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ORIGINAL PAPER

Nutritional, functional, thermal and structural characteristics of *Citrullus lanatus* and *Limonia acidissima* seed flours

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Abstract Seeds from fruits such as *Citrullus* (*C.*) *lanatus* (watermelon) and *Limonia* (*L.*) *acidissima* (wood apple) are not commonly utilized but could be suitable in numerous food formulations. It was shown that the protein content of defatted seed flours was 71.38 and 49.51 % and that these contained considerable amounts of minerals such as Na, Mn, Mg, K, Cu, Fe and Zn. The defatted *L. acidissima* seed flour was superior to *C. lanatus* in essential amino acids. The flours obtained from both seeds were also evaluated for functional properties and characterized by X-ray diffraction, differential scanning calorimeter and scanning electron microscope (SEM). Amorphous nature was observed in defatted *C. lanatus* and *L. acidissima* flours due to the low percentage of degree of crystallinity. Spherical morphologies were observed through SEM. The exothermic peak was recorded in defatted *C. lanatus* and *L. acidissima* flour.

Keywords *Citrullus lanatus* · *Limonia acidissima* · Functional property · SEM · XRD

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Introduction

Citrullus (*C.*) *lanatus* (watermelon) seeds are highly nutritive since they contain large amounts of proteins and many beneficial minerals such as magnesium, calcium, potassium, iron, phosphorous and zinc. In various parts of the world, *C. lanatus* seed extracts have been used in the treatment of diseases such as cancer, cardiovascular, hypertension and blood pressure and have also been used as a home remedy for edema and urinary tract infections [1]. According to FAOSTAT (2009), India ranked second in watermelon production amongst the Asian countries with an annual production of 4000 tons of fruit which generates 400 tons of seeds per year. Though technology exists for decorticating the seeds, only a small portion of this agricultural commodity is commercially processed and utilized. Numerous reports are available on utilization of fruit waste for development of various functional foods. An example is the use of watermelon rind in the preparation of pickle, preserve, pectin and also in the preparation of low glycemic index *thepla* [2, 3]. However, there are no reports available on utilization of the seeds from this industrially important fruit.

Limonia acidissima (wood apple) is native to and commonly found in dry plains of India and Ceylon and is used in the preparation of chutneys and for making jellies and jams [4]. It has been reported that every part of the fruit posse's medicinal properties: the flesh, leaves and stem bark of wood apples have been studied for their anti-tumor and antimicrobial activities, the pulp has anti-inflammatory, antipyretic and analgesic activities [5–7]. Wood apples have anti-diabetic and antioxidant potential since they can reduce blood glucose levels and malondialdehyde [8]. The fruit is much used in India folk medicine as a liver and cardiac tonic and when unripe, as a means of halting diarrhea and dysentery and for effective treatment for high cough, sore throat and disease of the gums. In addition to this, wood apples also have hypoglycemic, antitumor, larvicidal, antimicrobial and hepatoprotective activities [9]. Recently, it was shown that wood apples possess good antioxidant properties which can be preserved using drying technology [10, 11]. Although many beneficial effects of wood apples have been described, its use as a plant material, especially the seeds, has not been widely studied.

Seeds are unconventional sources of nutrition which could also provide functional properties after being incorporated in food products. The insertion of seeds in the human diet will be determined on their physical characteristics on their composition. Hence in the present work efforts were made to characterize the seeds of *C. lanatus* and *L. acidissima* for their nutritional (fat, protein, ash and carbohydrate, mineral analysis, amino acid analysis) and functional (WHC, OHC, emulsification capacity and stability etc.) properties as well as analyzing their structure and morphologies.

Materials and methods

Decorticated *C. lanatus* seeds and *L. acidissima* fruit were purchased from local market of Navi Mumbai, India and were stored at −20 °C until their final usage to prevent spoilage and to maintain uniform quality throughout the study. All other chemicals and reagents used in the present study were of analytical grade.

Preparation of *C. lanatus* and *L. acidissima* seed flour

L. acidissima seeds were isolated from pulp and washed under running tap water. A lab scale convective hot air dryer (Sakova Pvt. Ltd, Mumbai, India) was used for drying the seeds (60 °C). The seed flour was soaked in petroleum ether at room temperature with occasional stirring for a period of 6 h to remove the fat. The solvent was separated and extractions were carried out thrice with fresh solvent. After each extraction, the solvent was decanted. Later the fat free flour was air-dried at room temperature and ground to pass through 40 mesh sieve. All resulting flours were packed into clean airtight polyethylene bags and kept at 4 °C until utilization.

Nutritional characterization of *C. lanatus* and *L. acidissima* seed flours

Centesimal composition such as moisture, total ash, fat and protein contents of the flours were assayed by standard methods of Association of the Official Analytical Chemists [12], and the carbohydrate content was estimated by calculating the difference. The mineral content of the samples was determined using an Inductively Coupled Plasma Atomic absorption spectrophotometer (Perkin Elmer).

Amino acid composition was determined according to the method of Gratzfeld-Huesgen [13] were described as: 300 mg of defatted seed flour was dissolved in 3 ml of 6 N HCl and subjected to hydrolysis in boiling water bath for a period of 24 h. The tubes were cyclomixed for every 1 h for proper hydrolysis to take place. After 24 h of hydrolysis, the tubes were centrifuged at 3500 rpm for 15 min. The supernatant was filtered and was neutralized with 1 N NaOH. Then the filtered solution was diluted to 1:100 of the volume (1 ml diluted to 100 ml) with milli-Q water and was preceded for estimation of protein amino acids in HPLC. Instrumental analytic conditions of derivation and quantification were as described by Gratzfeld-Hüesgen

Functional characterization of defatted *C. lanatus* and *L. acidissima* seed flours

The water (WHC) and oil (OHC) absorption capacity, emulsion capacity and stability were determined as described by Akpata and Akubor [14]. Tap density and bulk density, Hausner's ratio and Carr's index were determined as described by Olayemi et al. [15]. Swelling power was determined as described by the method of Leach et al. [16]. Protein solubility (PS) was determined according to the method of Liang and Tang [17].

Thermal and morphological characterization of defatted *C. lanatus* and *L. acidissima* seed flours

Thermal properties of defatted seed flour samples were evaluated with a differential scanning calorimeter (DSC-60, Shimadzu, India). The instrument was calibrated with indium and an empty pan was used as reference. Defatted seed flour (approximately 4–6 mg) was weighed accurately into a stainless steel sample pan and then the pan was hermetically sealed and allowed to stand for at least 1 h prior to analysis. Samples were heated from 30 to 180 °C at a rate of 5 °C/min. Enthalpy, onset, peak, and conclusion temperatures were recorded.

Morphology of the powders was evaluated using a scanning electron microscope JSM-6380 after coating by gold sputtering.

X-ray diffraction measurements were performed on 1 g samples of defatted flour which were packed tightly in rectangular silicon cells. X-ray diffraction patterns were obtained with a diffractometer (Rigaku Miniflex) using monochromatic Cu-Kα radiation of 1.5406 Å. The diffractometer was operated at 30 kV, 15 mA and the spectra scanned over a diffraction angle (2θ) range of 2–40° with scanning rate of 3°/min.

Results and discussion

Nutritional characterization of *C. lanatus* and *L. acidissima* seed flours

The centesimal composition of whole and defatted *C. lanatus* and *L. acidissima* seed flours is represented in Table 1. The highest amount of protein content was found in defatted *C. lanatus* seed (71.38 %) as compared to whole watermelon seed flour (45.72 %). This could be due to the method of fat removal. Jyothi and Kaul previously found 61.29 % of protein content in defatted watermelon seed flour and 46.83 % fat in whole seed flour which was significantly different than our findings [18]. The *C. lanatus* seed, known as guna seeds in Nigeria, was shown to have 49 % fat in whole seed and the protein content was 36.58 % in whole seed and 50.93 % in defatted seeds which are closer to the values in this [19]. It was also shown that defatted *L. acidissima* seed contained 49.51 % protein, a value that has never been reported before. The variation in the protein content depends on the local growing conditions such as pH and composition of soils, climatic conditions (temperature, humidity, time of year), type of fertilizer used, etc.

Minerals play a key role in structural components of bones and teeth, regulating the activity of nerves, and maintaining acid- base balance [20]. Defatted *C. lanatus* contained good amount of minerals such as iron (15.50 mg/100 g), zinc (6.47 mg/100 g), manganese (3.38 mg/100 g), magnesium (669.34 mg/100 g), sodium (4.71 mg/100 g), potassium (448.3 mg/100 g), phosphorus (1769.1 mg/100 g) and copper (4.13) (Table 2). The highest amount of phosphorus and lowest amount of manganese were found in defatted *C. lanatus*. These results differ with those of Penuel et al. who found higher amounts of iron, sodium, manganese and lower amounts of magnesium and copper in guna seeds [19]. This difference could be due to the differences in the variety studied or environmental factors. In defatted *L. acidissima* seeds, mineral contents were as followed: iron (8.49 mg/100 g), zinc (5.76 mg/100 g), manganese (1.66 mg/100 g), magnesium (703.78 mg/100 g), sodium (9.55 mg/100 g), potassium (494.3 mg/100 g), phosphorus (1227.6 mg/100 g) and copper (7.18 mg/100 g). Based on these results and on the latest recommendations from world health organizations, both *C. lanatus* and *L. acidissima* seed flours possess very high levels of most essential minerals, hence these could be used p in the formulation of different food products in order to meet the requirements of growing population.

Nutritional qualities of proteins are based on their amino acid composition. Table 3 shows the amino acid content of defatted *C. lanatus* and *L. acidissima* seeds flours. Compared to the study by Jyothi and Kaul, the watermelon seeds evaluated in this study have significantly lower concentrations of most amino acids [18]. Yet again, this variation could be due to the weather conditions and the local environment which varies on season to season basis [21]. In *C. lanatus* seeds, the amino acid concentrations were significantly higher than those found in our *L. acidissima* seeds, and the concentrations are sufficient to able to meet the daily recommended intake if consumed in sufficient quantities. The mixture of either seed flours, or their use together with cereals would be necessary to develop nutrient-enriched foods.

Functional characterization of defatted *C. lanatus* and *L. acidissima* seed flours

*F*unctional properties have been categorized as the non-nutritive characters that food constituents play in a food system. Functionality of flour is important in the preparation, processing, storage, quality, and sensory attributes of foods. Knowledge of functional property is critical for the development of new products and the improvement of existing ones.

WHC was determined in defatted *C. lanatus* and *L. acidissima* seed powders. It was found to be 2.1 and 2.53 ml/g (Table 4) respectively. This value is lower than

Table 1 Centesimal composition of *C. lanatus* and *L. acidissima* seed flour

Composition (%)	Whole *C. lanatus* seed flour	Defatted *C.lanatus* seed flour	Whole *L. acidissima*	Defatted *L. acidissima*
Moisture	5.68 ± 0.25	4.14 ± 0.07	5.82 ± 0.12	4.62 ± 0.24
Fat	41.39 ± 2.81	7.26 ± 0.39	24.9 ± 1.81	1.53 ± 0.06
Protein	45.72 ± 3.46	71.38 ± 1.49	43.28 ± 4.65	49.51 ± 3.71
Ash	2.75 ± 0.32	3.22 ± 0.4	2.84 ± 0.04	3.29 ± 0.58
Carbohydrates	4.46	14	23.16	41.05

All the values are mean ± SD of three determinations

Table 2 Mineral content of defatted *C. lanatus* and *L. acidissima* seed flour

Parameters (mg/100 g)	Defatted *C. lanatus* seed powder	Defatted *L. acidissima* powder
Sodium	4.71 ± 0.09	9.55 ± 0.2
Potassium	448.3 ± 8.97	494.3 ± 9.87
Magnesium	669.34 ± 13.39	703.78 ± 14.08
Manganese	3.38 ± 0.07	1.66 ± 0.03
Copper	4.13 ± 0.08	7.18 ± 0.14
Iron	15.50 ± 0.31	8.49 ± 0.17
Zinc	6.47 ± 0.13	5.76 ± 0.12
Phosphorus	1769.1 ± 35.38	1227.6 ± 24.55

All the values are mean ± SD of three determinations

Table 3 Amino acid content of defatted *C. lanatus* and *L. acidissima* seed flour

Amino acids(mg/g)	Defatted *C. lanatus* seed powder	Defatted *L. acidissima* powder
Aspertic acids	25.48 ± 0.66	1.4 ± 0.05
Glutamic acid	10.80 ± 0.28	14.77 ± 0.68
Serine	8.96 ± 0.32	0.59 ± 0.02
Histidine	2.60 ± 0.09	1.24 ± 0.045
Glycine	12.96 ± 0.27	6.90 ± 0.28
Threonin	6.67 ± 0.2	1.61 ± 0.06
Arginine	10.53 ± 0.41	4.59 ± 0.18
Alanine	16.93 ± 0.66	4.99 ± 0.2
Tyrosine	6.34 ± 0.11	2.23 ± 0.08
Methionine	3.71 ± 0.07	0.69 ± 0.02
Valine	4.30 ± 0.08	1.09 ± 0.04
Phenylalanine	4.39 ± 0.08	2.87 ± 0.08
Isoleusine	4.59 ± 0.08	2.44 ± 0.06
Leucine	8.75 ± 0.32	5.19 ± 0.21
Lysine	3.57 ± 0.06	1.61 ± 0.05

All the values are mean ± SD of three determinations

Table 4 Functional properties of defatted *C. lanatus* and *L. acidissima* seed flour

Functional properties	Defatted *C. lanatus* seed powder	Defatted *L. acidissima* powder
Water holding capacity (ml/g)	2.10 ± 0.00	2.53 ± 0.12
Oil holding capacity (ml/g)	2.67 ± 0.06	2.17 ± 0.06
Tap density (g/ml)	0.39 ± 0.01	0.47 ± 0.00
Bulk density(g/ml)	0.26 ± 0.01	0.28 ± 0.01
Hausner's ratio	1.52 ± 0.04	1.67 ± 0.04
Carr's index (%)	34.17 ± 1.88	41.27 ± 2.79
Emulsifying activity (%)	47.58 ± 4.28	40.60 ± 1.13
Emulsifying stability (%) Heated at 80° for 30 min	45.22 ± 3.23	11.48 ± 0.82
Swelling power (g/g)	1.18 ± 0.01	1.23 ± 0.01

All the values are mean ± SD of three determinations

papaya, apple seeds flour and higher than guva, apricot and orange seed flour [22]. Lin and Zayas recorded WHC values for commercial protein concentrates between 1.90 and 2.20 ml/g which signifies that both watermelon and wood apple seed flours would have similar values to this product [23]. Water holding by proteins depends on several parameters, such as size, shape, amino acid hydrophilic–hydrophobic balance in the protein molecule, and compounds associated with the proteins. It also depend on physicochemical environment such as pH, ionic strength, temperature, and presence/absence of surfactants, etc. [24]. The proteins in our seed flours would thus have some regions that are responsible for the normal WHC observed.

OHC was analysed in defatted *C. lanatus* and *L. acidissima* seed powder which were determined to be 2.67 and 2.17 ml of oil per gram of powder respectively (Table 4). The capacity of the flour to absorb oil was lower to those reported for the Egyptian variety of watermelon, apricot, orange and paprika seed flour [22]. Butt and Batool reported that variations in oil absorption capacity that could be due to the variation in protein concentration, degree of interaction with water and oil and conformational characteristics [25].

Tap density (0.39, 0.47 g/ml), bulk density (0.26, 0.28 g/ml), Hausner's ratio (1.52, 1.67) and Carr's index (34.17, 41.27 %) of defatted *C. lanatus* and *L. acidissima* flour is shown in Table 4. In pharmaceuticals, Carr's index is used to determine flow ability of powder. For good flowing properties tap density and bulk density should be close which results in small carr's index. A Carr index greater than 25 is considered to be an indication of poor flow ability, and below 15, of good flow ability [26]. Therefore, defatted *C. lanatus* and *L. acidissima* seed flours showed a poor flowing ability.

Data on emulsion activity and stability of defatted *C. lanatus* and *L. acidissima* flour samples are presented in Table 4. Defatted *C. lanatus* and *L. acidissima* flour showed an emulsion activity 47.58 and 40.60 % respectively and stability were 45.22 and 11.48 % after 30 min. Akpata and Akubor reported emulsion activity 40.1 % and stability of 14.8 % for defatted orange seed flour which is comparably lower [14]. The defatted *C. lanatus* seed flour shows good stability in emulsion stability. The high emulsion activity and stability opens the door to the possible use of these flours in processed meat products like sausages and in stabilizing colloidal food systems [14].

The result of swelling power is presented in Table 4 which shows defatted *C. lanatus* seed flour has 1.18 and 1.23 for defatted *L. acidissima* seed flower which is lower than jackfruit seed flour (4.77) [27]. Swelling power is a measure of hydration capacity, because the purpose is a weight measure of swollen starch granules and their occluded water. Food eating quality is often connected with retention of water in the swollen starch granules [28]. The lower swelling power in defatted *C. lanatus* and *L. acidissima* seed flours is due to the lower amount of carbohydrates (14 and 41 %, respectively) whereas jackfruit seed contain 79.34 % carbohydrates [27].

The solubility of a protein may be the most important feature of functionality for a protein. In beverages, foams, and emulsions, a protein must be soluble to have functionality. Protein solubility of defatted *C. lanatus* and *L. acidissima* seed flour is shown in Fig. 1. The protein is more soluble at alkaline pH as compared to acidic or neutral pH. Protein solubility is complex and can be affected by many variables such as electrostatic interaction, hydrophobic interaction, and hydrogen bonding. The levels of these three major forces contributing to protein solubility by favoring protein- protein interactions or by favoring protein- solvent interaction [29]. It is important to note that in the neutral pH range, solubility of watermelon and wood apple seed flours proteins is very high (near 80 %) making them versatile for insertion in different food formulations.

Thermal and morphological properties of defatted *C. lanatus* and *L. acidissima* seed flours

DSC is utilized to measure the temperatures and heat flows associated with food products as a function of time and temperature in a controlled atmosphere. These measurements provide quantitative and qualitative information about physical and chemical changes that involve endothermic or exothermic processes, or changes in heat capacity. In thermodynamic systems, heat taken up by a system (gain) is considered positive known as endothermic, while heat given up (loss) is considered negative known as exothermic. In DSC analysis, the onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and enthalpies (ΔH) for defatted *C. lanatus* and *L. acidissima* flours were depicted in Fig. 2a, b. Defatted *C. lanatus* and *L. acidissima* seed flours show endothermic peak at (85.85, 82.09 °C), onset (61.32, 41.84 °C), endset (118.06, 125.02 °C) and ΔH (42.92, 186.01 J/g). In the cases of

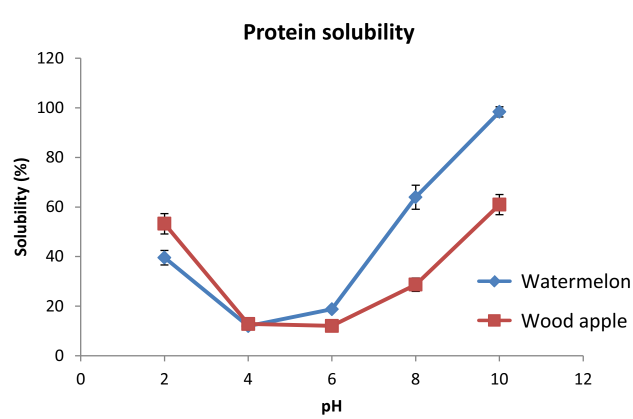

Fig. 1 pH dependant profile of defatted *C. lanatus* and *L. acidissima* seed flour

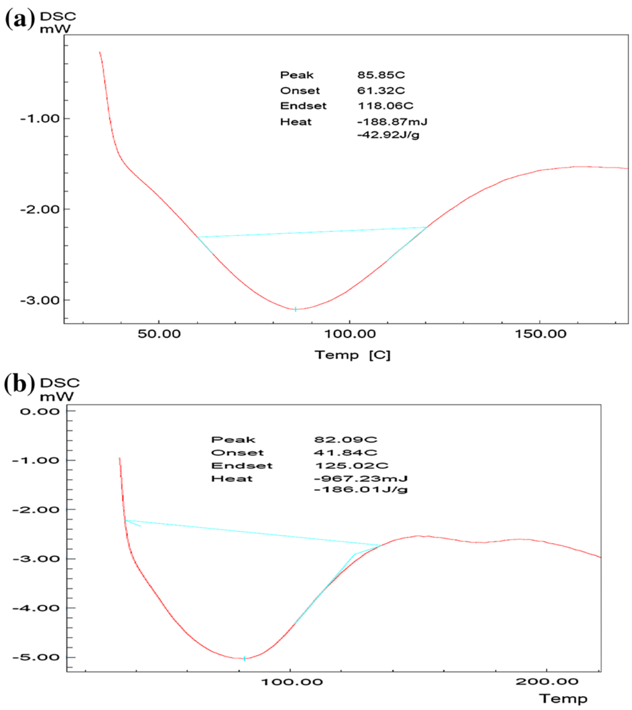

Fig. 2 **a** Differential scanning calorimetry thermograms of defatted *C. lanatus* seed flour. **b** Differential scanning calorimetry thermograms of defatted *L. acidissima* seed flour

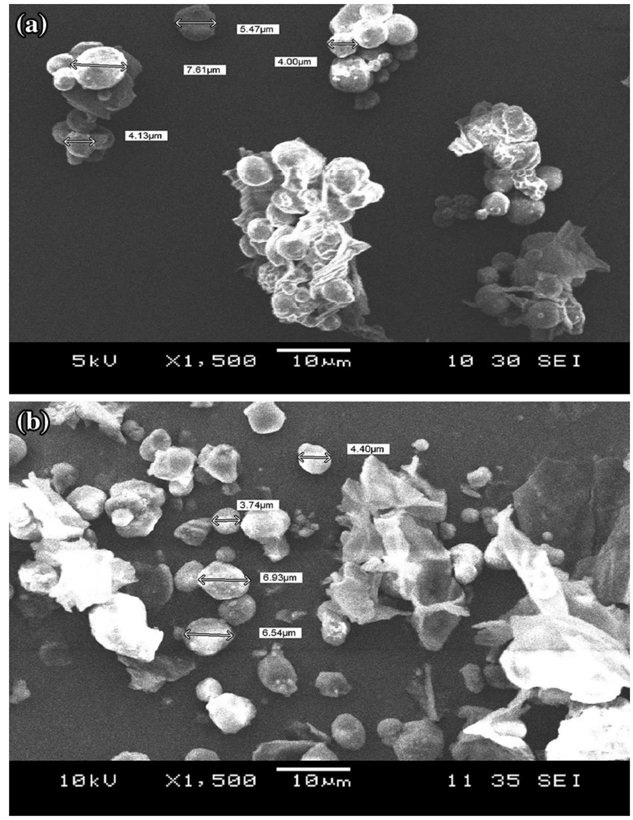

Fig. 3 **a** SEM images of defatted *C. lanatus* seed flour. **b** SEM images of defatted *L. acidissima* seed flour

sapota and papaya fruit powder exothermic peaks centered at 71.77 and 83.82 °C, whereas in guva powder endothermic peak centered at 68.64 °C [30].

SEM system was well-thought-out to be a powerful tool for determining and observing the caking occurrence on the powder surface. Measurements were achieved at magnifications of ×1500 and presented in Fig. 3a, b. It showed granules with spherical in shape and size and granule size varying from 4 to 7.61 μm in defatted *C. lanatus* and 3.74–6.93 μm in defatted *L. acidissima* flour.

Crystallization is of great significance for the stability of powdered samples which can be determined by means of X-ray diffraction analysis. Amorphous material shows diffuse and large peaks, whereas crystalline materials yield sharp and defined peaks in X-ray diffraction.

The X-ray diffraction patterns of defatted *C. lanatus* and *L. acidissima* seed flour are shown in Fig. 4. There is no sharp and define peak observed in *C. lanatus* and *L. acidissima* seed flours. The X-RD pattern of defatted *C. lanatus* flour recorded peak positioned at $2\theta = 18.26°$, 18.80°, 20.78°, 21.12°, and 21.5° which are typical of the A type of crystalline structure. There are four major types of X-ray diffraction patterns of native starches viz., A, B, C, and V [31]. In papaya, the peaks were found at 18° and 28° at 2θ angles indicating the presence of A-type starch. A peak was also recorded at 20° showing the possibility of C-type starch. In case of guava, a sharp peak at 18° was

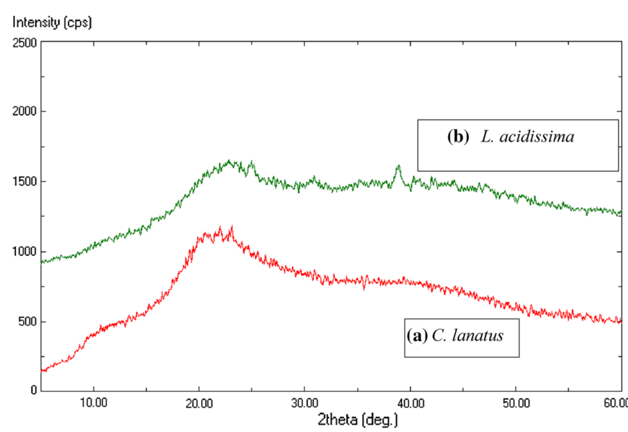

Fig. 4 X-ray diffractograms of defatted **a** *C. lanatus* and **b** *L. acidissima* seed flour

observed at 2θ angles. This shows the presence of type A starch. Other peaks were also noted at 19° and 25°. Similarly in the case of sapota, a sharp peak was observed at 15° which again stress the presence of A-type starch. The presence of A-type starch indicates that the semi-crystalline nature present in the fruit [29]. Whereas in case of defatted *L. acidissima* flour recorded peak positioned at $2\theta = 23.92°$, 35.14°, 36.70°, 38.96° and 40.38°. The jackfruit seed shows XRD diffraction peak pattern at around 15°, 17°, 23°, 31°and 38° [32]. Degree of crystallinity in defatted *C. lanatus* and *L. acidissima* seed flours were 1.72 and 2.22 % (Table 4) which is comparably lower than other fruit flours [33]. Sharon and Lis, hypothesized for the absence of sharp and define peak is that protein present in the flours have capacity to bind specifically and reversibly to monosaccharides and to form glycoproteins which prevent the organization of starch crystals and thus influence the crystallinity degree [34]. From the DSC and XRD results, it confirms that the defatted *C. lanatus* and *L. acidissima* seed flours are amorphous in nature.

Conclusion

The present study on defatted *C. lanatus* and *L. acidissima* seed flours revealed that these are good source of protein and nutrients such as minerals. This information is essential and suggests that these flours could be appropriate to be used as a matrix or included in different preparations for food fortification. This is very feasible due to the very good functional properties that were observed in these flours. Thermal stability and presence of spherical morphology and amorphous nature present in the defatted flours will be useful in the elaboration or inclusion in food systems.

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