 Date of Publication: 13th December 2024
ISSN: 2737-713X

NUTRITIONAL ANALYSIS OF SOURSOP SEEDS AND PULP COMPOSITE FLOUR

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Abstract

Soursop (Annona muricata) fruit, known for its potential health benefits, is underutilized in Ghana. This study aimed to investigate the impact of soursop seeds on the nutritional composition, total phenolic content and antioxidant activity of soursop pulp and seed flour. Oven drying was employed for sample preparation, and proximate composition analyses were conducted to evaluate the synergistic effects on the nutritional composition of the pulp + seed flour. The total antioxidant activity was also determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity method whereas the Folin-Coicalteau method was used to determine the total phenolics content. One-way ANOVA was used to identify significant differences. Soursop pulp + seed flour (made up of 88.82% pulp and 11.18% seed flour) was found to contain 8.21% moisture, 43.41% carbohydrate, 31.67% crude fibre, 10.05% crude fat, 3.81% crude protein and 2.86% ash. Additionally, soursop pulp and seeds exhibited antioxidant activity of 60-65 % and total phenolics of content 7-10 mgGAE, highlighting their potential for enhancing food products with health benefits.

Keywords

nutritional composition, total phenolic content, antioxidant activity, Annona muricata

Introduction

Soursop (*Annona muricata*), known by various names as guanabana, paw-paw, and graviola is a fruit commonly found in the western part of Africa. In Ghana, locallyit is known as "aluguntugui". It belongs to the Annonaceae family, which encompasses approximately 130 genera and 2300 species (Mishra et al., 2013). Soursop plant is an erect tree with relatively shallow roots enabling it to reach most nutrients in the soil. Its stem reaches 5–8 m in terms of height. Other characteristic nature of the tree is it roundish canopy with big, shiny, dark green leaves. This plant produces fruits which are edible and have a heart-shaped and green in color. The diameter of the fruit is within the range of 15 to 20 cm (Moghaddam et al., 2015). The pulp of this fruit is made up of an elongated receptacle surrounded by white fibrous juicy segments (Paull, 1998). These juicy segments if fertile, contain hard black seeds of oval shape (Morton, 1987).

Soursop fruit is underutilized especially in Ghana due to its short shelf life as a result of rapid senescence (Aderonke and Oreoluwah, 2018) and could also be attributed to the few scientific studies conducted on not only the fruit, but stem, leaves and the whole plant. As a result of this, *Annona muricata* has not been adequately exploited in the food industry in Western Africa but been used in the production of some medicines. In other Europeans countries, *Annona muricata* fruit is used in the production of fruit juices, drinks and incorporated into other foods. This has provided an alternate use for the plant.

Interestingly, among the few studies and utilization of *Annona muricata*, most of them have however neglected the seeds. This may be due to the fact that, some studies claim *Annona muricata* contains anti-nutrients such as oxalate, tannins and phytate and therefore will have negative impact on health of people when consumed. Soursop fruit can be used for the treatment of neuralgia, arthritis, diarrhea, dysentery, fever, malaria, rheumatoid disease, skin rushes and worms. Its leaves are used for treating cystitis, hypertension, nausea and sleeplessness. In addition, internal leaf decoction administration is thought to have anti rheumatic and neuralgic effects while cooked blades (leaves) treat abscesses and rheumatism in the most common way (De-Sousa et al., 2010). The crushed seeds are believed to have anthelmintic activities against external and internal worms and parasites. Soursop fruit pulp contains several phenolic compounds and is a good source of antioxidants (Moghaddam et al., 2015). Despite the fact that, other studies have suggested the concentration of anti-nutrients in fruits can be reduced by certain treatments and processing techniques, the state of commercialization of *Annona muricata* remains the same.

Various drying methods have been employed to convert soursop pulp and seeds into powdered form, each with varying effects on the nutritional composition. Additionally, soursop has been recognized for its essential nutrients and potential benefits.

Incorporating soursop seeds into soursop products or soursop flour could help supplement the lacking nutrients in soursop pulp flour. This could also have a positive impact on the health of humans and increase the utilization of this plant especially in the western part of Africa thereby, reducing number of foods and drugs imported into countries within this region. This study aimed to investigate the nutritional impact of soursop seeds on composite flour made from soursop pulp and seeds as well as the effect of oven drying on the phenolic and antioxidant contents of soursop pulp and seeds at varying temperatures.

Materials and Methods

Sample preparation

Soursop fruits were sourced from the *Asenema fruit farms* at Akuapem in the Eastern region of Ghana. The fruits were washed thoroughly and peeled. The pulp and seeds of the fruits were removed by cutting into smaller sizes with a stainless-steel knife and handpicking. The seeds were separated from the pulp also by handpicking. The pulp was steam blanched for 5 minutes. The pulp was dried using a conventional oven dryer at varying temperatures (50°C, 60°C and 74°C) for 24 hours, 7 hours and 6 hours respectively. The seeds were dried with a conventional oven at varying temperatures (50°C, 60°C and 74°C) for 5 h, 3 h and 2 h respectively. The dried samples were then milled separately into fine powder using a blender for the seeds and blender for the fruit pulp. Three different sample formulations were made whereby, sample A contained dried soursop pulp flour only, sample B contained dried soursop seed flour only whiles sample C contained 88.82% pulp and 11.18% seed flour (Badrie and Schauss, 2010). Total antioxidant activity as well as proximate composition of the various sample were determined.

Proximate composition analysis

The proximate compositions (moisture, ash, fat, fibre and protein contents) were determined according to AOAC (2010) method. The crude protein content was calculated by multiplyin ght percent Nitrogen content by the conversion factor of 6.25. The carbohydrate content was determined by a difference. Thus, % Carbohydrate content = 100 – [Moisture + ash + fat + fibre + protein].

Total phenolic content determination

This method is based on the oxidation of phenolic groups by using Folin-Ciocalteu's reagent. About 10 mg of gallic acid was weighed using the analytical balance. The gallic acid was transferred into a 100 mL volumetric flask. The gallic acid was topped up with distilled water to the mark on the volumetric flask. Five different standard solutions were prepared out of the stock solution. About 1 mL of the stock solution was transferred into a 50 mL volumetric flask and distilled water was added till it reached the calibrated mark. The process was repeated for 2 mL, 4 mL, 8 mL and 16 mL of the stock solution. About 20µL Folin-Ciocalteu's reagent was added 1mL of sample of varying concentrations and 20% Na_2CO_3. Absorbance readings were taken at 760 nm using the spectrophotometer and the results were recorded. The same procedure was repeated for all standard gallic acid solutions. The phenolic content was obtained from the gallic acid calibration curve and expressed as grams of gallic acid equivalent per 100 g of dry weight sample (gGAE /100g) (Patra et al., 2017).

Total antioxidant activity determination

Approximately 0.5g of sample was weighed and transfered into a 15 mL centrifuge tube. 10 mL distilled water was added and centrifuged at 10,000rpm for 15 min. Approximately 6.4 mL of reaction mixture was obtained. This mixture comprises 0.2 mL sample, 0.2 mL distilled water and 6 mL of 0.004% DPPH (1,1-diphenyl-2-picrylhydrazyl) solution, placed in separate tubes. The tubes were hand-shaked to mix the content thoroughly. The solution was allowed to sit undisturbed in a dark environment for 30 min at room temperature. Absorbance of both reaction mixture and blank were measured at 517 nm. The reaction mixture lacking sample develops the most intense colour. The colour decreases with increasing volume of extract added. The ability to scavenge the DDPH was calculated using the equation:

$$\%\ Inhibition = \left[1 - \left(\frac{As}{Ao}\right)\right]\ 100 \qquad (1)$$

where AS - Absorbance of sample; Ao - Absorbance of DPPH solution diluted to same volume of distilled water. Distilled water was used as blank

Statistical analysis

Experiments were conducted in triplicates and the outcomes were expressed as means and standard deviation. Experiment results were subjected to statistical analysis using IBM SPSS Statistical Tool version 22 and Microsoft Excel. The Tukey's Multiple Comparison Test of One-way ANOVA was used to compare means with p-values at 0.05.

Results and Discussions

From the results in Table 1 above, soursop pulp flour exhibited the highest concentrations of carbohydrates (48.09%), followed by crude fibre (30.36%) and lower concentrations of ash (2.64%), protein (1.13%) and fat (8.97%). In contast, the seed only flour was rich in crude fibre (43.42%), crude proteins (24.86%) and crude fats (18.27%). Soursop seed + pulp flour was observed to be rich in carbohydrates, crude fibre and crude fats.

Table 1. Proximate composition of soursop pulp, seed and pulp + seed composite flour

Parameters	Pulp Only	Seed Only	Pulp + Seed
Moisture (%)	$8.81^c \pm 0.00$	$3.06^a \pm 0.05$	$8.21^b \pm 0.05$
Crude Protein (%)	$1.13^a \pm 0.11$	$24.86^c \pm 0.53$	$3.81^b \pm 0.25$
Crude Fat (%)	$8.97^a \pm 0.08$	$18.27^c \pm 0.28$	$10.05^b \pm 0.06$
Ash (%)	$2.64^{a,b} \pm 0.14$	$1.86^a \pm 0.06$	$2.86^b \pm 0.54$
Crude Fibre (%)	$30.36^a \pm 0.28$	$43.42^b \pm 0.56$	$31.67^a \pm 0.17$
Carbohydrate (%)	$48.09^c \pm 0.12$	$8.54^a \pm 0.30$	$43.41^b \pm 0.41$

The soursop pulp + seed flour consists of 88.82% pulp and 11.18% seed flour. This ratio was done to enable comparison of this study to an average size soursop fruit. Alphabets in the same column signify

Lombor et al. (2014) reported that the moisture of the oven dried soursop pulp only flour was 8.10% which was within the same range as the moisture content observed in the sample in Table 1, attributable to the use of the same drying method. The difference in result is due to the longer drying duration (48 hours) used by Lombor et al. (2014). In this study the protein content of pulp only flour as presented in Table 1 was lower (1.13%) than that reported by Lombor et al. (2014) (21.31%), likely due to higher drying temperature approximately 73°C, causing protein denaturization. (Wijewardana et al., 2016) obtained similar results with a drying temperature of 73°C, showing a lower crude protein percentage of about 1.95%. It is worth noting that oven drying is effective for moisture

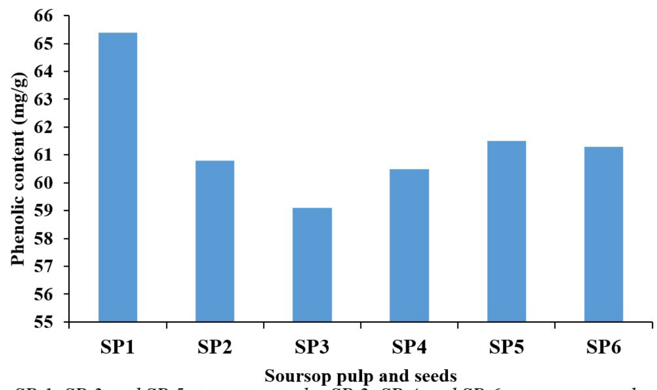
removal, which can positively impact the flour's shelf life.
Table 1 shows a similarityin the nutritional composition of soursop seed-only flour and the work of Fasakin et al. (2008). This similarity is attributed to the use of the same oven drying method , similar drying temperatures and drying times. The differences in moisture content, led to variations in carbohydrate content. Soursop seed-only flour had lower carbohydrate content compared to Fasakin et al. (2008) due to the difference in the moisture contents, leading to concentration of the other macronutrients in the flour. No significant differences were observed between tseed-only flour and the work of Fasakin et al. (2008) regarding crude protein, crude fat, fibre and ash contents. Additionally, Table 1 shows that seed-only flour had lower carbohydrate concentration compared to pulp-only flour, which aligns with findings by Sawant and Dongre (2014), indicating that soursop fruit pulps are typically rich in proteins whiles their seeds are rich in proteins and oils.

The results were analysed using IBM SPSS statistical tool with one-way ANOVA to identify significant differences between the values at 95% confidence interval. The analysis revealed significant difference between the three samples (pulp-only, seed-only, pulp + seed flours) in terms of their moisture, protein, fat and carbohydrate contents. Notably, soursop seed flour had lower moisture content than pulp flour despite a longer drying duration. The moisture content of soursop pulp generally ranges from 80-81%, requiring longer drying durations to remove moisture. Consequently, the pulp-only flour exhibited a relatively higher moisture content compared to seed-only flour, resulting in increased moisture content in pulp + seed flour compared to seed-only flour.

This shows the impact of the seed flours on the nutritional composition of the pulp + seed composite flour. It was observed that, soursop seed flour had a lower moisture content than pulp flour even though the pulp flour was dried for a much longer time. According to Onimawo (2002), the pulps of soursop fruits have moisture content of 80-81%, and will require longer drying durations to vaporize the present moisture. The higher moisture content of the pulp required a higher duration of drying. This however accounts for the relatively higher moisture content of pulp only flour as compared to seed only flour, and also responsible for the increased moisture content in pulp + seed flour as compared to the soursop seed only flour.

Soursop seed-only flour was found to have a high protein content leading to the increased protein content of soursop pulp + seed flour. Due to the very small quantity of seed only flour (11.18%) used in formulating the pulp + seed flour, the impact or influence of the seed flour on the pulp + seed flour was minimal. This reason applies to the other observations made with regards to crude fats, fibre, ash and carbohydrate contents of the three flours.

Figure 1, illustrate the phenolic composition of soursop pulp and seeds-only. Different drying temperatures and times were employed, impacting the phenolic composition of the soursop pulp and seeds. This ratio was chosen to facilitate a comparison with average-sized soursop fruit. In Figure 1 above, total phenolic content was determined by using the Folin-Coicalteau reagent method. Samples SP1, SP3 and SP5 indicate soursop pulp dried at 50°C, 60°C and 65°C within a time frame of 24 hours, 7 hours and 6 hours 10 minutes respectively. Samples, SP2, SP4 and SP6 indicate the phenolic content of soursop seeds dried at 50°C, 60°C and 65°C for 5 hours, 3 hours and 2 hours respectively. Soursop seeds are a good source of total phenolic compounds. SP 4 had the highest total phenolic content (10.12mg GAE) as compared to SP 2 and SP 6, 7.68 and 9.04 mg GAE respectively. SP4 was dried at 60°C for 3 hours, SP 2 at 50°C for 5 hours and SP 6 dried at 65°C for 2 hours. As the temperature was increasing, the time for drying was decreasing. The longer the food material is exposed to high temperatures, it may lead to loss of some essential compounds. Similarly, the higher the temperature, some essential compounds may also be affected (Abreu et al., 2014). Even though SP 6 dried for a short period, the seeds were exposed to high temperature. In sample, SP 2, even though the temperature for drying was quite lower as compared to SP 4 and SP 6, it took a longer period to dry.



SP 1, SP 3 and SP 5 = soursop pulp, SP 2, SP 4 and SP 6 = soursop seeds

Figure 1. Effect of drying time and temperature on total phenolic content of soursop pulp and seeds

In samples, SP1, SP3 and SP5, the total phenolic content increased with decreasing time. A greater portion was reserved in sample, SP5 (9.23 mg GAE) which was dried at a temperature of 65°C for 6 hours 10 minutes. At the end of the drying process, the samples SP1 and SP3 resulted in a TPC of 7.87 and 8.78 mg g GAE respectively. Similar studies conducted by Lombor et al. (2014) to determine the total phenolic content of mortiño fruit with varying temperatures and drying times showed that phenols were reserved in a greater portion at 60°C given the phenolic content to be 5.85 mg GAE with about 34% of its phenols reserved, whereas the samples dried at 40°C and 50°C had 3.87 and 6.50 mg GAE with about 25% and 29% retained respectively. A similar result was obtained in a study reported by Bennett et al. (2011) on Zante currant (7.5 mg GAE) and prune (5.69 mg GAE) dried by hot air as well as a research by (Bhouri et al., 2016) on Tunisian raisin varieties.

Prolonged heat treatment makes polyphenols thermolabile, which causes flavonoids and tannins to be destroyed during drying (Vashisth et al., 2011). Inadequate extraction capabilities of the currently available methods, binding of phenols to proteins, changes the chemical structures and other factors, may also contribute to the loss of phenolic content (Gumuşay et al., 2015). Enzymes that can oxidize endogenous polyphenolics may be released as a result of cell disturbance, including oxidative and hydrolytic enzymes. However, even a short period of exposure to high temperatures has the ability to deactivate these enzymes and protect the polyphenolics from further deterioration. Phenolic compounds decreased with increasing temperature and storage time. This may be related to phenolic compounds oxidation and polymerization (Swati and Poonam, 2015).

According to a hypothesis by Vashisth et al. (2011), phenolic acids may be released during heat processing as a result of the breakdown of cellular components and covalent bonds because they are mostly bound to carbohydrate and proteinaceous moieties. Phytochemical compounds may oxidize during hot air drying because the food material has more contact with oxygen. The color, vitamins and flavor of the fruit are also affected when exposed directly to hot air (Chong et al., 2013).

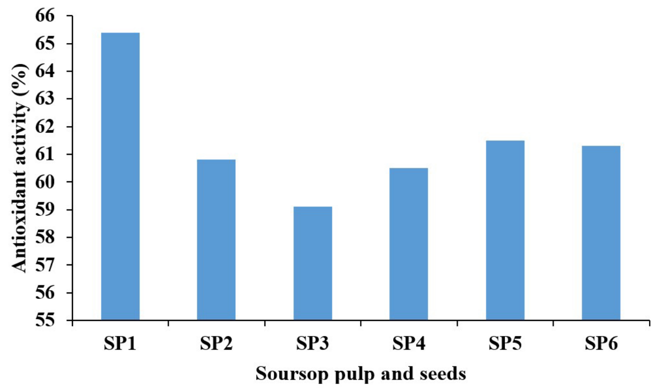
Figure 2 shows the total antioxidant content of soursop pulp and seeds only. These raw materials were dried at different temperatures and time. Therefore, the drying method had a significant influence on the phenol composition of the soursop pulp and seeds.

Temperature is one of the most important factors affecting antioxidant activity. Generally, heating causes an acceleration of the initiation reactions and hence a decrease in the activity of present or added antioxidants (Olakunle et al., 2014).

Table 2. Total antioxidant activity of soursop pulp flour, seed flour and pulp + seed flour at constant drying temperature and time

Soursop Part	Total Antioxidant Activity (%)
Pulp only	$10.48^a \pm 0.94$
Seed only	$35.62^c \pm 1.31$
Pulp + Seed	$30.10^b \pm 1.21$

As stated earlier, the composition of pulp flour and seed flour in pulp + seed flour remained unchanged in the evaluation of the total antioxidant capacity or activity. The seed flour is rich in antioxidants (30.10%) and therefore raise.d the antioxidant activity in the pulp + seed flour. Similar studies conducted by Olakunle et al. (2014) and Basker et al. (2007) showed that soursop stem bark and leaves respectively were rich in antioxidants. Also, the results in Table 2 shows that, *Annona muricata* pulp + seed flour had a higher antioxidant content than *Annona squamosal* and *Annona reticula* when compared to results provided by Basker et al. (2007). The findings suggest that, *Annona muricata* pulp and seed flour can act as a source of antioxidant and be useful in the food and pharmaceutical industries. From Figure 2, SP 1 had the highest antioxidant activity. As the temperature increased, there was a decrease in the antioxidant activity in SP 3. In SP 5, there was a rise in the antioxidant activity even though the temperature was higher (65°C). This is because, the drying time is a factor in affecting antioxidant activity (Banout et al., 2011). This behavior of rise and fall in antioxidant activity may be related to the drying process in relation to higher temperatures and low drying times or low temperatures and long drying times. This can promote a reduction or increase in antioxidant activity (Piga et al., 2003). Furthermore, in



SP 1, SP 3 and SP 5 = soursop pulp, SP 2, SP 4 and SP 6 = soursop seeds

Figure 2. Effect of varying drying time and temperature on total antioxidant capacity of soursop pulp and seeds

samples SP 2, SP 4 and SP 6 which represent soursop seeds dried at 50, 60 and 65°C for 5 hours, 3 hours and 2 hours respectively had increasing antioxidants capacity with increasing temperatures but decreasing drying times. There was no significant difference between SP 2 and SP 4.

According to (López-Vidaña et al., 2016), the antioxidant activity of mortino fruit extracts was evaluated using the FRAP assay at temperatures of 40, 50 and 60°C respectively. The results showed that the antioxidant capacity was higher in fruits dried at 60°C than those dried at low temperatures. However, as ddepicted in figure 2 SP 1, which was dried at a lower temperature, 50°C exhibited a higher antioxidant capacity, 65. 44% compared to samples SP 3 and SP5 which had antioxidant capacities of 59.17 and 61.61% respectively. Elevated temperatures are generally associated with having a negative effect on antioxidant capacity potentially due to irreversible oxidative processes during drying. Additionally, long drying times can affect antioxidant capacity.

Conclusion

The composite flour of *Annona muricata* pulp and seed comprises 3.81% crude proteins, 10.05% fats, 31.67% fibre, 2.86% ash and 43.41% carbohydrates with a low moisture content of 8.21%. Seed flour exhibits a substantial content of phenolic compounds and antioxidant activity contributing to the overall synergestic effect in the pulp and seed composite flour. When incorporated into foods and beverages, this composite flour could offer a wide array of health benefits to consumers.

Annona muricata, prevalent in tropical regions, holds the potential to generate income, revenue and foreign exchange when exploited properly due to its nutritional composition, uses and numerous health benefits.

Acknowledgement

The authors extend their appreciation to all those involved in this study particularly the laboratory technicians and research assistants in the Food Analysis Laboratory, Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana for their invaluable support and guidance.

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