Original Research Article

Effect of Time, Temperature and Size Reduction on Some Physico-Chemical Characteristics of *Sorghum bicolour* Leaf Sheath Extracts

Abstract

Sorghum bicolor leaf sheaths are a common dried ingredient used as a colourant for waakye, a popular Ghanaian dish made from rice and cowpea. The leaf sheaths are also used in traditional medicine due to its impressive bioactive composition. Its potential as a natural food colourant and antioxidant has been established but the effect of different processing conditions on the physicochemical composition and characteristics have not been adequately researched. The present study assessed the effect of size reduction (whole, coarse and fine), temperature (room temperature (28 °C) and 98 °C) and time (20, 40 and 60 min) on the colour, pH and ascorbic acid content of sorghum leaf sheaths. Samples were steeped in a measured amount of water and analysis conducted on the extracts. Size reduction significantly reduced the lightness (l*) and colour intensity (chroma and hue) of extracts steeped at both temperatures. The pH of all extracts was relatively neutral, ranging from 6.63 to 7.23 and was not significantly affected by size reduction or time. Extraction of ascorbic acid was more effective at 98 °C and did not degrade with constant heating within the experimental time. Average ascorbic acid content of extracts was 3.89 g/L. For effective utilization and value addition of *Sorghum bicolor* leaf sheaths, food producers should consider fine milling and late incorporation into food for optimum colour and phytochemical content preservation.

Key words: Sorghum, ascorbic acid, waakye leaves, colour, extract, size reduction

1.0 Introduction

Sorghum bicolor (L) Moench is the second most important cereal on the African continent (Mundia *et al.*, 2019; Jacob *et al.*, 2013) grown primarily for its energy rich grains (Are *et al*., 2019). In addition to its use as food and feed, other parts of the sorghum plant can be used for the production of bioenergy, syrup, building materials and brooms (Rashwan *et al*., 2021; Dahlberg *et al*., 2012). Some sorghum varieties (dye sorghum) are specifically grown for the pigments in their leaf sheaths (Akogou, 2018).

Sorghum leaf sheaths contain carotenoids, flavonoids, phenolic acids, chlorophyll, lycopene and β-carotene (Abugri *et al*., 2012). The rich bioactive composition has been found to induce homopoietic, anti-anaemic, anti-inflammatory and immune modulating properties (Okubena *et al*., 2018; Benson *et al*., 2013). They are also abundant in 3-deoxyanthocyanidins which are rare natural pigments the most abundant of which is apigeninidin (Akogou *et al*., 2018). The natural pigments have potential for use as a natural indicator for acid base titrations (Abugri *et al*., 2012) and a stable natural food colourant (Akogou *et al*., 2018).

Waakye leaves, as the leaf sheaths are commonly called in Ghana, are an ingredient in the preparation of waakye, a combination of cooked rice and cowpea. Dye sorghum leaf sheaths impart a signature reddish brown colour to the meal and are also used for colouring koko (a fermented cereal porridge), wagashi (a soft cheese) (Akogou *et al*., 2018), leather products (Abugri *et al*., 2012) and woven artefacts (Balole and Legwalila, 2006). Sorghum leaf sheaths have shown very high total antioxidant capacity which has been attributed to its anthocyanin content (Kayode *et al*., 2011).

The use of dye sorghum in food preparation has been found to substantially increase the phytochemical content and antioxidant capacity of waakye (Tsugli *et al*., 2019) and fermented maize dough (Akogou *et al*., 2019). Traditional methods of extraction involve the use of kanwu (saltpetre) with or without the application of heat (Akogou, 2018). Akogou (2018) proposed the use of leaf sheath powder for modernizing traditional preparations. Most processors employ the use of sorghum leaf sheath extracts (rather than the whole leaf) in food preparation (Akogou *et al.*, 2018) but there is little information available on how size reduction and time affect colour extraction from the leaf sheaths. The objective of this study, therefore, was to assess the effect of size reduction and cooking time on the extraction of colour and ascorbic acid into water extracts obtained from steeping sorghum leaf sheaths at different temperatures.

2.0 Methodology

2.1 Material

Leaf sheaths of *sorghum bicolor* without visible fungal infection were purchased from a local market in Ghana (Madina market).

2.2 Sample Preparation

The leaf sheaths were cut into different sizes to analyse the effect of size reduction on the colour, pH and ascorbic acid content of extracts. Sorghum leaf sheaths were cut in three different ways: leaf sheaths cut into lengths of 6 cm with an average width of 2 cm was designated as 'whole samples', 'coarsely shredded' (coarse) samples were leaf sheaths cut into sizes of 1 cm^2 and 'finely milled' (fine) samples were obtained by milling leaf sheaths with a hammer mill (mesh size 16).

15g of sample was placed into a 500 ml beaker containing 300ml of distilled water. Each designated size was steeped at room temperature (28 °C) and 98 °C for 20, 40 and 60 minutes. A water bath at ≈98 °C was used to maintain a constant temperature at 98 °C. After steeping for the required time the supernatant (extract) was strained, allowed to cool and then bottled prior to analysis.

2.3 Analysis

2.3.1 Colour

Colour analysis was conducted using a Minolta CR-310 Chroma Meter. The extracts were poured into petri dishes until half-filled to minimize spillage and for uniformity. Readings were done at the centre of the dish for accuracy. The Chroma meter was calibrated using a standard white calibration curve plate before measurements were taken.

The l*, a* and b* values were taken for each sample and the Chroma and Hue were subsequently calculated according formulas below:

$$Chroma = \sqrt{a^{*2} + b^{*2}}$$

$$Hue = \tan^{-1}(\frac{b^*}{a^*})$$

2.3.2 Ascorbic acid

Ascorbic acid (Vitamin C) content was determined by the Redox titration method using Iodine solution according to AOAC (2000). Iodine solution, 1% starch indicator and vitamin C standard solution were prepared in accordance with the standard. Ten (10) drops of 1% starch indicator was added to 25ml of sample in a 125ml Erlenmeyer flask. The sample was titrated against 0.005 mol/l iodine solution in a burette until the end point. Initial and final volumes of iodine were recorded and used to calculate the ascorbic acid content in mg/100g according to the following relation:

$$= 0.005 \times 176.12 \times average\ titre$$

Ascorbic acid content of a standard solution was determined and used to adjust obtained results.

2.3.3 pH

The pH of the extracts was measured using a pH meter according to AOAC (2000)

2.4 Data Analysis

All measurements were taken in triplicate.

Analysis of variance and mean separation were conducted using Statgraphics XVI employing multiple range test and LSD to assess differences between extracts at 95% confidence level. Pearson's correlation coefficients were calculated using Microsoft Excel.

3.0 Results and Discussion

3.1 Colour Analysis

From experimental observation, the sorghum leaf sheaths produced a rich reddish brown colour when steeped. Table 1 shows the l*, a*, b*, chroma and hue values for the different treatments.

Table 1: The effect of temperature, and material size on colour parameters of sorghum leaf extracts

	Material Size						
Temperature (°C)		98			28		
Time		20	40	60	20	40	60
l	Whole	39.52 ± 0.11^{Aa}	40.75 ± 0.17^{Aa}	39.73 ± 0.12^{Aa}	59.12 ± 0.11^{Aa}	61.83 ± 0.18^{Aa}	60.08 ± 0.12^{Aa}
	Coarse	38.64 ± 0.12^{Ba}	37.85 ± 0.15^{Ba}	37.79 ± 0.09^{Ba}	53.95 ± 0.05^{Ba}	56.68 ± 0.13^{Ba}	54.96 ± 0.21^{Ba}
	Fine	37.65 ± 0.10^{Ca}	37.42 ± 0.11^{Ca}	37.43 ± 0.18^{Ca}	39.67 ± 0.12^{Ca}	41.10 ± 0.12^{Ca}	38.04 ± 0.60^{Ca}
a	Whole	13.50 ± 0.11^{Aa}	15.75 ± 0.13^{Aa}	13.23 ± 0.09^{Aa}	10.56 ± 0.11^{Aa}	10.26 ± 0.19^{Aa}	11.31 ± 0.22^{Ba}
	Coarse	10.60 ± 0.25^{Ba}	7.33 ± 0.10^{Ba}	8.47 ± 0.12^{Ba}	18.32 ± 0.18^{Ba}	15.23 ± 0.29^{Ba}	15.88 ± 0.31^{Ba}
	Fine	3.92 ± 0.19^{Ca}	3.68 ± 0.02^{Ca}	3.65 ± 0.15^{Ca}	11.40 ± 0.31^{Aa}	14.74 ± 0.18^{Aa}	6.32 ± 0.09^{Ca}
b	Whole	2.27 ± 0.08^{Aa}	4.76 ± 0.13^{Aa}	2.84 ± 0.21^{Aa}	21.66 ± 0.07^{Ba}	18.71 ± 0.25^{Ba}	21.12 ± 0.31^{Ba}
	Coarse	0.88 ± 0.11^{Ba}	-0.47 ± 0.17^{Ba}	-0.18 ± 0.15^{Ba}	23.42 ± 0.12^{Ca}	23.28 ± 0.29^{Ca}	23.48 ± 0.32^{Ca}
	Fine	-1.44 ± 0.41^{Ca}	-1.48 ± 0.19^{Ca}	-1.46 ± 0.12^{Ca}	2.01 ± 0.28^{Aa}	5.36 ± 0.07^{Aa}	-0.35 ± 0.29^{Aa}
Chroma	Whole	13.69 ± 0.11^{Aa}	16.46 ± 0.16^{Aa}	13.53 ± 0.13^{Aa}	24.10 ± 0.07^{Aa}	21.34 ± 0.30^{Aa}	23.95 ± 0.37^{Aa}
	Coarse	10.64 ± 0.25^{Ba}	7.34 ± 0.10^{Ba}	8.47 ± 0.12^{Ba}	29.73 ± 0.17^{Ba}	27.82 ± 0.39^{Ba}	28.35 ± 0.0.43^{Ba}
	Fine	4.19 ± 0.05^{Ca}	3.97± 0.06^{Ca}	3.94 ± 0.11^{Ca}	11.58 ± 0.34^{Ca}	15.68 ± 0.15^{Ca}	6.33 ± 0.08^{Ca}
hue	Whole	9.53 ± 0.31 ^{Aa}	16.81 ± 0.33 ^{Aa}	12.13 ± 0.81 ^{Aa}	64.01 ± 0.25 ^{Aa}	61.25 ± 0.20 ^{Aa}	61.83 ± 0.14 ^{Aa}
	Coarse	4.75 ± 0.59 ^{Ba}	-3.64 ± 1.30 ^{Ba}	-1.22 ± 1.03 ^{Ba}	51.96 ± 0.24 ^{Ba}	56.82 ± 0.28 ^{Ba}	55.93 ± 0.24 ^{Ba}
	Fine	-20.25 ± 6.18 ^{Ca}	-21.82 ± 2.67 ^{Ca}	-21.81 ± 2.33 ^{Ca}	10.00 ±1.19 ^{Ca}	19.99 ± 0.43 ^{Ca}	-3.21 ± 2.62 ^{Ca}

LSD. For each measured parameter, means in the same column (per treatment time) with the same upper case are not significantly different (P>0.05) from each other for each steeping method. Means within the same row (per each material size classification) with the same lower case are not significantly different (P>0.05) from each other for each steeping method.

Lightness (l*), Redness (a*) and yellowness (*b) were generally lowest in milled sorghum leaf sheath extracts. No significant differences ($p>0.05$) were however observed between extracts of coarse and whole sorghum leaf sheaths. Size reduction provides an increased surface area for extraction of components which is likely to have caused the difference in lightness and colour intensity (Chauhan *et al.*, 2017; Onipe *et al.*, 2017; Zamri *et al.*, 2016; Maran *et al.*, 2015).

Visual observation showed that the colour of extracts became darker with heat application and had a cloudy, dull and almost dirty appearance. This corresponded with the lower lightness (l*), Chroma and hue values observed for samples steeped at 98 °C in comparison to those steeped at room temperature (28 °C). The application of heat has been used to aid extraction of components in processes of infusion and decoction due to its ability to increase the rate of extraction (Sultana *et al.*, 2023; Hidayat and Wulandari, 2021; Che Musa *et al.*, 2018; Chauhan *et al.*, 2017; Azwanida, 2015; Razak *et al.*, 2011). The increased extraction is evidenced by the lower l* value.

The present observation is however, also similar to that of Akogou *et al*. (2018) who observed that total anthocyanin content (the main colour components in sorghum leaf sheaths) reduced after heat treatments at 65, 95 and 121 °C for 30 minutes each and percentage losses of 17 ‑18%, 59 ‑66% and 60 ‑61% respectively. Chroma and hue were higher in sorghum leaf sheath extracts steeped at room temperature (28 °C) than in extracts steeped at 98 °C. The reduction in anthocyanins as a result of heat treatment is attributed to their breakdown into other compounds such as calchones (Sultana *et al.*, 2023; Hidayat and Wulandari, 2021; Yang *et al*., 2014) Whereas the a* value (redness) reduced, the opacity, indicated by a low l* value, increased.

The time of steeping, however played no statistically significant role in the measured lightness, redness and yellowness of brews suggesting that colour is stable within 60 minutes of constant heating. A more opaque brew would be preferred because of the likelihood of such a brew to impart more colour to waakye. A lower l* value was hence taken as a positive indicator. The reduction in Chroma and hue is however undesirable since it may connote a lower phytochemical content.

3.2 pH

The pH of waakye leaf extracts as depicted in figures 1 and 2 ranged from 6.63 to 7.23 implying that all the extracts were near neutral. The lowest pH was recorded by finely milled leaf sheaths infused for 60 minutes at room temperature (Fig 1) while the highest was recorded by finely milled and coarsely shredded leaf sheaths steeped at 98 °C for 40 minutes (Fig 2). The pH of brews followed no particular trend in relation to size reduction and temperature, as shown in figure 3. The relatively neutral range of pH is likely to have contributed to the stability of colour analysed. Apigeninidin, the main colour component in sorghum leaf sheaths (Owumi *et al*., 2022; Geera *et al*., 2012), has been found to be stable within a pH range of 6 – 10 (Akogou, 2018).

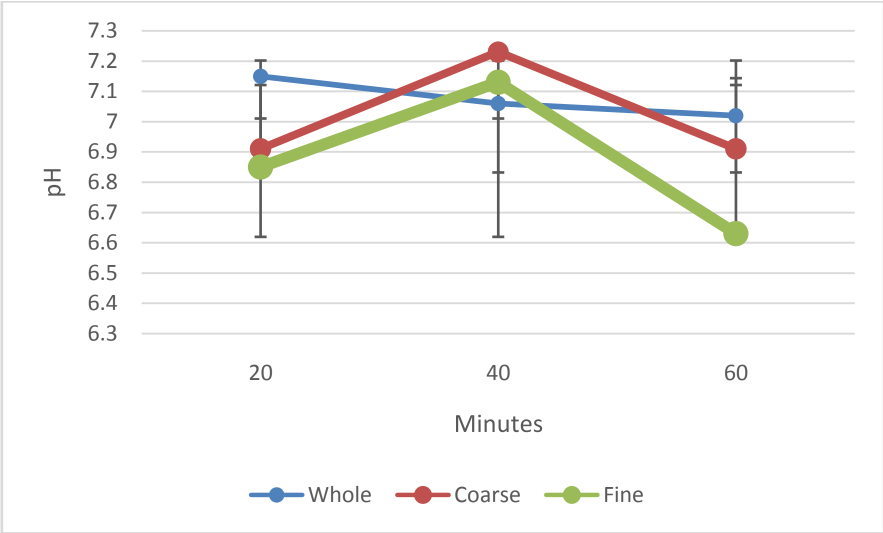

Figure 1: Effect of time on pH of sorghum leaf sheath extracts steeped at room temperature (28 °C)

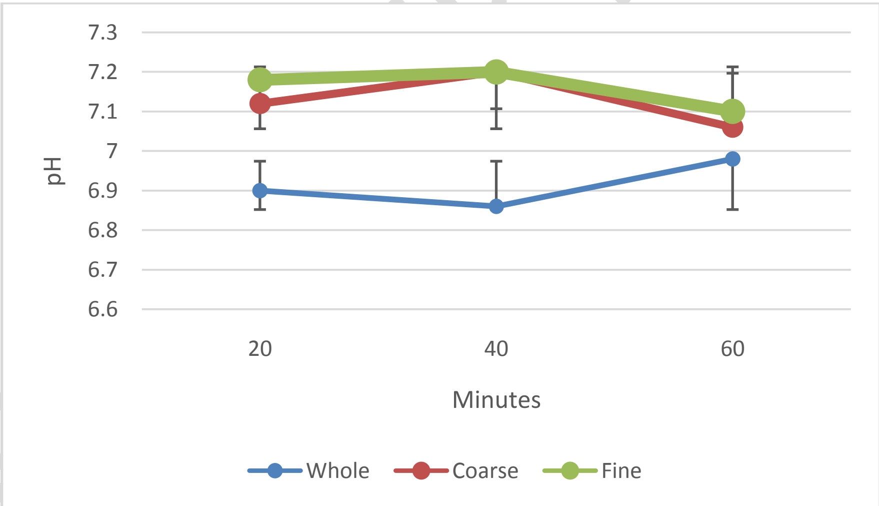

Figure 2: Effect of time on pH of sorghum leaf sheath extracts steeped at 98 °C

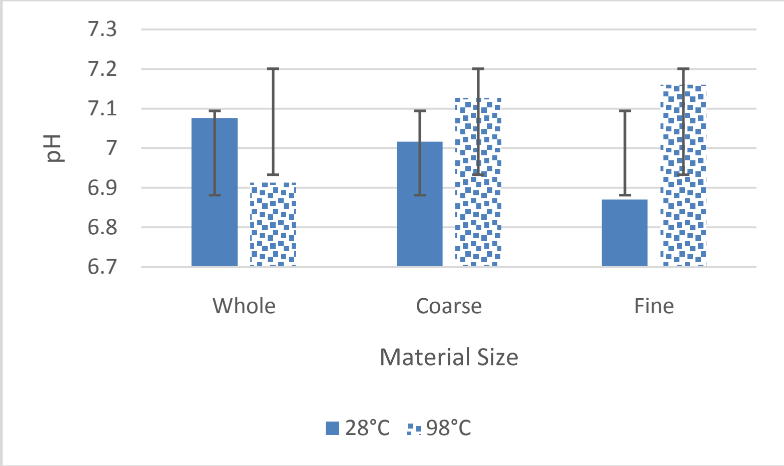

Figure 3: Effect of material size on the pH of sorghum leaves brews steeped at different temperatures

The range of pH values measured presupposes that the pH of sorghum leaf brews is relatively stable with respect to changes in material size and temperatures up to 98°C within a time of 60 minutes.

3.3 Ascorbic Acid Content (Vitamin C)

Ascorbic acid also known as vitamin C is a naturally occurring organic compound that is essential in the human diet (Taraj *et al.*, 2021; Hancock *et al.*, 2005). A lack of vitamin C in the diet can lead to the development of scurvy, a disease that affects the gums of humans (Taraj *et al.*, 2021; Magiorkinis *et al.*, 2011). Vitamin C may also act as an antioxidant against oxidative stress (Taraj *et al.*, 2021; Padayatti *et al.*, 2003). The change in ascorbic acid contents of the sorghum leaf extracts over time at different temperatures are shown in figures 4 and 5.

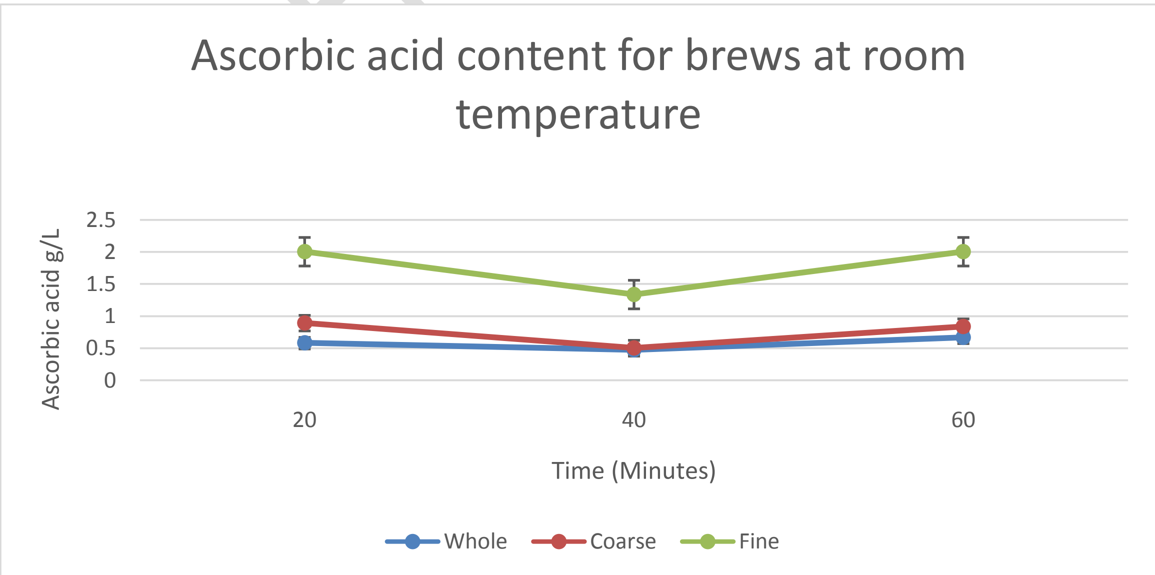

Figure 4: Effect of time on ascorbic acid content of sorghum leaf sheath extracts steeped at room temperature (28 °C)

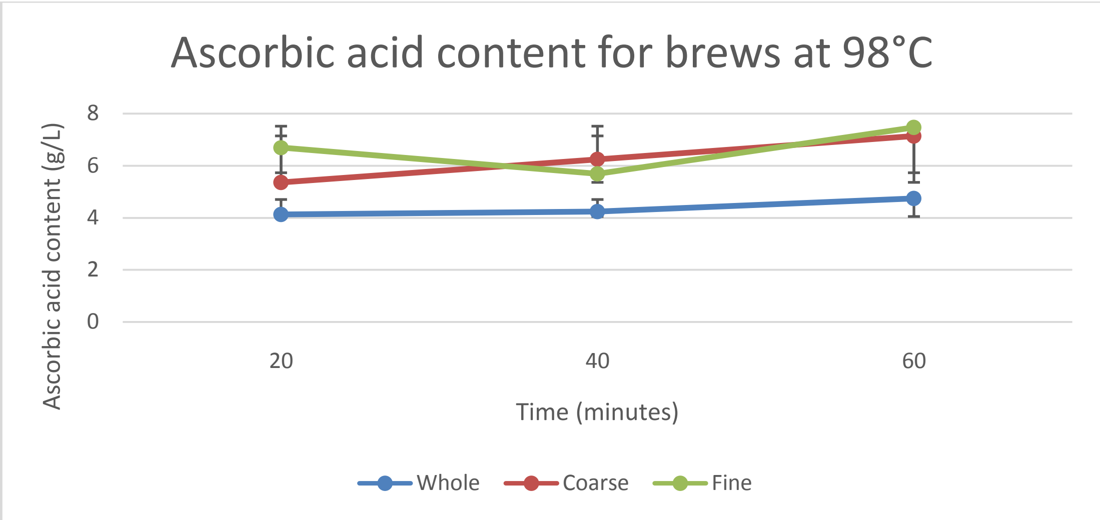

Figure 5: Effect of time on ascorbic acid content of sorghum leaf sheath extracts steeped at 98°C

Ascorbic acid content of the extracts ranged from 0.54 to 8.50g/l. Extracts of whole leaf sheaths steeped at 28 °C for 40 minutes recorded the lowest content and finely milled leaf sheaths steeped for 60 minutes recorded the highest ascorbic acid content. The ascorbic acid content of brews increased significantly with a reduction of material size. No significant differences ($p > 0.05$) were however observed between the ascorbic acid content of extracts from coarsely cut and finely milled sorghum leaf sheaths. Comminution increases the surface area of materials through the creation of new previously unexposed surfaces. The increased surface area leads to faster extraction of components (Azad, 2018). An increase in surface area has however also been found to cause depletion of ascorbic acid content as a result of increased exposure to the atmosphere (Gil *et al.*, 2006). Depletion due to comminution was not evident in this study.

The ascorbic acid content of extracts steeped at 98 °C contained higher contents of ascorbic acid in comparison to those steeped at 28 °C. Heat treatment of sorghum leaf sheaths aided the extraction of vitamin C from the leaves into the water as depicted in figure 6. Vitamin C is reported to be susceptible to heat and the extent of depletion is dependent on the holding time (Oyetade *et al.*, 2012). Igwemmar *et al.*, (2013) and Njoku *et al.* (2011) recorded reduction in vitamin C contents in fruits and vegetables after temperature treatments for short periods. This was not evidenced in the steeping of sorghum leaf sheaths at 98 °C within a period of 60 minutes.

The effect of heating on the ascorbic acid content is highly dependent on the food material it is contained in. While vitamin C in water is quite stable within a time of 1 hour, other materials such as fruit purees and leafy vegetables have their content depleted within a few minutes.

Furthermore, the vitamin C content of the brews remained stable, and instead increased with time, (though not statistically significant at the 95% confidence level) reaching a maximum concentration after a period of 60 minutes. Similar increases in vitamin C content were observed for rosehips which were extracted at 20 and 100 °C (Şendil, 2006). The vitamin C content increased with time and reached its maximum content at 4.5 hours before decreasing. Calyxes of *hibiscus sabdariffa* boiled in water for 5, 10 and 15 minutes also had an increase in the vitamin C content from 5 to 10 minutes but subsequently decreased again at 15 minutes (Bamishaye, 2011). Sendil (2006) suggested that the rosehip extract matrix contributed to the slow degradation of ascorbic acid. Three factors are key in ascorbic acid depletion: temperature, the form in which ascorbic acid occurs and the matrix (Stešková *et al*., 2006). The food matrix in which a nutrient is contained has a profound effect on the rate of reaction and its chemical behaviour because of the differences in interaction of the food components with unique chemical and structural features (Capuano *et al.*, 2017). While the exact mechanism that supports the stability of ascorbic acid in sorghum bicolor leaf sheaths is yet to be determined, it is evident that its composition has a role to play.

The quantity and stability of ascorbic acid in extracts suggests that sorghum leaf sheaths have the potential to supply a substantial amount of vitamin C to the foods they are used to prepare.

Table 2 shows the correlation between the different variables. A strong negative correlation (R^2 = -0.83, -0.87) was observed between the vitamin C content and the Chroma and hue of extracts (also observed between ascorbic acid and the l*, a* and b* values) as shown in Table 2. The negative correlation may be as a result of a depleting effect of ascorbic acid on the anthocyanin contents of the extracts. Previous studies in model solutions have shown a link between ascorbic acid addition and anthocyanin content reduction (Choi, Kim & Lee, 2002; Martí,, Pérez-Vicente & García-Viguera, 2002). While such interaction is unexpected in a natural matrix, extraction and steeping times may have influenced these observations.

Table 2: Correlation between pH, ascorbic acid, l*, a*, b*, Chroma and hue of sorghum *bicolor* leaf sheath extracts

	pH	*Ascorbic acid*	*l**	*a**	*b**	*Chroma*	*hue*
pH	1						
Ascorbic acid	0.311703	1					
l	0.045778	-0.80186	1				
a	-0.27323	-0.62594	0.459743	1			
b	-0.01198	-0.82229	0.960419	0.6185	1		
Chroma	-0.10209	-0.82607	0.877803	0.810162	0.961177	1	
hue	-0.09841	-0.86932	0.950018	0.685442	0.959615	0.957387	1

4.0 Conclusion

It can be concluded from the study that size reduction of sorghum leaf sheaths aids colour and ascorbic acid extraction in water. Extraction of finely milled leaf sheaths at 98°C produced the darkest water extracts with the highest ascorbic acid contents regardless of the time of steeping within (60 minutes). As such, the authors recommend that future research should look at the use of comminution and extraction to produce an ingredient that can be incorporated closer to the end of cooking rather than the beginning.

References

Abd Razak, N., Tumin, S. M., & Tajuddin, R. (2011). Effect of temperature on the color of natural dyes extracted using pressurized hot water extraction method. *American Journal of Applied Sciences*, *8*(1), 45-49.

Abou-Arab, A. A., Abu-Salem, F. M., & Abou-Arab, E. A. (2011). Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). *Journal of American Science*, 7(7), 445-456.

Abugri, D. A., Apea, O. B., & Pritchett, G. (2012). Investigation of a simple and cheap source of a natural indicator for acid-base titration: effects of system conditions on natural indicators. *Green and Sustainable Chemistry*, *2*(3), 117-122.

Abugri, D. A., Tiimob, B. J., Apalangya, V. A., Pritchett, G., & McElhenney, W. H. (2013). Bioactive and nutritive compounds in *Sorghum bicolor* (Guinea corn) red leaves and their health implication. *Food Chemistry, 138*(1), 718-723.

Akogou, F. U. (2018). *Natural food colouring with dye sorghum leaf sheaths* (Doctoral dissertation, Wageningen University).

Akogou, F. U. G., Kayodé, A. P. P., den Besten, H. M. W., Linnemann, A. R., & Fogliano, V. (2018). Effects of processing and storage on the stability of the red biocolorant apigeninidin from sorghum. *LWT*, *90*, 592-597.

Akogou, F. U., Besten, H. M. D., Kayodé, A. P., Fogliano, V., & Linnemann, A. R. (2018). Antimicrobial evaluation of red, phytoalexin-rich sorghum food biocolorant. *PloS one*, *13*(3), e0194657.

Akogou, F. U., Canoy, T. S., Kayodé, A. P., den Besten, H. M., Linnemann, A. R., & Fogliano, V. (2019). Application of apigeninidin-rich red sorghum biocolorant in a fermented food improves product quality. *Journal of the Science of Food and Agriculture*, *99*(4), 2014-2020.

Akogou, F. U., Kayodé, A. P., den Besten, H. M., & Linnemann, A. R. (2018). Extraction methods and food uses of a natural red colorant from dye sorghum. *Journal of the Science of Food and Agriculture*, *98*(1), 361-368.

Are, A. K., Srivastava, R. K., Mahalingam, G., Gorthy, S., Gaddameedi, A., Kunapareddy, A., Kotla, A & Jaganathan, J. (2019). Application of Plant Breeding and Genomics for Improved Sorghum and Pearl Millet Grain Nutritional Quality. In *Sorghum and Millets* (pp. 51-68). AACC International Press.

Azad, A. K. (Ed.). (2018). *Advances in Eco-fuels for a Sustainable Environment*. Woodhead Publishing.

Azwanida N. N (2015) A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants* 4: 196.

Balole, T. V., & Legwaila, G. M. (2006). Sorghum bicolor (L.) Moench. [Internet] Record from Protabase. *PROTA (Plant Resources of Tropical Africa/ Resources végétales de l'Afrique tropicale), Wageningen. http://database. prota. Org /search. htm*.

Bamishaiye, E. I., Olayemi, F. F., & Bamishaiye, O. M. (2011). Effects of boiling time on mineral and vitamin C content of three varieties of Hibiscus sabdriffa drink in Nigeria. *World Journal of Agricultural Sciences*, *7*(1), 62-67.

Benson, K. F., Beaman, J. L., Ou, B., Okubena, A., Okubena, O., & Jensen, G. S. (2013). West African Sorghum bicolor leaf sheaths have anti-inflammatory and immune-modulating properties in vitro. *Journal of Medicinal Food*, *16*(3), 230-238.

Capuano, E., Oliviero, T., & van Boekel, M. A. (2018). Modeling food matrix effects on chemical reactivity: Challenges and perspectives. *Critical reviews in food science and nutrition*, *58*(16), 2814-2828.

Choi, M. H., Kim, G. H., & Lee, H. S. (2002). Effects of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Research International*, *35*(8), 753-759.

Dahlberg, J., Berenji, J., Sikora, V., & Latković, D. (2012). Assessing sorghum [Sorghum bicolor (L) Moench] germplasm for new traits: food, fuels & unique uses. *Maydica*, *56*(2).

Geera, B., Ojwang, L. O., & Awika, J. M. (2012). New highly stable dimeric 3-deoxyanthocyanidin pigments from Sorghum bicolor leaf sheath. *Journal of food science*, *77*(5), C566-C572.

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food chemistry*, *54*(12), 4284-4296.

Hancock, R. D., & Viola, R. (2005). Improving the nutritional value of crops through enhancement of L-ascorbic acid (vitamin C) content: rationale and biotechnological opportunities. *Journal of agricultural and food chemistry*, *53*(13), 5248-5257.

Hidayat R., and Wulandari P. (2021). Methods of Extraction: Maceration, Percolation and Decoction. Eureka Herba Indonesia 2(1): 68-73.

Hiemori, M., Koh, E., & Mitchell, A. E. (2009). Influence of cooking on anthocyanins in black rice (Oryza sativa L. japonica var. SBR). *Journal of agricultural and food chemistry*, *57*(5), 1908-1914.

Igwemmar, N. C., Kolawole, S. A., & Imran, I. A. (2013). Effect of heating on vitamin C content of some selected vegetables. *International Journal of Scientific & Technology Research*, *2*(11), 209-212.

Jacob, A. A., Fidelis, A. E., Salaudeen, K. O., & Queen, K. R. (2013). Sorghum: Most under-utilized grain of the semi-arid Africa. *Scholarly Journal of Agricultural Science*, *3*(4), 147-153.

Jamini, T. S., Islam, A. K. M. A., Mohi-ud-Din, M., & Saikat, M. M. H. (2019). Phytochemical composition of calyx extract of roselle (Hibiscus sabdariffa L.) genotypes. *J Food Tech Food Chem*, *2*(102), 2.

Kayodé, A. P., Nout, M. R., Linnemann, A. R., Hounhouigan, J. D., Berghofer, E., & Siebenhandl-Ehn, S. (2011). Uncommonly high levels of 3-deoxyanthocyanidins and antioxidant capacity in the leaf sheaths of dye sorghum. *Journal of agricultural and food chemistry*, *59*(4), 1178-1184.

Magiorkinis, E., Beloukas, A., & Diamantis, A. (2011). Scurvy: past, present and future. *European journal of internal medicine*, *22*(2), 147-152.

Maran, J. Prakash, et al. "Extraction of natural anthocyanin and colors from pulp of jamun fruit." *Journal of Food Science and Technology* 52.6 (2015): 3617-3626.

Martí, N., Pérez-Vicente, A., & García-Viguera, C. (2002). Influence of storage temperature and ascorbic acid addition on pomegranate juice. *Journal of the Science of Food and Agriculture*, *82*(2), 217-221.

Mundia C. W., Secchi S., Akamani K. and Wang G. (2019). A Regional Comparison of Factors Affecting Global Sorghum Production: The Case of North America, Asia and Africa's Sahel. Sustainability 11, 2135; doi:10.3390/su11072135.

Njoku, P. C., Ayuk, A. A., & Okoye, C. V. (2011). Temperature effects on vitamin C content in citrus fruits. *Pakistan Journal of Nutrition*, *10*(12), 1168-1169.

Ogwumike, O. O. (2002). Hemopoietic effect of aqueous extract of the leaf sheath of Sorghum bicolor in albino rats. *African Journal of Biomedical Research*, *5*(1-2).

Okubena O., Makanjuola S., Ajonuma L. C., Dosunmu A., Umukoro S., Erah P. O. (2018). The West African Sorghum bicolor leaf sheath extract Jobelyn and its diverse therapeutic potentials. MOJ Drug Design Development & Therapy 2(1): 20-28.

Onipe O. O., Beswa D., Jideani A. I. O. (2017). Effect of size reduction on colour, hydration and rheological properties of wheat bran. Food Science and Technology (Campinas).

Owumi, S.E., Kazeem, A.I., Wu, B. Ishokare L. O., Arunsi U. O. and Oyelere A. K. (2022). Apigeninidin-rich Sorghum bicolor (L. Moench) extracts suppress A549 cells proliferation and ameliorate toxicity of aflatoxin B1-mediated liver and kidney derangement in rats. Sci Rep 12, 7438.

Oyetade, O. A., Oyeleke, G. O., Adegoke, B. M., & Akintunde, A. O. (2012). Stability studies on ascorbic acid (Vitamin C) from different sources. *Journal of Applied Chemistry*, *2*(4), 20-24.

Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., Chen, S., Corpe, C., Dutta, A., Dutta, S.K. & Levine, M. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. *Journal of the American college of Nutrition*, *22*(1), 18-35.

Rashwan AK, Yones HA, Karim N, TahaEM, Chen W. (2021). Potential processing technologies for developing sorghum-based food products: An update and comprehensive review. Trends Food Science and Technology 110: 168–182.

Şendil, O. (2006). Effect of some parameters on the extraction and decomposition of ascorbic acid in the rosehip. *Turkish J. Pharm. Sci*, *3*(2), 61-72.

Stešková A., Morochovičová M. & Lešková E. (2006). Vitamin C degradation during storage of fortified foods. *Journal of food and nutrition research*, *45*(2), 55-61.

Sultana H., Chetia A., Saikia A., Khan N. J. (2023). An Updated Review on Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Sch Acad J Pharm, 12(7).

Taraj K., Hasa A., Muca A. (2021). Sources and benefits of vitamin C. Technium BioChemMed 2(1): 23-31.

Tugli, L. S., Essuman, E. K., Kortei, N. K., Nsor-Atindana, J., Nartey, E. B., & Ofori-Amoah, J. (2019). Bioactive constituents of waakye; a local Ghanaian dish prepared with Sorghum bicolor (L.) Moench leaf sheaths. *Scientific African*, 3, e00049.

Zamri, Tengku Khamanur Azma Tg Mohd, Mimi Sakinah Abd Munaim, and Zularisam Ab Wahid. "Extraction Optimization of Natural Dye from Clitoria Ternatea Flower by OFAT." *The National Conference for Postgraduate Research, Universiti Malaysia Pahang*. 2016.