Effects of Temperature and Solid-liquid-ratio on the Extraction of Total Phenolic Content of Hibiscus sabdariffa leaves

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Abstract

Hibiscus sabdariffa leaves (HSL) can be used as tea leaves because of its bioactive compounds. This study demonstrates the effects of extraction temperature and solid liquid extraction ratio on the total phenolic content (TPC) of HSL. The HSL was harvested, washed dried with vacuum dryer at 40 °C and ground. The resultant powder was characterized in terms of tapped and bulk density, Carr's index and Hausner ratio to ascertain its flow characteristics prior to the extraction using distilled water as the extraction medium at 70, 80and 90 °C and at the solid-liquid ratio of 1:9 and 3:7. The bulk and tapped density of 212m were 20.29 ± 0.1753 g/ml and 25.58 ± 0.2700 g/ml respectively .The carr index and Hausner ratio were 20.67 ± 1.154 and 1.26 ± 0.0185 respectively. Maximum extraction TPC was observed at 90 °C and at the solid-liquid ratio of 1:9 while the least TPC was observed at 70° C at 3:7 solid-liquid ratio.

Introduction

Hibiscus sabdariffa is a plant of Malvaceae family, a blooming shrub found mostly in the tropical regions of Africa and Asia (Edo et al., 2023). The English name for this plant is Roselle while Nigerians call it Zobo. The leaves and calyx are the most commonly used parts of the plant for culinary and medicinal purposes (Agunbiade et al., 2022). Its Culinary use include as ingredient in soups, stews, cakes, wine, drinks, jams, pudding, tea etcetera while medicinally, the plant is believed to poses abilities to manage a plethora of diseases ranging from age related to degenerative diseases such as cancer, diabetes, high blood pressure etcetera. Teas are mostly made from plants parts to extract bioactive compounds such as polyphenols for use in the human body as it enhances many human physiological functions. The plant's therapeutic uses have been documented in (Riaz & Chopra, 2018). Roselle leaves potentials for use for medicinal purposes are due to its high content of polyphenols.

Polyphenols are naturally found bioactive compounds in plants' parts such as leaves, flowers, fruits and seeds. Polyphenols are secondary metabolites and a broad group of bioactive compounds with high antioxidant effects (Chen et al., 2024) . Polyphenols are primarily responsible for the healing capabilities of most plant parts the reason they are used in supplements due to their bioactivities (Feng & Zhang, 2023; Sahraeian et al., 2023). As consumers' choices and lifestyle shift towards healthy choices, this study presents the effects of temperature and solid-liquid ratio on the extraction of total phenolic content of Hibiscus sabdariffa leaves.

Material and Methods

The Hibiscus Sabdariffa leaves used for this experiment were harvested form the experimental farm of the Agricultural and Food Engineering University of Uyo, Nigeria. They were processed and dried as described in (Oladejo et al., 2023) with some modifications. Briefly, they were harvested, washed and dried using vacuum dryer oven at 40 °C and record of the mass every 15 minutes until constant mass was obtained. The resulting dried leaves were ground to powder and used for the experiments.

Determination of flow properties

Bulk and tapped densities, Carr's index and Hausner ratio were the flow properties that were determined to characterize the powder sample.

Bulk and tapped density

The tapped density of zobo was determined by hitting a 500ml cylinder filled with zobo leaves powder to the 500 ml level mark. The initial mass was read before tapping 200 times. Bulk density was determined with

the initial mass and volume before tapping. Tapped and bulk density was calculated using Equation 1.

$$Density = \frac{Mass}{Volume} \quad 1$$

Carr's index and Hausner ratio determinaton

Carr's index and Hausner ratio were derived from the bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) values of zobo powder using the following Equations 2 and 3 (Carr, 1965; Hausner, 1967)

$$Carr's\ Index = 1 - \frac{\rho_{bulk}}{\rho_{tapped}} \times 100 \quad 2$$

$$Hausner\ ratio = \frac{\rho_{bulk}}{\rho_{tapped}} \quad 3$$

Where ρ_{bulk} and ρ_{tapped} are bulk and tapped densities respectively.

Determination of moisture content

Moisture content was determined as found in (Akpan et al., 2021). Five grams (5g) of the sample was placed in an oven at 105 ^{0}C for 24 hours.On cooling, the sample was weighed again and the weight loss recorded. The moisturecontent was calculated using Equation 4

$$Moisture\ Content = \frac{M_1 - M_2}{M_1} \times 100 \quad 4$$

Where; M_1 = initial mass of the ground sample, M_2 = Mass of dried sample.

Extraction

Distilled water was used as the extraction liquid for total phenols from Roselle (Hibiscus sabdariffa) leaves at temperatures of 70, 80 and 90°C and extraction time of 150min readings were taken at15 mins intervals. Two solid-liquid ratios were used for extraction: 1:9 and 3: 7. For 1:9 extraction procedure, 10 milligrams of ground sample was measured and placed in a 500 ml cylindrical beaker and 90 ml of distilled water was added and placed on a magnetic stirrer at level 5 stirring magnitude to extractfor 15 min interval till 105 mins. The same procedure was done for 70ml of distilled water and 30 milligram of sample (7:3 liquid-solid ratio). Each experiment was done in triplicate.

Determination of Total Polyphenol Content

The analysis of total phenolic content was carried out as described by (Chen et al., 2024).
1 ml sample solution was mixed with 4 ml sodium carbonate solution (75 g/l) in a 10 ml measuring flask. The flask containing the mixture was shaken vigorously. A 2 ml solution of folinciocalteu reagent was then added into the measuring flask and the mixture was shaken. Distilled water was continuously added until homogeneity was obtained. This mixture was left in a dark room at room temperature for one hour. The absorbance of the mixture was then measured at a wavelength of 760 nm using a UV-spectrophotometer. The total phenolic content was expressed as gallic acid equivalent in mg per gram of dry extract.

Results and Discussion

The bulk and trapped density of the Rosella leaves at 212 µm particle size were 20.29 ± 0.1753and 25.58 ± 0.2700 respectively .The car index and Hausner ratio at 212µm were 20.67 ± 1.154 and 1.26 ± 0.0185 respectively.

Effect of Temperature on yield of total phenolic content of Roselle leaves

Extraction temperature played a major role in the extraction of total phenolic content (TPC) in Roselle leaves. Increase in the extraction temperature of distilled water and extraction time increased the amount of total phenolic content extracted from Roselle leaves. The maximum concentration of phenol was obtained at extraction temperature 0f 90°C and extraction time of 150min. This trend was observed at all solid-liquid ratios studied (Figures 1& 2).

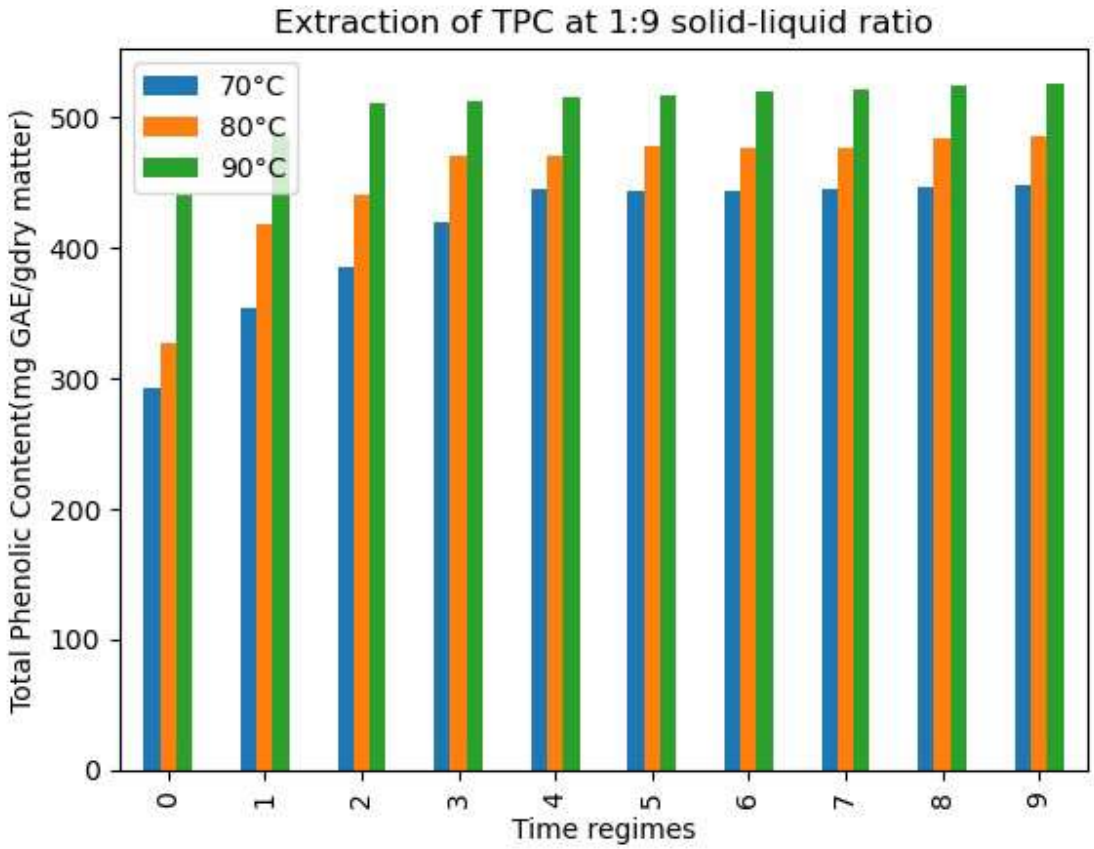

Figure 1: Extraction of TPC (mgGAE/gdry matter) at 1:9 solid-liquid ratio for time regimes of 0=15 mins, 1=30 mins, 2=45 mins, …….9= 150 mins.

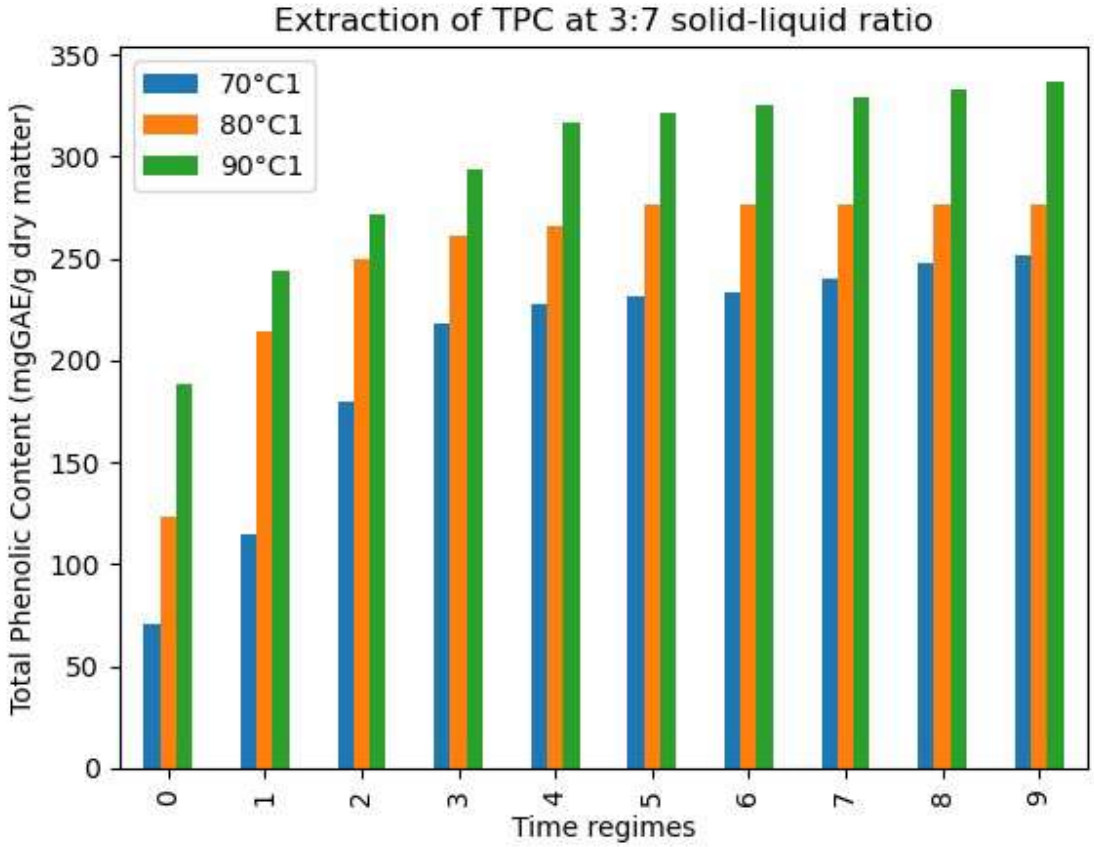

Figure 2: Extraction of TPC (mgGAE/gdry matter) at 3:7 solid-liquid ratio for time regimes of 0=15 mins, 1=30 mins, 2=45 mins, …….9= 150 mins.

Figures 1 and 2 show the extraction of TPC from Roselle leaves at the solid-liquid ratio of 1:9 and 3:7 at time regimes of 0-9 corresponding to 15 mins – 150 mins (at 15 mins increment). At every time regime, an increase in temperature resulted in an increase in the TPC extracted. The minimum TPC was obtained at 70 °C for both solid-liquid ratios and the maximum TPC was obtained at 90 °C this is due to the consequent increase of the diffusion rate and solubility of the extracted substances. Additionally, Roselle being rich in vitamin C doesn't degrade its anthocynins at high temperature as Vitamin C as an inhibitor, though other fractions of TPC may degrade, the rate of degradation is lower than the rate of production (Fiacre et al., 2023). The effect of time on the TPC yield as seen in Figures 1 and 2 is due to the accumulation of the TPC over time and the continuous embrittlement of the leaves particles over time to release the polyphenols.

Table 1: Effect of Solid-Liquid ratio on the distil water extraction of TPC of Roselle Leaves.

	Time	70°C	80°C	90°C	70°C1	80°C1	90°C1
count	10.000000	10.000000	10.000000	10.000000	10.000000	10.000000	10.000000
mean	82.500000	412.167220	452.748650	506.848970	201.627954	249.790630	296.116720
std	45.414755	53.055021	48.878707	26.221054	61.465468	48.576150	48.058401
min	15.000000	292.072900	327.532300	439.820700	70.980440	123.286500	188.815900
25%	48.750000	393.464800	447.762125	510.801250	189.587275	252.288375	277.596550
50%	82.500000	443.760600	473.802750	515.787700	229.882000	271.388900	319.066700
75%	116.250000	444.991850	477.681100	520.774225	238.256875	276.531300	327.992550
max	150.000000	448.193100	484.883800	525.760700	251.259900	276.678300	336.918400

Footnotes: 70°C, 8°0 and 9°0 are for the extraction at 1:9 solid-liquid extraction while 70° C1, 80 °C1 and 90° C1 are for the extraction at 3:7 solid-liquid extraction

Total phenolic content was observed to decrease when the solid-liquid content was increased as shown in Table 1. This is contrary to the observation of (Fiacre et al., 2023). Table 1 shows the mean, standard deviation, minimum values (at 15 minutes) , IQR and the maximum values for all the temperature run of this experiment and for the the two solid-liquid ratios studied. Maximum values were obtained with the extraction ratio of 1:9 solid-liquid at 90 °C (525.76 mg GAE/g dry matter). This can be explained with saturation of solutes in solution.

Conclusion

This study evaluated the effects of process parameters like the solid-liquid ratio, temeparature and time on the yield of total polyphenol content (TPC) of Hibiscus sabdariffa leaves. The temperatures used were 70, 80 and 90° C , solid-liquid ratio of 1:9 and 3:7 were used for time of 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150min. It was observed that Temperature was an important factor during the process: 90 °C had the highest TPC at both Solid-liquid ratios (525.76mgGAE/g dry matter and 336.92 mgGAE/g dry matter for 1:9 and 3:7 solid liquid ratios respectively. The least TPC was observed at 70 °C at 15 mins for both Solid-liquid extraction ratios. This was due to the high solubility rate at high temperature and the higher rate of generation of phenolic compounds than the degradation rate. Increase in Solid-liquid ratio adversely affected the extraction of TPC of Roselle leaves.

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