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Variation of reducing and total sugars starch, total phenolic contents in unripe and ripe jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*) fruits

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Abstract: The change in starch, total phenolic contents (TPC) and reducing (RS) and total reducing sugars (TRS) contents were investigated in two starch-containing fruits, namely jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*) at unripe and ripe stages. Results showed that starch content increased in jackfruit but decreased in West Indian locust from unripe to ripe stage. In jackfruit, both RS and TRS increased while in West Indian locust RS decreased but TRS did not vary significantly. On the other hand, results showed that TPC increased significantly in ripe jackfruit, while West Indian locust TPC decreased in ripe fruit. Conclusively, results demonstrated that these two fruits seem having different ripening biochemistry**.**

Keywords: Starch; Sugars; Phenolics; *Artocarpus heterophyllus; Hymenaea courbaril*

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Introduction

The ripening of fruit is a complex programmed process, controlled genetically, and this process culminates in profound changes in the fruit's traits such as colour, texture, flavour, and aroma. Due to the nutritional and economic importance of fruit species, these processes have been, and still continue to be studied extensively at both biochemical and genetic levels. Fruits with different ripening mechanisms can be divided into two groups; climacteric, in which ripening is accompanied by a peak in respiration and a concomitant burst of ethylene, and non-climacteric, in which respiration shows no dramatic change and

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ethylene production remains at a very low level [1-4].

The domesticated jackfruit tree, *Artocarpus heterophyllus*, is an important in tropical and sub-tropical regions. The tree is a major component of subsistence and small farmers' farming systems and the fruit often assumes the role of a secondary staple food as well as contributing to the livelihoods of the poor. Fruit growth and maturation normally takes 5 months after fruit set but harvesting can be done even after 4 months. In the West Indies, jackfruit ripens in June. In the tropics, the fruit ripens normally at ambient temperatures (20-35 ºC) in three to ten days depending on the stage of maturity at harvest. Starch is the principal storage biochemical compound of the fruit, and during ripening it is converted to sugars, giving a sweet and sticky pulp. The colour of the bulbs changes from pale green-light yellow to an attractive golden yellow colour and this change is accompanied by a sweet aroma characteristic. In Jamaica, the apex of jackfruit is often cut to speed ripening and improve flavour. West Indian locust, *Hymenaea courbaril*, is thought to be indigenous to the Amazon rainforest and parts of tropical Central America. Although hardly tasty, the fruit is edible, however, many people do not like it because of its smell and taste that describe one of its common names "stinking toe" [5,6]. The fruit is an indehiscent oblong pod of 8-15 x 3-5 cm, and the pericarp dull dark brown, hard, woody, of about 5 cm thick. The seeds (1 to 6) are light to dark brown, hard, flattened, obovoid to ellipsoid, 1-2 cm long, surrounded by a dry, creamy brown or greenish pulp. Pods weigh 10-50 g and the pulp accounts for less than 20% of the total weight [5,7].

With the development of analytical chemistry and technology tools, the development and maturation of fruits is being receiving further scientific scrutiny because of both the uniqueness of such processes to the biology of plants and the importance of fruits as a significant component of the human diet. Therefore, biochemical analysis of fruit development, and especially ripening of fleshy fruits, has resulted in significant gains in knowledge over recent years. Nevertheless, this knowledge is still limited due the numerous traits of fruits that are locally found, and also the discovery of the nutritional qualities of many "indigenous" fruits that are under or not utilized. Some studies have reported on the chemical variation of jackfruit during maturation [8-11]. However, no study reported on the biochemical variation of unripe and ripe jackfruit. On the other hand, no study is referenced on the chemical variation of West Indian locust during maturation, ripening or storage of the fruits except the work of Contreras-Calderón et al. [12] reporting on the phenolic content of *Hymenaea courbaril* fruit. To fill this gap, the aims of this paper were to reports on the variation of starch, total phenolic contents (TPC) and reducing (RS) and total reducing sugars (TRS) contents in two starch-containing fruits, namely jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*) at unripe and ripe stages.

Materials and Methods

Plant material: Both fruits, jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*) were obtained from the local market was harvested from the Botany Garden, Department of Life Sciences, UWI, Mona. The fruits were harvested at two different ripening stages: unripe and ripe (Figure 1). For analysis, fruit of jackfruit was peeled and the seeds discarded. The fresh pulp was diced and stored at -20°C until use. The pericarp and the seeds of West Indian locust were also discarded, and the powdery pulp was stored at -20°C until. Sugars extraction and analysis: Sugars were extracted as described previously [13]. Briefly, samples of 50 were homogenised in 50 ml of water using a blender, and the homogenate was then heated for 30 min in a boiling water bath After cooling, the

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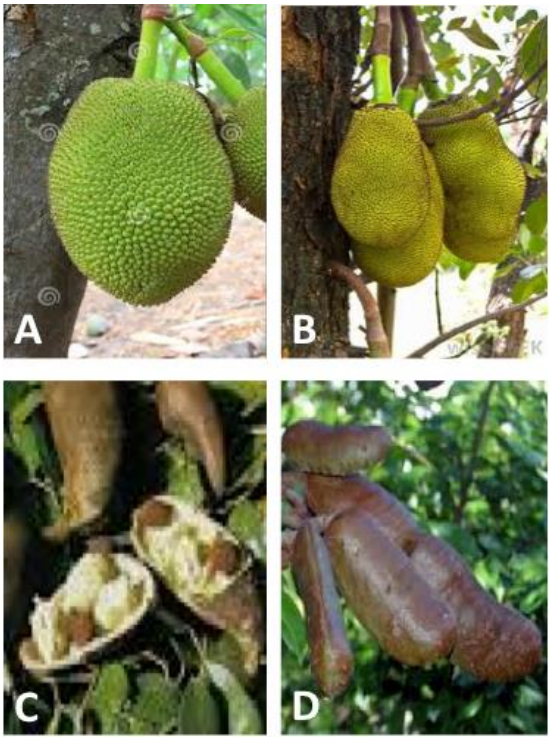
homogenate was centrifuged for 15 min at 3,000 rpm and the clear supernatant was collected and used for reducing and total reducing sugars, while the precipitate is used for starch analysis. Reducing sugars of the extracts were quantified by the method of Somogyi and Nelson [14,15]. Total sugars were quantified by the same method after acid hydrolysis of the extract with HCl (1 N).

Figure 1: Unripe (A,C) and ripe (B,D) jackfruit (*Artocarpus heterophyllus*) (Top) and West Indian locust (*Hymenaea courbaril*) (Bottom).



Starch analysis: Starch content was determined according to the method of Nielsen [16]. Briefly, the precipitate obtained from sugars extraction is dissolved in perchloric acid (0.3 g in 5.7 ml), and the volume brought to 25 ml with distilled water. A sample of 7 ml was mixed with iodate (mixture of 0.1% KI and 0.02% KIO_3) and the optical density was determined at 660 nm. A calibration curve was made with pure starch and results are expressed in mg per grad dry weight (mg/g D.W).

Total phenolics extraction and assay: Total phenolics were extracted as described by Kalt et al. [17]. Total phenolics were determined using the Folin-Ciocalteu method and gallic acid was used as standard [18]. Samples (50 g) were homogenized in 70% ethanol containing Na-metabisulfite ($Na_2S_2O_5$, 20 g/L) and ultrasound assisted extraction (UAE) using an ultra sound sonicator at room temperature. The extracts were centrifuged at 3000 rpm (round per minute) for 10 min and the supernatant collected for TPC assay. Total phenolic compounds (TPC) of extracts were quantified colorimetrically using Folin–Ciocalteu reagent and chlorogenic acid as a standard. Five millilitres of Folin–Ciocalteu (diluted ten-fold in distilled water), 2 mL of sodium bicarbonate (200 g/L) and 2 mL of distilled water were added to 1 mL of extract. After15 min incubation at room temperature, the absorbance was read at 730 nm, and results expressed in chlorogenic acid equivalents per fresh weight (mg CAE/g F.W). Statistical analysis: All the analyses were carried out in triplicate and the experimental work run in triplicate (n=9). Data were expressed as the means±SD and analysed statistically by determination of t-test (at $P \leq 0.05$) using GraphPAd Prism 4.03(GraphPad Software, Inc., 2236 La Jolla, CA, USA).

Results and Discussion

As shown in Table 1, reducing sugars (RS) contents increased significantly by two folds in ripe jackfruit, while RS decreased slightly in ripe WI locust. Reducing sugars increased by 112% but decreased by 9% in jackfruit and WI locust, respectively. Total reducing sugars (TRS) varied less significantly in ripe fruits compared to RS (Table 1). TRS decreased by 2% in ripe jackfruit, but in WI locust, TRS increased slightly by 10% in ripe WI locust. On the other hand, starch content increased in both jackfruit and WI locust, although the increase was more significant in the first fruit (Table 2).

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In ripe jackfruit, starch content increased by 2.5 folds, while in ripe WI locust, starch content increased by 32%. The ratios TRS/RS was estimated and was 2.1 and 098, and 1.65 and 2.0 in unripe and ripe jackfruit and unripe and ripe WI locust, respectively.

Table 1: Variation of reducing sugars (RS) (mg/g D.W) of unripe and ripe jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*). Different superscript letters indicate significant difference.

	Reducing Sugars (RS)		Total Reducing Sugars (TRS)	
	Unripe	Ripe	Unripe	Ripe
Jackfruit	104.40±31.19[a]	221.64±55.47[b]	221.64±55.47[a]	216.35±37.94[a]
WI Locust	124.03±20.4[a]	113.69±49.44[b]	205.77±22.18[a]	226.08±47.43[b]

Table 2: Variation of starch Content (mg/g D.W) of unripe and ripe jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*). Different superscript letters indicate significant difference.

	Unripe	Ripe
Jackfruit	142.7±32.5[a]	376.5±21.5[b]
WI Locust	215.4±84.6[a]	284.4±33.9[b]

Table 3: Variation of total phenolic Contents of unripe and ripe jackfruit (*Artocarpus heterophyllus*) and West Indian locust (*Hymenaea courbaril*). Results are expressed in µg/g F.W). Different superscript letters indicate significant difference.

	Unripe	Ripe
Jackfruit	41.91±10.07[a]	109.85±23.85[b]
WI Locust	556.48±40.39[a]	499.75±4 9.44[b]

Total phenolic contents (TPC) showed similar pattern to those of RS and TRS (Table 3). In ripe jackfruit, TPC increased significantly from 41.91 to 109.85 µg g^{-1} fresh weight, while in ripe WI locust, TPC decreased from 556.40 to 499.75 µg g^{-1} dry weight. This weak variation of TPC in WI locust could be due to the fact the fruit is dry and the variation observed would be significantly based on the dry weight. Ong et al. [9] investigated the chemical composition changes during ripening of jackfruit and found that total sugars increased significantly during the ripening process, but they noticed that total soluble solids at the top portion of the fruit were significantly higher than the middle and bottom portions. Similar results have been reported by Azizur Rahman et al. [19] who investigated the variation of carbohydrate composition of two forms of jackfruit. The authors found that free sugars and starch increased with maturity, and glucose, fructose and sucrose were the major sugar constituents with varying proportions. For example, these authors found that starch content of the perianth samples increased from 7.8 to 50.5% of dry matter. Matior Rahman et al. [8] also reported an increase in total sugars but noticed a decrease in starch contents in soft jackfruit. Jagadeesh et al. [10] also investigated the chemical composition of bulbs from 24 different firm-type jackfruit and found a wide variation in the sugars and starch contents depending on the cultivar. No referenced data reporting the total phenolic compounds of jackfruit was found except the work of Jagtpap et al. [11] who reported varying concentrations of TPC depending on the extraction solvent used. The authors reported concentrations ranging from 180 to 460 µg g^{-1} dry weight, and ethanol was the best solvent for extracting phenolic compounds followed by water,

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methanol, and acetone. These values are close to our finding as values are estimated on fresh weight basis. Nonetheless, great variations in the TPC of fruit and fruits are reported, and this variation is due to the chemical complexity of phenolic compounds, their type and the extraction solvent used [17], the extraction method [20]. Moreover, TPC of plants are also influenced by internal (cultivar) and external (agronomic and environmental) factors [21].

Although extensive literature exists on the composition of leaves, seeds and bark of *Hymenaea courbaril*, scarce referenced work describing the physico-chemical composition of WI locust fruit is available. Dias et al. [22] assessed the nutritional composition of the fruit and the physico-chemical and bioactive properties of WI locust (*Hymenaea courbaril* L.) pulp and seed oils, and found that the main macronutrient in pulp and seed was crude fiber (51.87 and 121.45 mg 100 g^{-1}) and considerable amounts of Vitamin C (121.45 mg 100 g^{-1}). Contreras-Calderón et al. [12] reported the content of total phenolics of Colombian WI locust and found that the concentration averaged 97.2 mg 100 g^{-1} fresh weight. Recently, Veggi et al. [20] used supercritical fluid extraction (SFE) with CO_2 and co-solvents to extract and assess TPC in WI locust bark. They found that the maximum total phenolic compounds (TPC) of 335.00 mg g^{-1} dry weigh, and Mello-Peixoto et al. [23] found that TPC in WI locust seeds were 464.34 mg g^{-1} of dry extract, and these values are much higher that what is found in the fruit.

Conclusion

Conclusively, our data showed that during ripening total and reducing sugars increased more significantly in ripe jackfruit, but less in West Indian locust. Results also showed that starch content increased significantly in both jackfruit and West Indian locust, although the increase in ripe jackfruit was much higher compared to that observed in ripe WI locust. On the other hand, total phenolic contents increased much significantly in ripe jackfruit, while in WI locust the variation was not significant showing a slight decrease. Jackfruit and WI locust are well know as starchy produce, and starch content could be used as a maturity and ripening index. However, further investigation is needed to determine exactly how starch varies during ripening by considering different stages for example three or four and monitoring the starch content on a large number of samples, as well as how starch forms vary.

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