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SHORT COMMUNICATION

Structural and Elemental Analysis of *Waraka* and *Wala* Jackfruit Seed Flour Samples by SEM-EDX Method

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ABSTRACT

Diversification of jackfruit seed flour depends on its phenotypic and organoleptic characteristics. Seed flour is of good source of starch, dietary fiber, and minerals. Scanning electron microscopy-energy dispersive X-ray spectroscopy analysis (SEM-EDX) provides a quick non-destructive determination of the elemental composition of the sample readily identifying some elements present in the biological materials. Therefore, the objectives of this study were to determine the structural morphological characteristics and mineral elements present in the seed flour samples of two Jackfruit varieties, *waraka* and *wala*. Jackfruit seeds flour samples of *waraka* and *wala* were prepared by using de-coted dried seeds. The SEM-EDX method was used to identify the element profile within the ultra-structure. SEM observation of the two jackfruit seed samples showed different structure morphology. Bell-shape starch granules were observed in *waraka* seed flour. Spherical shape starch granules were observed in *wala* seed flour. The mean starch granule size of the *waraka* and *wala* seed flour were 8.00±0.78 µm and 8.93±0.13 µm, respectively. *Wala* flour showed comparatively higher macro-element content than *waraka* flour. The macro elements present in *wala* were 47% oxygen, 41% carbon, 8% nitrogen, 2% sodium and 0.7% potassium. *Waraka* seed flour contained 53% of oxygen, 41% of carbon, and 0.5% of potassium. Study confirmed that structural morphology and element composition of seed flour are different between two jackfruit varieties, *waraka* and *wala*.

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INTRODUCTION

Jackfruit (*Aartocarpus heterophyllus* Lam), also known as 'Kos' in Sri Lanka, is a non-seasonal fruit-bearing tree. Seeds of Jackfruit are reorganized as bio-waste (Hossain, 2014). According to Hettiaratchi et al. (2011), jackfruit is rich in digestible carbohydrates (10% in the bulb and 22% in the seeds). Jackfruit contains minerals, polyphenols, carotenoids, and flavonoids (Nair et al., 2017; Swathi et al., 2019). Jackfruit is also widely known for its medicinal properties such as antimicrobial, anti-diabetic, anti-inflammatory, and antioxidant characteristics (Waghmare et al. 2019). Jackfruit seed has different sources of carbohydrates which result in a low glycaemic index in seed meal (Hettiarachchi et al., 2011). Jackfruit seed flour can be employed as alternative flour in baking and confectionary items by combining it with other flours (Hossain, 2014). However, it is known as an underutilized starch source. Lack of understanding on the flow characteristic behaviour of jackfruit seed flour and the differences in properties as affected by the jackfruit varieties (e.g. between *waraka* and *wala*) are limiting the use of jackfruit seed flour in food industry.

The rheological behaviour of starch depends on the characteristics of starch granules. Previous studies have shown that the mean granule size of the jackfruit seed starch granule size laid between 5 to 10μm (Kittipongpatana and Kittipongpatana, 2011). The bell-shaped structure of the jackfruit seed starch granules (SG) was observed using scanning electron microscopic (SEM) images previously (Kittipongpatana and Kittipongpatana, 2011). According to Maurya and Mogra (2016), jackfruit seed is a rich source of several minerals, including N, P, K, Ca, Mg, S, Zn, and Cu etc. Most of the minerals are rich in seed kernel than in the pulp of jackfruits.

The SEM coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) is a newly introduced method for macro and microelement analysis of biological samples. There are some traditional ways for element analysis such as atomic absorption spectroscopy, and ion chromatography, which are more complicated and time-consuming methods. The SEM-EDS facilitates rapid detection of the elements present in a sample using a non-destructive method. It provides a scale pattern graph for the element presence in the sample. Therefore, it can be used to differentiate the samples that belong to the same species or variety (Reimer, 2000). X-ray productions with EDX coupled with SEM has become a solution for detecting the changes in the element concentration of biological samples within the system (Terzano et al., 2019). The SEM-EDS method is developed based on three phenomena. Firstly, the creation of low energetic electron beams for imaging the sample in the SEM. Then the generation of X-ray beams to ionize the atom and move the electrons into a higher energy level. This can cause the photons to form the excited elements as a peak in the spectrum graph at a specific energy level. The third phenomenon is the generation of Auger electrons due to the production of X-ray beams (Terzano et al., 2019).

Most of the previous studies on jackfruit are on jackfruit seed properties and value-added products. Lakmali (2021) reported the production of pasta from jackfruit variety *waraka*. There are very limited studies are on the varieties of jackfruits such as *wala* and *waraka* and their respective properties in relation to food industry. There is no proven evidence of structural and element analysis of seed flour of *wala* and *waraka* to date. Therefore, this study was focused on identifying the structural morphological characteristics and elements that are present in the seed flour samples of two jackfruit varieties, *waraka* and *wala*. The results obtained from this study may lead to enhancing the future usage of these two varieties in both the pharmaceutical and food industries.

METHODOLOGY

Collection and preparation of sample

Seeds of the two types of Jackfruit varieties *waraka* and *wala*, were obtained from a local market in Anuradhapura. Each seed sample weighing about 1.5 kg was thoroughly cleaned, and the outer white aril was removed. Then seed samples were dried in an oven for 24 h at 60 ^{o}C followed by grinding the seed samples into flour and storing in a refrigerator (4 ^{o}C) until use for further analyses.

Scanning Electron Microscopy-Energy Dispersive Spectroscopy Analysis

The SEM-EDS analysis was performed using an electron microscope established at the Faculty of Technolog,y University of Rajarata, Sri Lanka. The seed flour samples of *Waraka* and *Wala* were examined under SEM-EDS (Zeiss Evo 18, Germany). SEM analysis was conducted by placing the sample on a sample holder followed by plasma gold–coating and then processing the image at 10 kV accelerating voltage. All the samples were used in triplicate. The image was magnified 1000 times to 5000 times throughout the scan. The starch granule (SG) size of both seed flour samples were measured by using Image J software. The average granule size for each sample was calculated using the following equation (1).

$$Average\ SG\ \ size = \frac{\sum Total\ line\ length\ of\ observed\ SG}{Number\ of\ SGs} \quad (1)$$

The SEM-EDS method provided a chromatogram of the composition of mineral elements with the atomic number, atomic percentage, and mass percentage. Apart from that, it provided the specific peak pattern for each sample with the presence of an element. It was drawn with energy region vs. voltage of the EDX. Electron excited X–ray peaks indicates the major elements in the periodic table except for H, He, and Li (Newbury and Ritchie, 2012). Major elements are observed at a concentration >0.1 mass and microelements are observed in concentrations ≤0.1-0.01 mass and the minimum detection limit is 0.001- 0.003. Starch granule size and element concentration data were analyzed using a two sample t-test ($P<0.05$) using Minitab 17 version software.

RESULTS AND DISCUSSION

SEM observation of the two jackfruit seed samples showed different structural morphology. Bell and spherical shaped SG structures were observed in flour samples during this study (Figure 1). Bell-shaped starch granules were observed in *waraka* seed flour but spherical shape starch granules were observed in *wala* seed flour. This may related to the different pasting behavior of *wala* and *waraka* starch and cooking properties. Previous findings suggested that jackfruit seed SG shape varied with round, dome-shaped, trigonal, and tetragonal granules (Dutta et al., 2011). The physical and chemical characteristics of seed flour depend on the shape and size of the SGs (Khang et al., 2020). The granule shapes of the starch derived from Jackfruit seeds was similar in morphology to the granules in starch from tapioca (Khang et al., 2020). Tapioca SGs are smooth in texture and a spheroid or half-oval SG structure. Despite the shape of SG, average size of tapioca SG was approximately 1.5 fold larger than the size of Jackfruit seed SG, which was in the range of 7-9 µm (Khang et al., 2020). Therefore, Jackfruit seed starches can be blended with tapioca starch in value-added product processing and also get the different cooking characteristics.

Waraka and *wala* seed starch average granule sizes were in the range of 7.81±1.0 µm and 8.78±0.54 µm ($P>0.05$) respectively. Previous findings recorded that SG sizes are different with the variety of the Jackfruit (Tongdang, 2008). However, previous studies have noted that Jackfruit seed SG size was in the range of 6-12 µm (Dutta et al., 2011; Kittipongpatana and Kittipongpatana, 2011). This study also indicated that the sizes of SG of Jackfruit seeds were comparable to the previous finding (Dutta et al., 2011). Types of starch granules can be classified according to their size. The SG size between 5-25 µm is classified as B-type SG. Therefore, SG of *Waraka* and *wala* seeds belongs to the B-type SG (Cornejo-Ramírez et al., 2018). Previous studies have showed that the B-type SG has low amylose content and higher starch crystallization behavior. Therefore, *wala* and *waraka* seed starch may also contain low amylose starch with higher capacity of starch crystallization behavior and may have lesser digestibility (Planchot et al., 1997). The results of the element analysis of this study are shown in Table (1) with the weight percentage of each element.

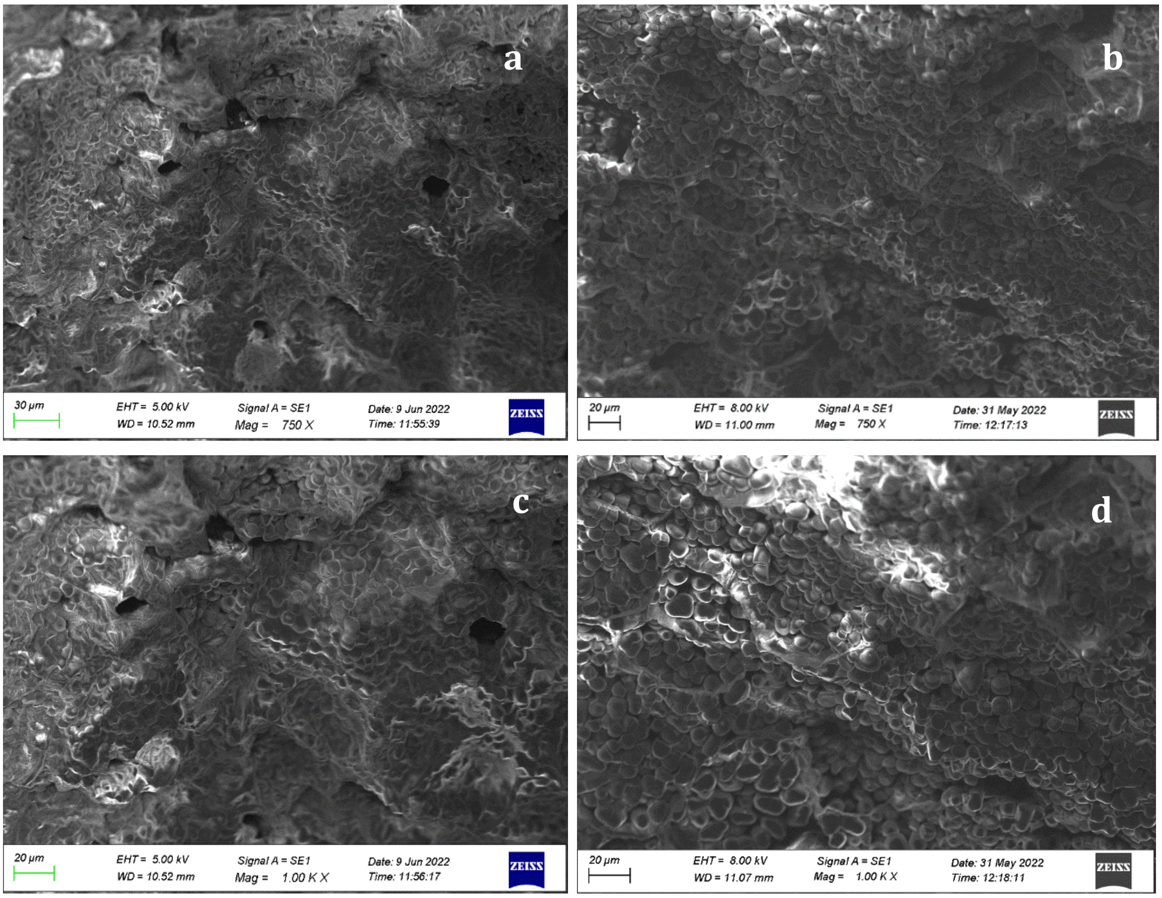

Figure 1. Starch granule structure of two jackfruit types; cultivars *wala* seed starch granule structure *(a and c)* and, *waraka* seed starch granule structure (b and d)

Table 1. Element analysis of *wala* and *waraka type Jackfruit* seed flour

Element	Atomic number	*Wala* seed flour (g/100 g)	*Waraka* seed flour (g/100 g)
Carbon	6	49.94±0.06a	46.32±1.40b
Oxygen	8	47.28±0.65a	53.15±1.55b
Nitrogen	7	8.75±0.13	-
Sodium	11	2.03±0.14	-
Potassium	19	0.73±0.68a	0.52±0.10a

Elemental analysis indicated that significantly higher amounts of carbon and oxygen ($P<0.05$) contained in the *wala* seed flour sample compared to the *waraka* seed flour sample. However, considerable amount of nitrogen was detected only in *wala* seed flour sample. Nitrogen and sodium elements were not detected in the *waraka* seed flour. That could be related to comparatively low concentration of nitrogen and sodium in the *waraka* seed flour than *wala* seed flour and also the contentions were out of the SEM-EDS detection level. However, both flour samples contained similar amount potassium content ($P>0.05$). High percentages of carbon and oxygen were suggesting that significantly high carbohydrates contents in both seed flour samples but low nitrogen may be related to the very low protein content in the *waraka* seed flour sample. The amount of trace elements presents in the seed flour samples suggested that both flour samples contained different proportions of elements in the tissues indicating the compositional differences of the *waraka* and *wala* seeds. A previous study has conducted to the SEM-EDS analysis for the comparison of elements in commercially available rice samples (Gee et al., 2019). In another study, SEM-EDS analysis was carried out to identify the xylem cavitation susceptibility in olive trees (Sabella et al., 2019). Therefore, SEM-EDX method can be considered a reliable method for element analysis of food samples (Scimeca et al., 2018) similar to this study. This SEM-EDS diction method of element in tissue sample being widely used in biomedical research, which is closely related to the food industry (Scimeca et al., 2014; Scimeca et al., 2016; Scimeca, Giannini, et al., 2014). However these findings suggested that further studies are needed to confirm these element analysis results by SEM-EDX method.

CONCLUSIONS

Although both *wala* and *waraka* Jackfruit seed flour has a similar starch granule sizes, shape of the starch granules were in different in the *wala* and *waraka* seed flour samples. The *wala* flour contained comparatively higher macro and micro-element content. This study clearly reveals that these two cultivars of jackfruit seed flour may exhibit different starch characteristics irrespective of similar SG size. Therefore, when considering the food applications of Jackfruit seed flour or starch in the food industry, it is essential to take into account the differences between *wala* and *waraka* seed flour characteristics.

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