Effect of boiling on the pH, glucose and soluble proteins contents of waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*) and fluted pumpkin (*Telfairia occidentalis*)

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ABSTRACT

The study aimed at evaluating the effect of boiling on the pH, glucose, and soluble protein contents of waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*), and fluted pumpkin (*Telfairia occidentalis*). The fresh extract was prepared by homogenizing 10 g of fresh extracts using a mortar and pestle in 100 mL of distilled water while the boiled samples were prepared by boiling 100 g of fresh samples in 500 mL of water for 20 min. 10 g of the boiled leaf extract was then homogenized in 100 mL of distilled water. The results showed that boiling affected the parameters studied as pH decreased significantly ($p>0.05$), glucose increased significantly ($p > 0.05$), and soluble protein contents decreased significantly ($p > 0.05$) in the boiled sample compared with the control. It is shown from the results that boiling has the ability to alter a plant's nutrients and reduce its pH.

Key words: Glucose, soluble protein, boiling, waterleaf, bitter leaf, fluted pumpkin.

INTRODUCTION

The provision of sufficient and nutritionally adequate food is one of man's basic requirements. The National Food Security Agency strives to supply food in sufficient quantities and quality to keep up with population growth. However, the extensive post-harvest practices led to inconsistent food quality (Oboh, 2003). Vegetables are horticultural crops that can be grown annually or perennially. Some of their parts, such as their roots, stalks, flowers, fruits, and leaves, can be eaten raw or cooked (Welbaum, 2015). Cooking, a crucial step in food preparation, can affect the nutritional quality of food in both negative and positive ways (Fabbri and Crosby, 2016). Processing has a different impact on different nutrients depending on how sensitive they are to heat, oxygen, pH, and light (Reddy and Love, 1999).

The herbaceous, perennial, glabrous plant *Talinum fruticosum* (TF), often known as "water leaf" is a member of the Portulacaceae genus (Odujebe, 2017; Fatoye et al., 2020). It is widely cultivated as a leaf vegetable in tropical areas and commonly referred to as a "water leaf" because of its high moisture content, which is around 90.8 g per 100 g of the edible leaf (Atibita et al., 2021). It is a 30–100 cm tall upright perennial herb with swelling roots and juicy stalks. Two lateral basal buds may be seen on the branches. Nearly opposite leaves are spirally arranged and frequently clustered at the top of the stem. Waterleaf grows quickly and readily self-seeds after being established. *T. fruticosum* is primarily self-pollinating and blooms early throughout the year. It spreads easily, does well in the shade and in cloudy weather, and is quickly turning into an invasive species in agriculture. Due to their propensity to adopt a crassulacean acid metabolism (CAM) pathway, waterleaf is comparatively tolerant to drought conditions, resulting in efficient utilization of available moisture, carbon dioxide assimilation during the night, and increased growth (Swarna, and Ravindhran, 2013). Most of the humid tropical nations, including those in West Africa, Asia, and South America, cultivate the plant extensively. *T. fruticosum* is a common vegetable in many African nations because of its slimy texture. In Nigeria, some tribes use it to make traditional soups like Gbure, Edikaiko, and Afang (pronounced differently in Yoruba, Ibibio, and Efik). According to Ezekwe et al. (2013), *T. fruticosum* is high in beta-carotene, minerals (such as calcium, potassium, and magnesium), pectin, protein, and vitamins and is thus useful as a vegetable soup.

Bitter leaf, or *Vernonia amygdalina*, is a 2–5-meter-tall shrub or small tree that thrives in a variety of ecological zones throughout Africa. It belongs to the Asteraceae family and popularly called bitter leaf in Africa, ewuro in Yoruba, 'etidot' in Ibibio, onugbu in Igbo, ityuna in Tiv, ilo in Igala, oriwo in Edo, grawa in Amharic, omubirizi in South-western Uganda, ndole in Cameroun, chusar-doki and shawaka in Hausa (Ezekiel et al., 2015) and it is called origbo in Urhobo language (Eraga et al., 2020). It produces a large amount of forage and is drought-resistant. Both humans and animals consume bitter leaves. It is washed to remove or reduce the bitter flavor before being used as a vegetable for human consumption. In addition to promoting digestion, it also lowers fever. It can also be used locally as a natural remedy or parasite repellent. An initial phytochemical analysis of bitter leaf revealed the presence of steroids, alkaloids, terpenes, tannins, and saponins (Ali et al., 2019, Anigboro et al., 2014). Alkaloids, saponins, and tannins, which are antinutritional components, are responsible for the bitter leaf's superior flavor (Oboh, 2005). Bitter leaves are used by the locals in soup and stew as a tonic to build strength after being cooked and soaked in water. It has been discovered that boiling and heating reduce secondary plant chemicals and improve the feed's flavor (Oboh, 2005). Due to the presence of antinutritional elements and secondary plant products, the leaves are extremely bitter.

In Nigeria, fluted pumpkin (*Telfairia occidentalis*) is regarded as a staple food (Oluranti, 2021). Among plant foods, vegetables are a broad group with a range of energy and nutrient levels (Slavin and Lloyd, 2012; Egbune et al., 2021). One of the many crops that are grown in the rainforest zones of the majority of West and Central African nations is the fluted pumpkin. In Nigeria, it is primarily abundantly grown in the south. It is a member of the Cucurbitaceae family, a perennial shrub that is dioecious, can tolerate some drought, and has succulent dark green leaves (Imosemi, 2020). It is one of the most commonly grown vegetables in the Nigeria due to its flavor, nutritional value, therapeutic properties, and marketability. Iroko or aporoko in Yoruba, ugu in Igbo, ume in Urhobo, umeke in Edo, and ikong in Efik/Ibibio are the native names for the vegetable (Imosemi, 2020). The root, stem, leaves, fruit, and seed of the vegetable make up its entire body. The fruits are pale green and weigh between 3 and 10 kilograms. The seeds are 3–5 cm broad. The stem contains spreading tendrils. The leaves are divided into 3–5 leaflets. These vegetables are consumed by a sizeable portion of Nigerians after it has undergone some sort of processing.

Pumpkin is a highly nutritious vegetable with many health benefits. It is rich in vitamins A, C, E, and K, as well as minerals such as potassium, magnesium, and iron (Hussain et al., 2022). These nutrients play important roles in maintaining overall health, including supporting immune function, bone health, and heart health. Pumpkin is also high in antioxidants, particularly beta-carotene, which gives it its bright orange color. Antioxidants help protect the body against oxidative stress, which can cause cell damage and contribute to the development of chronic diseases such as cancer and heart disease (García-Sánchez et al., 2020).

In addition, pumpkin may help regulate blood sugar levels due to compounds that improve insulin sensitivity and lower blood glucose levels (Tasya et al., 2022). It also contains fiber, which supports digestive health by promoting regular bowel movements and reducing the risk of constipation. The anti-inflammatory properties of pumpkin may also improve symptoms of irritable bowel syndrome (IBS) (Singh et al., 2018). Pumpkin may also have potential benefits for heart health. The antioxidants and fiber in pumpkin may help reduce inflammation and lower cholesterol levels (Valdez-Arjona et al., 2019). Studies have shown that consuming pumpkin seed oil can lower blood pressure and improve cardiovascular health in postmenopausal women (Majid et al., 2020).

Therefore, in order to get relevant information, it is necessary to assess how boiling affects the pH, glucose, and soluble protein concentrations of waterleaf (*T. fruticosum*), bitter leaf (*V. amygdalina*), and fluted pumpkin (*T. occidentalis*).

MATERIALS AND METHODS

Plant materials

Waterleaf (*T. fruticosum*), bitter leaf (*V. amygdalina*) and fluted pumpkin (*T, occidentalis*) were purchased from the Abraka Main Market Ethiope East Local Government Area of Delta State, Nigeria. The leaves were identified and authenticated at the Department of Botany, Faculty of Science, Delta State University, Abraka (DELSU 118). The leaves were washed in flowing water to remove debris and rinsed in distilled water. They were air dried before weighing.

Preparation of extracts

Preparation of fresh extract

Exactly 10 g of fresh extracts were homogenized using a mortar and pestle in 100 mL of distilled water. After homogenization, 10 mL of homogenate was centrifuged at 3500 rpm for 10 min to obtain supernatant. The supernatant was used as the crude extract or sample for the various assays which was carried out in triplicates.

Preparation of boiled extract

Exactly 100 g of fresh samples were weighed and boiled in 500 mL of water for 20 min. 10 g of the boiled leaf extract was then homogenized in 100 mL of distilled water. After homogenization, 10 mL of homogenate was centrifuged at 3500 rpm for 10 min to obtain supernatant. The supernatant was used as the crude extract or sample for the various assays which was carried out in triplicates.

Determination of biochemical parameters

pH measurement

The pH of both fresh and boiled extracts was determined using a Mettler Toledo pH meter.

Glucose determination

Glucose was determined according to the method described in the Randox glucose kit. 20 µl of standard and 20 µl of the various samples were pipetted into a series of test tubes labeled in triplicates, and then 2 ml of the reconstituted glucose working reagent was added to each test tube. Distilled water was used as blank. The contents of the test tubes were mixed and incubated for 25 min at 15-25°C. After the incubation time elapsed, the spectrophotomer was zeroed using blank and the absorbance of the standard and samples was read at 500 nm.

Glucose concentration (mg/dl) was then calculated using the formula below.

$$Glucose\ Conc. = \frac{Abs\ sample}{Abs\ standard} X\ Standard\ conc.\ i.e\ 1.02mg/ml$$

Determination of soluble protein

Total soluble proteins were determined according to the method described by Gornall et al. (1949). Series of protein standards ranging in concentration from 0.5 to 10 mg/ml was prepared such that the final volume for the assay was 0.5 ml. The various samples were also prepared in a series of test tubes such that the final volume is 0.5 ml. Distilled water (0.5 ml) was used as blank. Thereafter, 2.5 ml of Biuret reagent was added to each sample and standard, vortexed and allowed to react for 30 min. A graph of absorbance of standards was plotted against concentration 0.5 to 10mg/ml to obtain a standard calibration curve of bovine serum albumin. The amount of protein present in the sample can then be estimated directly from the standard graph.

Statistical analysis

Data obtained were subjected to statistical analysis using one-way ANOVA (analysis of variance) and Fischer's test of least significance (LSD); values are presented as Mean ± Standard deviation. Results were considered significant at p-values less than 0.05, that is, at 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

The effect of boiling on the pH levels of Waterleaf (*T. fruticosum*), Bitter leaf (*V. amygdalina*), and Fluted pumpkin (*T. occidentalis*) was investigated. The findings showed that there was a slight decrease ($p > 0.05$) in the pH levels of all the leaves after boiling when compared to their fresh control, as depicted in Figure 1. Since pH is a direct measure of acid content ([H+]), it obviously has a significant impact on the processing of food. pH is the most common analytical measurement used in industrial processing. Among the reasons for monitoring pH in food processing are: to make products with consistent, well-defined qualities; to effectively produce products at an optimal cost; to avoid end users developing health issues; and to satisfy regulatory criteria. Although most vegetables may withstand a little more or less than this pH range, it is recommended that vegetables have a pH between 6 and 7 (Shapiro, 1990). The outcomes demonstrate that, despite being reduced after boiling, the pH of the vegetables is still within the range that is suitable for consumption. The denaturation and aggregation of proteins have been demonstrated to be influenced by pH (Andlinger et al., 2021; Egbune et al., 2023; Ndego et al., 2023). The solubility and digestion of dietary protein, as well as internal acid-base homeostasis, have been speculated to be impacted by meal pH and/or buffering capacity in earlier research (Massie, 1981; Van Herreweghe, 1974; Ojo et al., 2022). In contrast to Kamrunnaher et al. (2019)'s study on the red amaranth potato, the results of this study are consistent with those shown by Roy et al. (2020) research on dark red kidney beans.

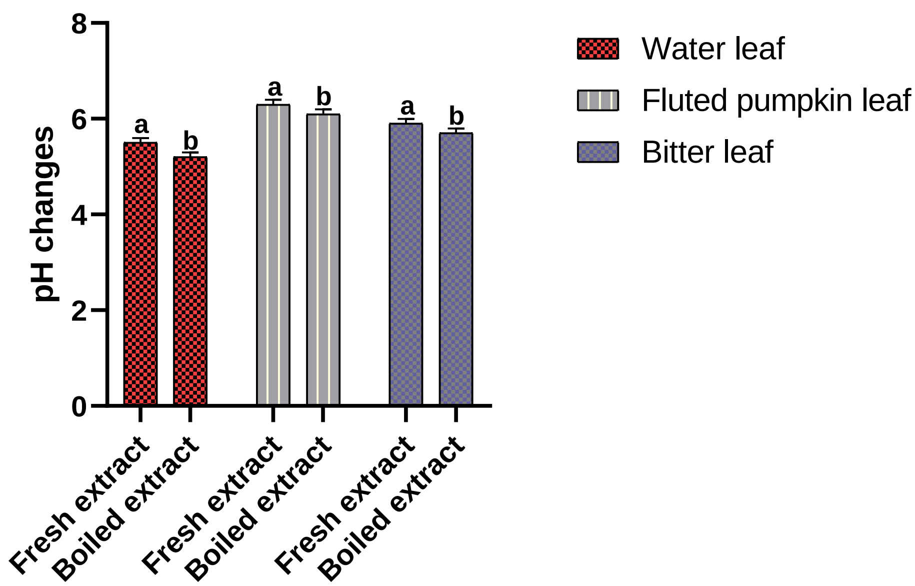

Figure 1. Effect of boiling on the pH of waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*) and fluted pumpkin (*Telfairia occidentalis*). Values with different superscripted letter is significantly different (p>0.05) from the control.

In comparison to the fresh sample, the boiled samples of fluted pumpkin *(T. occidentalis*), bitter leaf (*V. amygdalina*), and waterleaf (*T. fruticosum*) showed considerably higher glucose concentrations (p > 0.05) (Figure 2). This is in contrast to the findings of José et al. (2020), who showed that boiling decreased the amount of glucose in cladodes. According to Bernhardt and Schlich (2006) the process utilized for food preparation could greatly affect the phytochemical properties of vegetables. Different foods have varying amounts of starch granules and structural arrangements (Chakraborty et al., 2020; Egbune et al., 2022). In the course of food preparation, the addition of heat and water causes the starch granules in foods that contain starch to inflate. If this swelling and rupturing occur too frequently, the outcome is internal cell disarray or even cell rupture (Qi et al., 2021). Heat tends to burst interior cells, releasing the glucose concentration of the extracts, which contributes to the sweetness of the vegetable (Hashemi, and Jafarpour, 2020). By doing this, the starch can become much more accessible for enzymatic digestion. As a result of heating, which raises food temperatures to about 100 °C, some foods can become too swollen, which is likely what caused the test subjects' blood sugar levels to spike so quickly after consuming cooked potatoes (Lal et al., 2021). These alterations in processed vegetables may be caused by the breakdown and release of low-molecular-weight carbohydrates into the cooking water (Rodrguez-Sevilla et al., 1999; Anigboro et al., 2022). The findings of this study are at odds with those of Rodrguez-Sevilla et al. (1999) study, which found that heat treatment significantly affected the loss of soluble sugars in cladodes.

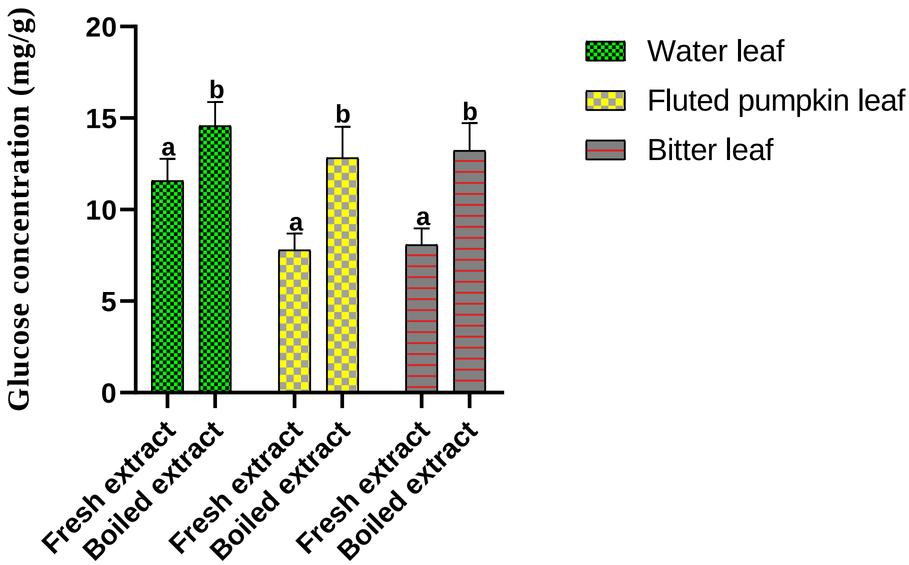

Figure 2. Effect of boiling on the glucose concentration of waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*) and fluted pumpkin (*Telfairia occidentalis*). Values with different superscripted letter is significantly different ($p>0.05$) from the control.

The soluble proteins concentration of boiled waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*) and fluted pumpkin (*Telfairia occidentalis*) were significantly reduced ($p>0.05$) compared with the control (Figure 3). This result is in accordance with the work of Tonukari et al. (2015) which showed that soluble protein of *V. amygdalina* decreased significantly. Boiling has been shown to have effect on the concentration of soluble proteins in various plant-based foods, including waterleaf (*T. fruticosum*), bitter leaf (*V. amygdalina*), and fluted pumpkin (*T. occidentalis*).

Studies have reported that boiling can lead to denaturation, degradation, and/or loss of soluble proteins in these plant materials. For example, a study by Adeyemo et al. (2010) found that boiling of bitter leaf led to a decrease in the concentration of soluble proteins, as well as the total phenolic content and antioxidant activity of the plant. Similarly, a study by Adetunji et al. (2011) showed that boiling of fluted pumpkin resulted in a significant reduction in the concentration of soluble proteins and other nutrients, such as ascorbic acid, total phenols, and total flavonoids.

In general, the effect of boiling on the soluble protein concentration of these plant materials appears to be complex and dependent on various factors, such as temperature, time, and cooking method.
The study by Kristian and Oystein (2013) found that boiling kidney beans can lead to a decrease in their protein content over time and according to them this decrease is due to the denaturation of proteins during boiling, as the heat causes the structure of the starch-protein complex to be altered and the hydrophobic groups of the protein to be exposed to water. This exposure can lead to a reduction in the level of water-soluble protein.

These results are supported by the study by Runyon et al. (2015), who investigated the effect of heat treatment on the soluble protein content of oats. The authors found that heat treatment led to a decrease in the soluble protein content, suggesting that boiling can have a similar effect on the protein content of other plant-based foods.

The study by Southon and Faulks (2002) suggests that cooking vegetables can affect their nutritional composition due to the diffusion of water and low molecular compounds during cooking. These compounds can either degrade or be transformed into bioavailable structures, which can impact the overall nutritional quality of the food. Boiling has the potential to dissolve and reduce the protein content of plant-based foods, such as kidney beans, oats, and vegetables.

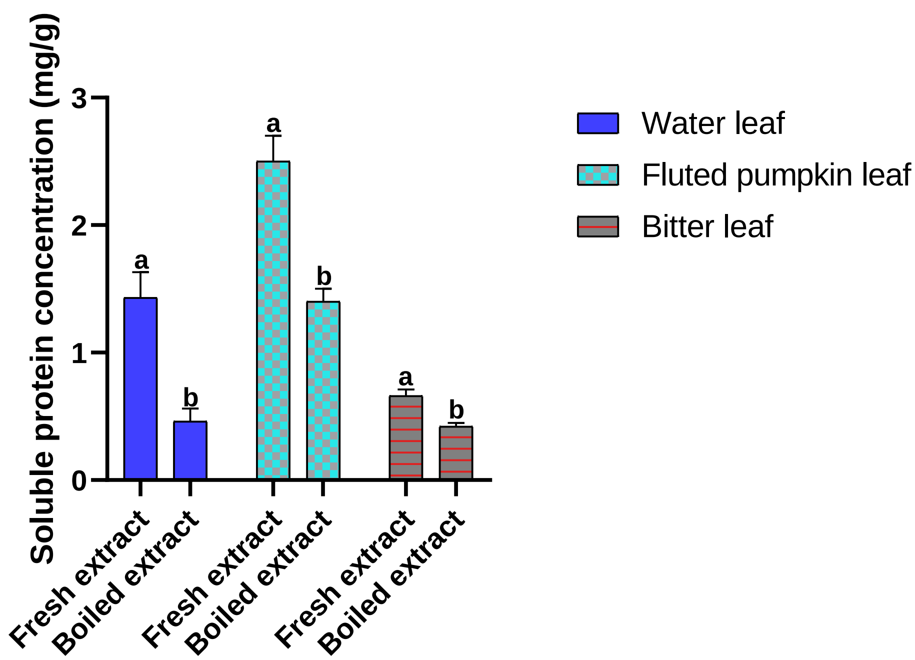

Figure 3. Effect of boiling on the soluble proteins concentration of waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*) and fluted pumpkin (*Telfairia occidentalis*). Values with different superscripted letter are significantly different ($p>0.05$) from the control.

CONCLUSION

From this study, boiling has been shown to have an impact on the pH, glucose and soluble protein contents of waterleaf (*T. fruticosum*), bitter leaf (*V. amygdalina*), and fluted pumpkin (*T. occidentalis*). It is shown that boiling can lead to denaturation, degradation, and/or loss of soluble proteins in these plant materials.

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