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RESEARCH ARTICLE

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Morphological Assessment of Annatto (*Bixa orellana* L.) Fruit and Seed for the Development of Mechanical Seed Separator

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ABSTRACT

Background: The development of post-harvest equipment or machinery is vital for agricultural productivity. Bixin, a natural colourant derived from annatto seeds, is still separated manually, which may result in pigment loss. The engineering properties of annatto fruits and seeds must be analyzed in this note for the development of a mechanical seed separator.

Methods: The fruits were collected from Forest College and Research Institute, TNAU, Mettupalayam, Tamil Nadu. Engineering properties such as physical, gravimetric and frictional properties, colour and bixin content were determined for two dried annatto fruit and seed varieties (A_1 and A_2). The Z-test and t-test ($p<0.05$) were used to statistically analyze the data.

Result: The morphological characteristics of annatto fruits A_1 and A_2 differed noticeably and significantly. The surface area and volume of the A_1 fruit were higher. The fruit and seed were merely spherical and conical. The lowest static friction was observed for stainless steel in both fruit and seeds. The mean bixin content of the A_1 and A_2 seeds was 1.5±0.4% and 1.84±0.57%, respectively. These engineering properties will be used to design a mechanical separator without much loss of pigment.

Key words: Annatto, Bixin, Engineering properties, Fruit, Seed.

INTRODUCTION

Annatto or lipstick tree belongs to the family Bixaceae (Kumaran *et al*., 2014) It is a perennial and tropical tree, that originated in Central and South America (Pandey *et al*., 2019). The trees are extensively distributed in parts of Africa and Asia. In India, it is widely cultivated in the forest areas of Madhya Pradesh, Orissa Andhra Pradesh, Kerala, Tamil Nadu, West Bengal, Gujarat, Maharashtra and Chhattisgarh (Math *et al*., 2016; Bindyalaxmi *et al*., 2022). The trees can reach heights up to 10 m. The flowers are pinkish-white in colour. The fruits are of different shapes such as ovoid and flattened, ellipsoid, or conical. The fruits are capsules that may contain seeds from 20 to 60 on average. (Akshatha *et al*., 2011; Vilar *et al*., 2014). The trees are cultivated for their seeds.

In general, fruiting starts after 3 to 4 years of planting. A 3-year-old tree may yield 0.5-1.0 kg of seeds annually. An average of 300-600 kg of seed may be expected to yield per hectare (Math *et al*., 2016). A gradual decline in the yield may be found after 10 years of planting. The shapes of the seed differ from pyramidal to nearly conical (Vilar *et al*., 2014). Once, the fruit gets matured and dried, the capsules split open, showing the seed out.

The seed produces yellow-red-orange pigments. The main pigments are bixin and norbixin. Bixin is a di-apocarotenoid, an oil-soluble compound, extracted from the pericarp of the seed. While norbixin is a water-soluble pigment. Both bixin and norbixin are safe, eco-friendly and approved food colorants for their antioxidant and antibacterial properties (Balakrishnan *et al*., 2021). It is widely used in the food industries, cosmetics, pharmaceutical and textile sectors (Vilar *et al*., 2014).

The bixin content of the seed may vary depending on the cultivar and the climatic conditions (Vilar *et al*., 2014). Heavy rain and dehiscence of pods are the major issues that may cause injury to the fruit and leads to the loss of seed. Due to the lack of a single cultivar in abundance, in most countries, different cultivars are planted in a single area. In India, Southern states like Andhra Pradesh, Tamil Nadu, Karnataka and Kerala are the major producers of annatto seeds. Different cultivars of annatto are produced through advanced biotechnological or plant breeding technology to meet the demand for quality bixin (Aksatha *et al*., 2011).

Post-harvest operations such as drying, separation of seed, cleaning, packaging and storage plays a vital role to

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retain the quality of the seeds. In general, the seeds are separated manually by beating the fruits. The pressure applied to the seed may cause pigment loss (Kumaran, 2014; Math *et al*., 2016). The use of power operated seed separator or decorticator may reduce the loss of pigment and minimize the time of processing. Insight from the above-mentioned content, the study aims to analyze the engineering properties of two different variety annatto (*Bixa orellana* L.) fruit. This aid to understand their morphological features that are useful to design a mechanical decorticator for annatto seed separation.

MATERIALS AND METHODS

Annatto fruits were collected from Forest College and Research Institute, TNAU, Mettupalayam, Tamil Nadu, India from November to February 2021-2022. The engineering properties such as physical, gravimetric and frictional properties were analyzed for two varieties (A_1 and A_2) of dried annatto fruit (100 nos.) and their seeds. The moisture content of the fruit and seeds was determined as per the standard method for the analysis of oil seed, IS:3579; using a hot air oven at a temperature of 105±1°C for 24 hours (Math *et al*., 2016). The basic dimensions like length (l), breadth (b) and thickness (t) of fruits and seeds were measured using a digital vernier caliper having 0.01 mm accuracy. Using the basic dimensions of the fruits and seeds, the arithmetic mean diameter (D_a), geometric mean diameter (D_g) and equivalent mean diameter (D_e) were calculated by the standard formulae stated by Mohsenin (1986). The projected area of the fruits and seeds such as long (length), intermediate (breadth) and short (thickness) dimensional axes were calculated as per the standard formulae given by Mohsenin, 1986. From the values of the projected area of length, breadth and thickness, the criteria projected area was calculated. To determine the shape of the fruits and seeds, parameters like sphericity, aspect ratio and flakiness ratio were calculated using standard formulae (Mohsenin, 1986). The surface area and volume (oblate, spheroid and ellipsoid) were also calculated by definite formulae. The pod and seed ratio was determined by measuring the mass of the seeds and pods of the fruit. The gravimetric properties such as true density, bulk density and porosity were determined using the water displacement method, mass-by-volume ratio method and the relationship between true and bulk densities of the samples, respectively (Mohsenin, 1986). In frictional properties, the angle of repose was determined by pouring the materials from a set height on a circular base to form a heap to find the angle formed. The static coefficient of friction was determined for four different base materials like stainless steel, aluminum, mild steel and rubber (Kaliniewicz *et al*., 2015). The colour of the seed was determined using a handheld tintometer which gives L*(Lightness-darkness), a*(red to green), b* (yellow-blue) and h° (0-45°, redness) values (Pathak *et al*., 2020). Bixin content was analyzed by the spectrophotometric method given by FAO (2006). The significant difference between the two varieties of fruits and seeds was statistically analyzed by Z- the test and t-test ($p<0.05$) based on the sample size.

RESULTS AND DISCUSSION

Physical properties

The mean values of the physical properties of annatto fruits and seeds (A_1 and A_2) (Fig 1) are presented in Table 1 and Table 2. The mean moisture content of fruits and seeds was 9.27±1.2% (w.b.) and 6.6±1.50% (w.b.), respectively, an optimum and safer moisture level for storage and easy removal of seed from the pod (Math *et al*., 2016). The mean mass of A_1 and A_2 fruit was 2.39±0.48 g and1.35 ±0.38 g, respectively. The mass of the A_1 fruit was 77.03% higher than the A_2 fruit. The pod ratio of A_1 and A_2 fruit was 57.09% and 43.14%, respectively. The number of seeds observed in the A_1 and A_2 fruit was around 15-20 and 20-40, respectively. This observation was on par with Vilar *et al*., (2014), Math *et al*., (2016) and Umadevi *et al*., (2020).

The mean length (38.45±4.65 mm), breadth (27.64±3.10 mm) and thickness (24.34±2.26 mm) of the A_1 fruits were found to be larger than the A_2 fruits but it was contrary in the case of the A_1 variety seeds. There was no significant difference in breadth and thickness between A_1 and A_2 seeds. The geometric mean diameter (D_g), equivalent mean diameter (D_e) and arithmetic mean diameter (D_a) of A_1 and A_2 fruit were 29.43±2.71 mm, 29.49±2.73 mm and 30.15±2.86 mm; 23.00±2.39 mm, 23.24±2.37 mm and 23.00±2.39 mm, respectively. The equivalent and arithmetic mean diameter of the fruit and seed exhibited very close resemblance in values but showed significant ($p<0.05$) difference between the variety (Pathak *et al*., 2020), this helps to design the hopper, selection of screen size and shape, provision of concave clearances between the screen and the beaters for the separation of seed from the pod based on the shape of the fruit and seed.

The sphericity of A_1 and A_2 fruits and their seeds were 76.89%, 70.7%; 80.78% and 76.50%, respectively. This shows that fruit and seed were merely spherical or oblong (Vilar *et al*.,2014; Pandey *et al*., 2019). The sphericity and aspect ratio defines the good flowability of the fruit and seed. The flakiness ratio is relative to the sphericity and aspect

Fig 1: Annatto fruit (A_1 and A_2) with seeds.

ratio; the obtained value shows that the fruits and seeds of both varieties were not flaked. These properties indicate the rolling or sliding nature of the fruit and seed. There were no significant differences in sphericity, aspect ratio and flakiness ratio between the seeds (p>0.05).

There was a significant difference in the projected area perpendicular to the length, breadth and thickness between the fruits. Since the A_1 variety fruits are large, the criteria projected area value was higher. In seed, the criteria projected area showed a significant difference based on the projected area perpendicular to the length of the seeds. These parameters help to determine the water loss during drying and forecasting of harvest time (Pathak *et al*., 2020). The surface area and volume of the A_1 fruit were 38.78% and 52% higher than the A_2 fruit. Whereas the A_2 fruit seeds showed 28.68% and 39.28% higher surface area and volume. This help to calculate the capacity of a machine, storage structures, *etc*. A_1 fruits were large and required more space than A_2 (Pathak *et al*., 2020).

Gravimetric properties

The gravimetric properties like bulk density, true density and porosity were determined to find the storage volume, resistance to the airflow, *etc* (Table 3 and Table 4). The bulk

Table 1: Physical properties of annatto fruit variety A_1 and A_2.

Physical properties		Mean	SD	Z-value	p-value
Mass (g)	A_1 fruit	2.39	0.48	1.95	0.00**
	A_2 fruit	1.35	0.38		
Length (mm)	A_1 fruit	38.45	4.65	9.35	0.00**
	A_2 fruit	32.70	4.07		
Breadth (mm)	A_1 fruit	27.64	3.10	48.71	0.00**
	A_2 fruit	22.96	2.96		
Thickness (mm)	A_1 fruit	24.34	2.26	16.79	0.00**
	A_2 fruit	16.45	2.20		
Geometric mean diameter (mm)	A_1 fruit	29.43	2.71	4.68	0.00**
	A_2 fruit	23.00	2.39		
Equivalent mean diameter (mm)	A_1 fruit	29.49	2.73	13.78	0.00**
	A_2 fruit	23.24	2.37		
Arithmetic mean diameter (mm)	A_1 fruit	30.15	2.86	12.57	0.00**
	A_2 fruit	23.00	2.39		
Sphericity (%)	A_1 fruit	76.89	4.58	9.80	0.00**
	A_2 fruit	70.71	4.36		
Aspect ratio	A_1 fruit	0.72	0.08	1.46	0.14 (NS)
	A_2 fruit	0.71	0.07		
Flakiness ratio	A_1 fruit	0.89	0.09	13.32	0.00**
	A_2 fruit	0.72	0.09		
Projected area perpendicular to the length (mm^2)	A_1 fruit	840.94	176.59	11.7	0.00**
	A_2 fruit	594.15	115.95		
Projected area perpendicular to the breadth (mm^2)	A_1 fruit	607.04	137.84	11.80	0.00**
	A_2 fruit	417.97	83.09		
Projected area perpendicular to the thickness (mm^2)	A_1 fruit	530.71	92.82	21.26	0.00**
	A_2 fruit	298.59	58.47		
	A_1 fruit	530.71	92.82		
Criteria Projected Area (mm^2)	A_1 fruit	659.56	130.47	14.52	0.00**
	A_2 fruit	436.91	82.03		
Surface area (mm^2).	A_1 fruit	2741.61	501.49	17.90	0.00**
	A_2 fruit	1678.16	324.04		
Oblate spheroid volume (m^3)	A_1 fruit	13809.78	3777.22	17.26	0.00**
	A_2 fruit	6621.66	1800.10		
Ellipsoid volume (m^3)	A_1 fruit	15810.93	5035.19	11.67	0.00**
	A_2 fruit	9254.25	2251.52		
Pod ratio (%)	A_1 fruit	57.09	6.72	9.48	0.00**
	A_2 fruit	43.14	8.07		
Seed ratio (%)	A_1 fruit	42.91	6.72	9.48	0.00**
	A_2 fruit	56.86	8.07		

*Significant at p<0.05, **Significant at p<0.01 and NS-not significant.

and true density of A_1 and A_2 fruit were 38.26±0.28 kg m^{-3}, 44.98±0.14 kg m^{-3}; 1033±10.73 kg m^{-3} and 515.00±13.2 kg m^{-3}, respectively. The porosity value of A_1 and A_2 fruit was 96.26% and 91.26%, respectively. The higher porosity was due to the expansion and splitting of dried pods (Kumaran, 2014; Pandey *et al*., 2019). The true density of the seed was higher than the pod because of the individual mass of the seeds. The mean porosity of the A_1 and A_2 seeds was 61.16±0.006% and 69.08±0.016%, respectively. All the gravimetric properties between the variety of fruits and seeds showed significant differences with $p<0.05$.

Frictional properties

The mean angle of repose of A_1 and A_2 fruit was 40.44±0.74° and 43.53±0.62°, respectively. The higher repose angle represents the higher friction between the fruits, the presence of bristles on the outer surface resulted in higher internal friction. A similar angle of repose value was observed in both varieties of seeds (37.2±0.4°). Internal friction between the seeds was minimum than in fruits. The lowest static friction (0.039, 0.121) was observed for stainless steel (Murakonda *et al*., 2022) followed by other materials like aluminium, rubber and mild steel for fruits. Stainless steel can be suggested for conveying systems for fruit with minimum friction that reduces energy losses. There was a significant difference in frictional properties between annatto fruit due to their difference in mass, shape and texture. In terms of seeds, mild steel and stainless steel showed the lowest static friction. Higher static friction was observed in materials like rubber and aluminium. Higher frictional value was observed in seeds because of their resinous pericarp (Bindyalaxmi *et al*., 2022). There was no significant difference ($p>0.05$) in the angle of repose between the seeds.

Colour and bixin content

The annatto seed colour was represented by L*, a*, b* and h° values; the mean colour values were 21.70, 18.81, 6.16 and 40.60°, respectively. The positive a* and b* value

Table 2: Physical properties of annatto seeds variety A_1 and A_2.

Physical properties		Mean	SD	t-test	p-value
Length (mm)	A_1 seed	4.14	0.37	5.08	0.00**
	A_2 seed	4.85	0.36		
Breadth (mm)	A_1 seed	2.97	0.19	2.15	0.05 (NS)
	A_2 seed	3.18	0.56		
Thickness (mm)	A_1 seed	3.79	0.30	1.15	0.29 (NS)
	A_2 seed	2.85	0.28		
Geometric mean diameter (mm)	A_1 seed	3.23	0.20	3.71	0.00**
	A_2 seed	3.70	0.35		
Equivalent mean diameter (mm)	A_1 seed	3.23	0.19	3.72	0.00**
	A_2 seed	3.70	0.35		
Arithmetic mean diameter (mm)	A_1 seed	3.27	0.20	4.41	0.00**
	A_2 seed	3.78	0.30		
Sphericity (%)	A_1 seed	80.78	5.57	1.24	0.23 (NS)
	A_2 seed	76.49	9.33		
Aspect ratio	A_1 seed	0.74	0.09	1.13	0.28 (NS)
	A_2 seed	0.69	0.13		
Flakiness ratio	A_1 seed	0.96	0.12	0.078	0.93 (NS)
	A_2 seed	0.95	0.11		
Projected area perpendicular to the length (mm^2)	A_1 seed	9.37	1.22	5.06	0.00**
	A_2 seed	12.62	1.62		
Projected area perpendicular to the breadth (mm^2)	A_1 seed	6.95	0.90	2.14	0.05 (NS)
	A_2 seed	8.85	2.66		
Projected area perpendicular to the thickness (mm^2)	A_1 seed	6.64	0.83	2.04	0.06 (NS)
	A_2 seed	8.44	2.67		
Criteria projected area (mm^2)	A_1 seed	7.65	0.86	3.05	0.01*
	A_2 seed	9.97	2.25		
Surface area (mm^2)	A_1 seed	32.81	4.02	3.50	0.00**
	A_2 seed	43.22	8.42		
Oblate spheroid volume (m^3)	A_1 seed	17.83	3.30	3.28	0.00**
	A_2 seed	27.17	8.36		
Ellipsoid volume (m^3)	A_1 seed	18.66	3.51	3.53	0.00**
	A_2 seed	28.42	7.99		

*Significant at $p<0.05$, **Significant at $p<0.01$ and NS-not significant.

Table 3: Gravimetric and frictional properties of annatto fruit variety A_1 and A_2.

Gravimetric properties		Mean	SD	t- value	p-value
Bulk density (kg m^{-3})	A_1 fruit	38.26	0.28	36.90	0.00**
	A_2 fruit	44.98	0.14		
True density (kg m^{-3})	A_1 fruit	1033.33	57.73	15.15	0.004**
	A_2 fruit	515	13.22		
Porosity (%)	A_1 fruit	0.96	0.001	28.57	0.00**
	A_2 fruit	0.91	0.002		
		Frictional properties			
Angle of repose (°)	A_1 fruit	43.53	0.62	5.77	0.004**
	A_2 fruit	40.47	0.74		
		Coefficient of static friction (μ)			
Stainless steel	A_1 fruit	0.039	0.001	272.28	0.00**
	A_2 fruit	0.121	0.001		
Mild steel	A_1 fruit	0.230	0.002	16.6	0.00**
	A_2 fruit	0.200	0.001		
Aluminium	A_1 fruit	0.116	0.002	3.20	0.04*
	A_2 fruit	0.122	0.001		
Rubber	A_1 fruit	0.118	0.001	5.55	0.00**
	A_2 fruit	0.123	0.001		

*Significant at p<0.05, **Significant at p<0.01.

Table 4: Gravimetric and frictional properties of annatto seeds variety A_1 and A_2.

Gravimetric properties		Mean	SD	t- value	p-value
Bulk density (k gm^{-3})	A_1 seed	393.14	7.33	18.44	0.00**
	A_2 seed	463.68	2.17		
True density (k gm^{-3})	A_1 seed	1011.67	2.58	66.68	0.00**
	A_2 seed	1499.67	1.57		
Porosity (%)	A_1 seed	61.11	0.006	19.44	0.00**
	A_2 seed	69.08	0.001		
		Frictional properties			
Angle of repose (°)	A_1 seed	37.25	0.42	0.08	0.9 (NS)
	A_2 seed	36.91	0.45		
		Coefficient of static friction (μ)			
Stainless steel	A_1 seed	0.40	0.001	5.95	0.00**
	A_2 seed	0.42	0.005		
Mild steel	A_1 seed	0.41	0.021	7.39	0.00**
	A_2 seed	0.39	0.003		
Aluminium	A_1 seed	0.41	0.002	18.02	0.00**
	A_2 seed	0.54	0.013		
Rubber	A_1 seed	0.47	0.004	2.06	0.11 (NS)
	A_2 seed	0.46	0.008		

*Significant at p<0.05, **Significant at p<0.01 and NS- not significant.

signifies the reddish and yellowish nature. A huge angle of less than 45° confirms that annatto falls under the red-orange colour group (Grillitsh, 2019). The primary pigment in annatto seeds are bixin and norbixin (carotenoids) a reddish-yellow colour (Cevallos *et al*., 2021). A_1 and A_2 seeds had a bixin concentration of 1.5±0.4% and 1.84±0.57%, respectively. Location, soil, environment and weather all have an impact on bixin content (Math *et al*., 2016; Umadevi *et al*., 2020).

CONCLUSION

Assessment of the engineering properties of two different annatto fruit and seed varieties showed a significant difference in morphological characteristics. The A_1 fruit was long and wide, with thick pods. The A_1 variety fruit was large while the A_2 fruit was small. A_2 variety fruit possessed a large number of seeds than A_1. The fruit and the seed were spherical or oblong and merely heart-shaped or flattened. There was a significant difference in the morphological characters between the variety. The variety showed a difference in bixin content. These parameters will help to design a mechanical decorticator to separate the seeds from the pods without much loss in the pigments.

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