column

(Phenomenex, Torrance CA). The mobile phase was composed of two solutions: Solvent A (99% distilled water, 1% glacial acetic acid v/v) and solvent B (99% acetonitrile, 1% glacial acetic acid v/v) with a flow rate of 1.0 ml/min and the column temperature maintained at 40°C. A solvent gradient was used from 90% A at time 0 to 70% A at 20 minutes. Detection for anthocyanins was conducted at 520 nm (Gradinaru et al., 2009).

Aroma compounds analyses by SPME GC-MS

The aroma compounds of the hibiscus juice were extracted by Headspace Solid-Phase Microextraction (HS-SPME) and analyzed with Gas Chromatography-Mass Spectrometry (GC-MS). The method described by Sheibani et al. was used. For the extraction of the volatiles, a Shimadzu QP-2010 ultra GCMS equipped with an AOC-5000 Plus SPME auto-sampler (Shimadzu Scientific, Columbia, MD, USA) was used (Gradinaru et al., 2003). The extraction injection and identification procedures followed the method described by Sheibani, et al. A Shimadzu SH Rxi-5MS column with 30.0 m length, 0.25 µm film thickness and internal diameter of 0.25 mm was used. All data were analyzed by Analysis of Variance (ANOVA) with a level of 5% (α=0.05) as a criterion of statistical significance using JMP® Pro 11.0.0 statistical analysis software (SAS, Cary, NC, USA). Follow up statistical analyses were conducted on all significant effects by performing mean comparisons using Tukey's HSD and Slice tests [Hopkins AL et al., 2013].

RESULTS AND DISCUSSION

The pH was almost the same for all extracts, between 2.44 -2.50. This pH is quite a bit lower than most juices such as apple, orange, grape etc. and is in the range of cranberry juice. The soluble solids (degrees brix °Bx) was lower for the cold extract compared to the hot. This can be explained by the fact that heat helps disintegration of soluble particles and may increase solids, including acids, in the juice (Patel et al., 2004).

Effect of freeze-drying on total anthocyanins and hibiscin contents for hot extraction

The results from the HPLC analyses showed there was a significant difference ($p<0.05$) in anthocyanin contents between the hot extract (1448 ± 20 µg/mL) and its reconstituted freeze-dried powder (1334 ± 14 µg/mL). A significant difference ($p<0.05$) was also observed for hibiscin contents between the hot extract (798 ± 15 µg/mL) and its reconstituted freeze-dried powder (744 ± 15 µg/mL). These results for the fresh extract and for the reconstituted freeze-dried powder in anthocyanins and hibiscin were not expected because among the steps (freezing and lyophilization) used to obtain the freeze-dried powder from the extract, none of them are expected to affect anthocyanins stability. This result might be due to the fact that hot extraction makes extracts less stable over the time. Perhaps, part of the total anthocyanins was lost during the time (3 days) it takes to freeze and then lyophilize the product.

Earlier studies have also reported this: for instance, Cisse et al. have demonstrated that heating and/or pasteurization make hibiscus extracts less stable and induced color changes during storage. Using CIELAB system to evaluate the color stability of freeze-dried hibiscus anthocyanins, Duangmal et al. have demonstrated that the total anthocyanins were responsible for the changes in Chroma (intensity of color). When maltodextrin and trehalose were used as stabilizers, they observed that the anthocyanin degradation rate decreased (Ramirez-Rodrigues et al., 2011).

Effect of freeze-drying on total anthocyanins and hibiscin contents for cold extraction

Our results showed there was no significant effect of freeze-drying on the anthocyanins in the cold extract ($p>0.05$). The average amount of anthocyanin content in the fresh extract (1466 ± 4 µg/mL) was about 15% higher ($p>0.05$) from the amount in the reconstituted freeze-dried powder (1279 ± 66 µg/mL). The amount of hibiscin (848 ± 32 µg/mL) in fresh extract was a bit higher than the freeze-dried powder (820 ± 29 µg/mL). These results can be explained by the fact that no heat was applied during the processes of extracting the juice and freeze-drying.

As explained earlier, heat treatments affect the stability of anthocyanins in hibiscus. For the cold extraction, no heat was applied, and anthocyanins are reportedly more stable under these conditions. These authors reported that the anthocyanin degradation during storage followed first-order reaction kinetics. Anthocyanins in the extract obtained at 100°C then pasteurized at 90°C for 5 min had the highest rate of degradation, followed by those in the extract obtained at 100°C but not pasteurized and finally those in the extract obtained at 30°C then pasteurized. The effect of extraction temperature was predominant on the deterioration of anthocyanins (hibiscus anthocyanins degraded more quickly during storage when the temperature of extraction was high).

Effects of freeze-drying on the volatiles of freeze-dried hibiscus powder from cold extraction

Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify the aroma compounds present in the cold extracted hibiscus and its freeze-dried powder. Mass spectra were compared to NIST and Wiley libraries. Hydrocarbon standards (C5, C6, C7, C8, C10, C12, C14, C16, C18, C20, C22) were also run to calculate Kovats Indexes, also known as LRI (Linear Retention Index), to facilitate identification of the aroma compounds. Identification of the different flavor compounds was aided by consulting online databases. Compounds were considered identified if

the compound matched the mass spec library and Kovats index.

This big difference in aromas composition content between the two products can be explained by the different in soluble solids present in the both product while running the GC-MS analyses. After freeze-drying, the amount of powder weighed to reconstituted solution did not generate the same amount of soluble solid content as in the fresh extract. This limiting factor may be the reason of the small amount of aroma in the instant powder.

Effects of freeze-drying on the volatiles of freeze-died hibiscus powder obtained from hot extraction

Similar to the cold extracts, aroma compounds present in the hot hibiscus extracts and freeze-dried powder were identified using GC-MS. Twenty-seven compounds were identified in the fresh hot extract and twenty-three in its freeze-dried powder. Table 1 below shows these different aroma components.

Table 1: Aroma compounds found in the hibiscus samples.

Compound	Cold extract	Freeze-dried cold extract	Hot extract	Freeze-dried hot extract	LRI literature (DB5)	Calculated LRI (DB5)	Odor description
Acetic acid	X	X	X	X	600	660	Sour, vinegar
Hexanal	X	X	X	X	801	822	Grass, tallow, fat
Furfural	X	X	X	X	829	838	Bread, almond, sweet
Heptanal	X		X		903	903	Fat, citrus, rancid
(E)-2-Heptenal			X	X	951	959	Apple, green
5-Methylfurfural	X	X	X	X	978	967	Almond, caramel, burnt sugar
1-Octen-3-ol			X		982	980	Mushroom
1-Octen-3-one	X				999	981	Soap, gasoline
6-Methyl-5-hepten-2-one	X		X	X	985	988	Mushroom, earthy, woody
Octanal	X		X	X	1006	1005	Fat, soap, lemon, green
D-Limonene			X		1033	1035	Lemon, orange
Phenylethanal			X	X	1049	1048	Hawthorne, honey, sweet
(E)-2-Octenal	X		X	X	1060	1060	Green, nut, fat
Nonanal	X	X	X	X	1104	1106	Fat, citrus, green
(E,Z)-2,6-Nonadienal			X	X	1154	1156	Cucumber
(E)-2-Nonenal	X		X	X	1154	1162	Cucumber, fat, green
Nonanol			X		1187	1172	Cucumber
Dodecane	X	X			1200	1192	Alkane
alpha-Terpineol	X		X	X	1195	1198	Oil, anise, mint
Decanal	X		X	X	1209	1207	Soap, orangepeel, tallow
(E,E)-2,4-Nonadienal				X	1217	1217	Fat, wax, green
5-Hydroxymethylfurfural	X		X	X	1225	1226	Butter, caramel
Nonanoic acid			X	X	1275	1263	Green, fat
Eugenol			X	X	1364	1365	Clove, honey

The results show that many of the aroma compounds detected in the hot extract were also detected in the freeze-dried powder from the same extract. Statistical analysis was performed by one-way ANOVA to do the comparison of their respective areas in each of the samples.

Many of the aroma components detected in the samples in this study have been identified in previous studies. Among the aroma compounds we observed, the following were previously reported: Hexanal, heptanal, limonene, octanal, nonanal, 1-octen-3-ol, acetic acid, decanal, and E-2-nonenal. As mentioned earlier, many authors have reported that differences in the number of aroma compounds in the hibiscus or dried hibiscus can be related to factors such as heat

treatment, variety and extraction methods used to isolate the volatile (solvent used, time, solute concentration).

CONCLUSION

This work focused on evaluating the effect of freeze-drying on composition of hibiscus extracts. There was no significant effect of freeze-drying on the anthocyanins in the cold extract, but in contrast, a difference was noticed in anthocyanin contents between the hot extract and its freeze-dried powder. This was not expected because among the steps (freezing and freeze-drying) applied to get the freeze-dried powder from the extract, none is known to have an effect on the anthocyanins of hibiscus. This result might be due to the fact that hot extraction makes extracts less stable over the time. Earlier studies have also reported that anthocyanins were less stable. The pH was almost the same in all extracts. The soluble extract was less when cold extract was made and higher in hot despite the presence of the two other variables (temperature and source).

This can be explained by the fact that heat makes easy the disintegration of some soluble particles and this increase their quantity in the juice. GC-MS analysis of the volatiles showed different profiles when comparing cold and hot extracts to their respective instant powder as well as when comparing hot to dry extract. But many of the aroma compounds were found in both hot and cold extraction. Globally, the results of this study can help in the optimization when processing hibiscus derivatives such as juices, concentrates and instant powder in regards of the growing interest worldwide in functional food product like hibiscus. The latter is a ready to use and easy to commercialize and would be a good alternative for industrial as far as hibiscus has many potentials applications in the food, pharmaceutical, cosmetic industries, etc. Expanding these investigations on hibiscus powder obtained from spray-drying and monitor the self-life of both dried products over the time when stored at specific temperatures in future work can help in activities carried around hibiscus. Likewise, the evaluation of consumer acceptability on the different products through sensory evaluation tests also needs to be done.

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