Isolation of *Annona cherimola Miller* starch from low-quality fruits

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Abstract*– The study aimed to take advantage of Annona cherimola Miller that represents low quality and post-harvest calibre for obtained starch. For that, the freshly collected fruits were manually conditioned to obtain their pulp and later obtain isolate starch by moisture extraction technique. The native starch (ANCh) was physic chemically characterized, and total starch content was determined. The ANCh shows values of moisture and starch content of 13.15 ± 0.30% and 72.35 ± 0.51%, respectively. Respecting to starch thermal properties as water solubility index (WSI) values of 1.92 ± 0.08% to 7.06 ± 0.4%; swelling powder (SP) 8.13 ± 0.28 to 31.04 ± 0.3 g of water / g of starch; water absorption capacity (WAC) 7.97 ± 0.27 to 28.64 ± 0.16 of water / g of starch. In conclusion, Annona cherimola miller native starch could be applied in jellies, sweets, sauces, mayonnaise and sausages improving their rheological properties, as water retention capacity and solubility, especially in products that require being subjected to temperatures around 90°C.

Keywords*-- Annona cherimola Miller, low quality, post-harvest wastages, native starch.

I. INTRODUCTION

Annona cherimola Miller is a unique Annonaceae known as cherimoya. It is a fruit that inhabits the western slopes of Peru, around 7 ° 59'54.2 "S, 78 ° 40'09.1" W and 1632 m elevation [1] having as main production centres the valleys of Lima provinces: Huarochirí, Huara, Canta, Huaral, among others; although it is also produced in the Cajamarca, Apurímac and Junín regions.

Rape *Annona cherimola Miller* pulp is characterized by carbohydrate content above 20%, where the predomination of fructose (4.45%), glucose (11.75 %) and saccharose (9.4 %). Respecting to protein and fat content is low, for that is demanded its energy, vitamins and minerals [2] [3] [4]. *Annona cherimola Miller* also has been studied for its nutraceutical properties as phenolic content (28.50± 1.92 to 174.90 ± 11 mg GAE per 100 g of fresh weight), which is related to biological actions as antioxidant, anti-inflammatory, antidiabetic, antihypertensive, among others. Their proanthocyanidins content (28.54 ± 7.98 mg PAC-A equivalent per 100 g de fresh weight) is related to positive effects on gastrointestinal diseases treatment [5].

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The production of cherimoya in the country reaches 20 thousand tons per year, but only 2% of the national production is exported [6]; perhaps because it is a delicate fruit and sensitive to handling, requiring both harvesting and transport carefully. [7] [8]. Furthermore, because it is a climacteric fruit, it reaches maturity quickly which, makes the logistics processes for its conservation and trade more expensive due to the cold chain requirements [9]. These factors, added to the commercial quality criteria, can generate post-harvest loss or discard around 60% of the national production [10].

Several botanic species produced has been studied in Peru for starch extraction, like potato, corn, oca, mashua, ulluco and lucuma. [11]. Even agro-industrial residues, such as lucuma seeds, can be significant starch sources [12].

Starch is a structural biopolymer produced and stored for different plant species. According to their properties, the species could be used in papermaking as adhesive and as a structural component to modify the texture and consistency of products in the food, cosmetic and pharmaceutical industry. In the last years, starch has been using in the elaboration of biodegradable and compostable plastic.

Starch has been a compound for amylose and amylopectin polysaccharides. Amylose is composed of a linear chain of glycosidic units between 200 and 500 units. While amylopectin is composed of a ramified structure which bound by α (1-6) bond of every ten linear units of glucose [13].

Starch properties and their applications depend on their composition, grain form, size and source [14] [15]. For that, is necessary to study new native starch with specific properties sources for specific uses in the industry, weighing those low-cost sources or agro-industrial waste that can harm the environment.

The study aimed to extract and value some physicochemical and functional *Annona cherimola Miller* starch properties to evaluate their potential industrial application, improving the fruit value chain.

II. METHODOLOGY

2.1 Plant material

Annona cherimola Miller cv. Cumbe were collected from Collo productive community, Arahuay district, Canta province (Lima, Peru), on the harvest period of June- August. The next day of harvest, the fruit was

transfer to the Industrial Process Laboratory of Pontifical Catholic University of Peru (Lima) to process.

2.2 Biometric and physicochemical evaluation of chirimoya fruit.

The selection of low-quality fruit was established by visual inspection and calibre determine by unitary mass of fruits following the scale from table 1. Those post-harvest discard fruits from II and III categories (according to table 1, codes from 6 to 10) or those, which not classified were considered on the study calibre. They show shape, development and colour defects, and epidermis defects cause by friction and stipple (above 10% of the surface), but no pulp affection.

Tabla I. Classification scale for the calibration of Cherimoya

Code/ Caliber	*Range of mass in grams*	*Commercial category*		
		Extra	*I*	*II y III*
0	*851 and more*			
1	*701 to 850*			
2	*551 to 700*			
3	*401 to 550*			
4	*301 to 400*			
5	*228 to 300*			
6	*176 to 225*			
7	*126 to 175*			
8	*96 to 125*			
9	*71 to 95*			
10	*50 to 70*			

Source: Infoagro [16]

Then, *Annona cherimola Miller* fruits were subjected to non-destructive physical tests to parameterize their biometric, measuring their height and diameter (in cm) with a digital Vernier (Figure 1). Additionally, the firmness was measured by an analogue penetrometer (Agriculture Solutions LLC, GY-03, USA), which has a scale from 1 to 24 kg/ cm2 and an 8mm lead size.

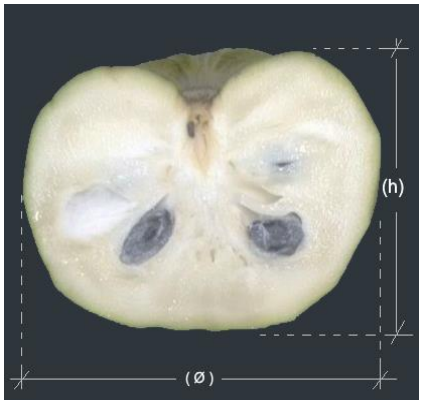

Fig. 1 Annona cherimola Miller cv. Cumbe longitudinal section

Regarding the destructive tests, the manual separation of the peel, pulp and seeds was carried out to establish their yields. The following analyzes were carried out on the pulp obtained:

Soluble solids measurement (°Brix): using the refractometer method with a digital refractometer (Kruss, DR201-95, GERMANY) [16]

pH measurement: through a potentiometer method [16] using a Crison potentiometer (Basic 20, Spain) previously calibrated with buffer 4 and 7.

Acidity determination expressed in sulfuric acid: through titration method [16], using a NaOH (0.1 N) solution as a titrant and phenolphthalein as an indicator.

Water activity measurement: Through direct measurement, using a water activity analyzer, aqua Novasina (LabSwift-aw, Spain).

Moisture determination: Through indirect gravimetric method by desiccation [16], using a Memmert oven (UN30, Germany) at 130°C per 3 hours or until constant weight.

Ash determination: by incineration gravimetric method [16], using a Thermconcept muffle (KLE 05/11, Germany) at 550 °C per no less than 2 hours or until evidence of white or grey ashes.

2.3 Isolation of native starch.

The fruit was conditioned (wash, disinfected, peeled and extracted the seed) and the pulp was cut into cubes of 3 ± 0.5 cm of the side for *Annona cherimola Miller* native starch (ANCh) extraction. Immediately, the fruit immersed in a sodium sulfite solution (0,1%, w/v); then was mashed for 2 minutes in a food processer (BLSTBC4129-053, Oster®, Mexico), and let rest for 6 hours. Through a 100 mesh (150 µm) nylon fabric, the suspension was filtered for separated residue (fiber) from the liquid substance (protein and starch). The residue was subjected to successive washing with distilled water until obtaining clear water to extract the maximum starch content. The suspension (protein and starch) was filtered through nylon fabric 200 mesh (75 µm) and the liquid fraction was let to precipitate at 5 ± 1 °C for 2 hours, then that supernatant was discarded. The precipitated content onto starch was washed 3 times by resuspension in distilled water and the starch was recovering until the last wash by centrifugation at 3500 rpm for 10 minutes. After the last extraction, starch was dried in a convective force oven (Memmert, Germany) at 50±5 °C until constant weight. Later, it was grounded in a hammer mill (national origin) whit a 0, 5 mm sieve size. Then, it was sieved through a mesh 100 (150 µm) (Retsch, ASTM E11, GERMANY). Finally, starch was stored in Nylon-Polyethylene bags until the analysis.

2.4 Evaluation of Annona cherimola Miller native starch physicochemical characteristics.

Annona cherimola Miller native starch (ANCh) obtained was subject to these assays:

pH measurement: through a potentiometer method [16] using a Crison potentiometer (Basic 20, Spain) previously calibrated with buffer 4 and 7.

Water activity measurement: Through direct measurement, using a water activity analyzer, agua Novasina (LabSwift-aw, Spain).

Moisture determination: Through indirect gravimetric method by desiccation [16], using a Memmert oven (UN30, Germany) at 130°C per 3 hours or until constant weight.

Ash determination: by incineration gravimetric method [16], using a Thermconcept muffle (KLE 05/11, Germany) at 550 °C per no less than 2 hours or until evidence of white or grey ashes.

Total starch content: Starch content was determinate by the total starch assay kit from Megazyme International Ireland Limited [17]. A 100mg of sample was weighed into a centrifuge tube, which was suspended with 5 ml of ethanol (80% v/v) and it was incubated at 80-85 ° C per 5 min. Then, it was centrifuged at 3000 rpm, and the supernatant was discarded. Later, a 2 mL of KOH at 2M was added and mixing for 20 minutes by constant shaking, keeping the tubes on the inverted water bath. Moreover, an 8 ml of sodium acetate buffer 1.2M (pH 3.8) was added to each tube on constant agitation; immediately, 0.1 mL of α-amylase and 0.1mL of amyloglucosidase were placed, mixed with a vortex. It was brought to incubation at 50 °C for 30 minutes. Then, it was quantitatively transferred into a fiola adjusting the volume with distilled water to100 ml. An aliquot is centrifuged at 3000 rpm per 10 min. Subsequently, a 0.1 ml aliquot is transferred to test tubes, adding 3.0 mL of GOPOD reagent to each tube (including D-Glucose controls and blank reagent), incubating the tubes at 50 ° C for 20 min. Finally, the absorbance was read at 510 nm for each sample, while the D-glucose control read is against the blank reagent.

2.5 Evaluation of Annona cherimola Miller native starch functional properties.

The measurement of water solubility index (WSI) (1), swelling power (SW) (2) and water absorption capacity (WAC) (3) was carried out by modifying the method purpose by Anderson R. C et al., (1969) [18]. A 25 mL of a 2% (w / v) starch suspension was used in a centrifuge tube immersed in a thermostatic water bath, maintaining the temperature at 60, 70, 80 and 90 ° C for 30 min with constant stirring. Subsequently, it was cooled at room temperature and centrifuged at 3500 rpm for 15 min. The supernatant was decanted and carefully, transferred to a previously weighed crucible, for drying in a Memmert oven at 120 ° C until constant weight. The paste (swollen granules) was weighed and used for the corresponding calculations:

$$WSI = \frac{soluble\ material\ weight\ (g)}{starch\ weight\ (g)} \times 100\ . (1)$$

$$SP = \frac{pasta\ weight\ (g)}{starch\ weight\ (g) - soluble\ material\ weight\ (g)}\ . (2)$$

$$WAC = \frac{pasta\ weight\ (g)}{starch\ weight\ (g)}\ . (3)$$

2.6 Statistical analysis.

All the measurement and analysis was realized by triplicate. The results were expressed as mean and standard deviations, for which MS Excel was used.

III. Results and discussions

3.1 *Biometry and physicochemical properties of Annona cherimola Miller fruit.*

From the sample provided by the farmers from Collo-Canta, the fruits were classified according to the criteria indicated in Table I. It was found that the fruits considered post-harvest losses presented little uniformity, damage to the epidermis due to common diseases, bird bites or sun exposure, and of a non-commercial size (Fig. 2). The collected samples were found in sizes 6, 8 and 10, with weights of 61, 107 and 206 g / fruit, respectively. For these sizes, an average height of the fruit of 4.7, 5.5 and 6.7 cm was found, and an average diameter of the fruit of 4.8, 5.9 and 8.1 cm, respectively (Table II). The hardness or texture of all the fruits is greater than 24 Kg/cm.

Table II. Biometric analysis of Annona cherimola Miller classified as postharvest loss

Parameter	Caliber / Code		
	6	8	10
Weight (g)	206.76 ± 11.04	107.83 ± 8.31	61.06 ± 7.27
Height (cm)	6.71 ± 0.48	5.53 ± 0.62	4.78 ± 0.63
Ø (cm)	8.08 ± 0.66	5.92 ± 0.62	4.83 ± 0.49
Texture (Kg/cm2)	> 24	> 24	> 24
% Peele	22.5 ± 0.82	12.9 ± 2.11	14.72 ± 2.48
% Seeds	6.83 ± 1.07	4.08 ± 1.29	3.75 ± 1.39
% Pulp	70.67 ± 1.27	83. 02 ± 3.17	81.53 ± 3.70
N° Seeds	17 ± 1	6 ± 1	3 ± 1

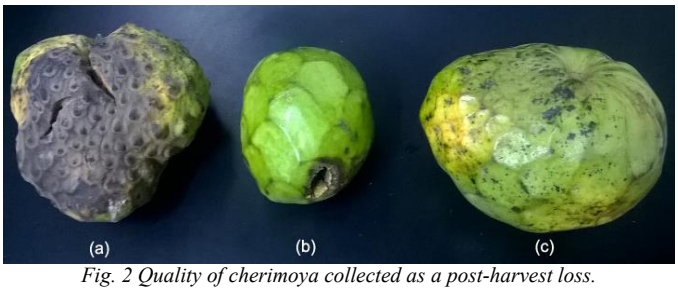

Fig. 2 Quality of cherimoya collected as a post-harvest loss.
(a) Discarded fruit due to severe damage to the epidermis, (b) usable, undeveloped and heterogeneous fruit, and (c) usable fruit with epidermis slight damage.

Regarding the pulp yield of the collected fruits, a yield of over 70% was obtained in all sizes, being higher in sizes 8 and 10, where the peel yield was 12.9 and 14.7%, respectively; compared to what was found for calibre 6, which presented a peel yield of 22.5%

From the analysis of its physicochemical attributes of the pulp, a ° Brix of 6.30 ± 2.11 was found, pH of 6.47 ± 0.35, Acidity (expressed in malic acid) of 0.15 ± 0.02%, aw of 0.96 ± 0.01, the humidity of 71.52 ± 2.12 % and an ash content of 0.65 ± 0.12%. Similar results was obtained in other study in terms of moisture and ash with 71.5-78.3% and 0.372-0.556% respectively [19].

3.2 *Physicochemical characteristics of Annona cherimola Miller starch.*

From the native starch isolation process, a yield of 10.83 ± 1.02% was obtained, about the pulp that starts the process. Table III shows the results of the physicochemical analysis of native *Annona cherimola Miller* starch (ANCh). The moisture content obtained was 13.15%, a value is within the humidity range of 4 to 16.96% reported for other native starches [20] [21] [15] [22] [23] [24]. This is favourable since at lower humidity, better storage is achieve and the possibility of microbial growth, mainly moulds, decreases, prolonging its useful life. In this line, it is reinforce by the water activity value (aw) obtained of 0.52. At that level of water activity, starch behaves as intermediate moisture food; therefore, it is difficult to susceptible to microbial alterations [25] [26]

Table III. Physicochemical composition of Annona cherimola Miller starch

Attribute	X ± SD
pH	6.39 ± 0.23[a]
a_w	0.520 ± 0.049[a]
Moisture (%)	13.15 ± 0.30[a]
Ashes (%)	0.045 ± 0.004[a]
Starch (%)	72.35 ± 0.51[a]

The purity of the ANCh represented 72.35% of total starch content, a value higher than that reported for two varieties of banana: *Musa cavendishii* with 73.42% and *Musa paradisíaca* with 68.13% [27]. In comparison with *Annona squamosa* (25.6%) and *Annona muricata* (27.3%), ANCh has a higher value [28].

Regarding the ashes of the ANCh, Melian (2010) [27] indicates that the ash content present in the starches represents the number of minerals and remaining salts that resulted from the extraction and the mineral content of the raw material of origin. For the ANCh sample, a value of 0.045% was found, which was lower with different sources of native starch such as Sago (*Metroxylon sagu*), square banana (*Musa balbisiana*), Sweetsop (*Annona squamosal)*, and soursop (*Annona muricata)* with values of 0.16% from 2.30% [28] [29] [30]. Low ash content can also mean high purity of native starch [31] [32]. On the other hand, a high ash content may be due not only to the starch extraction method or to the source of origin. In addition, the level of maturity of the raw material and the phosphorus content present in its starch as in tubers [33].

Regarding the pH of the ANCh, it presented a value of 6.39, which is within the pH range of 6.18-7.8, values reported for yam starches, Ramphal fruit (*Annona reticulata Linn.* (*Annonaceae*)); oca (*Oxalis tuberosa mul*), ulluco (*Ulucus tuberosus loz*), mashua (*Tropaelum tuberosum*) and velvet grain (*Mucuna pruriens*) [21] [34] [22] [35]. The native starches pH expected to be close to neutral. If it is above pH 7, it may indicate that the starch was exposed to a NaOH purification process. That process is a common practice to increase the purity or starch content in the product.

3.3 Functional properties of native Annona cherimola Miller starch.

According to Table IV, swelling power, water absorption capacity and solubility index of the ANCh increase with the increase in the temperature of the starch solution.

The solubility index increases from 1.92% to 7.06% when the ANCh solution increased from 60 ° C to 90 ° (Fig. 3). This maximum value is similar to that found in the starch of Zaragosa beans (*Phaseolus lunatus L.*) and that of green bananas (*Musa sp. Square variety*) [6] [36]. These variations can be explained by the structural differences in the granules of different starch sources and their different amounts of amylose are released from the interior of the starch granule when the latter begins to lose its structure due to the absorption of water [15] [6] [23]. For Liu, et al., (2007) [37]. The increase in the solubility of starch can be attributed to the greater solubilization of its polymers from granules with weaker rigidity when they are subjected to increases in temperature. In this way, starch with a higher solubility index could be used in sauces, mayonnaise and other semi-viscous products, improving its texture; in bakery and pastry products, in beverages and jellies

as a thickener; in ice cream as a stabilizer and sausages and sausages as a binder [36] [38]

Table IV. Water solubility index (WSI), water absorption capacity (WAC) and swelling power (SP) of native Annona cherimola Miller starch.

T (°C)	WSI (%)	SP (g of water / g of starch)	WAC (g of water / g of starch)
60	1.92 ± 0.08[a]	8.13 ± 0.28[a]	7.97 ± 0.27[a]
70	2.73 ± 0.04[b]	11.71 ± 0.16[b]	11.39 ± 0.15[b]
80	4.68 ± 0.09[c]	16.77 ± 0.06[c]	15.99 ± 0.04[c]
90	7.06 ± 0.45[d]	31.04 ± 0.37[d]	28.64 ± 0.16[d]

* Average of at least 3 repetitions. Values of the same property that share the same letter are not statistically significant ($p<0.05$).

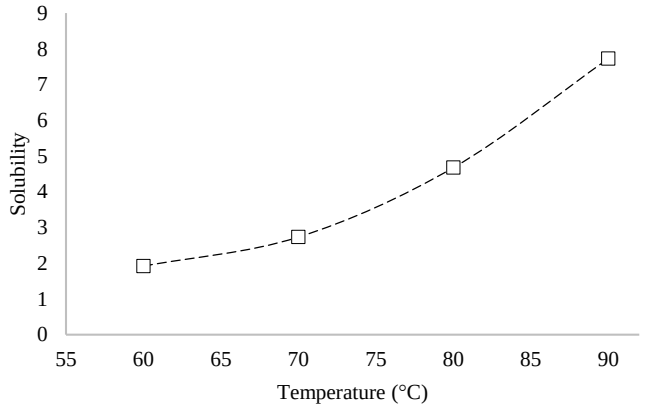

Fig. 3 Solubility index of native Annona cherimola Miller starch.

The swelling power (SP) of ANCh increases with increasing temperature since at high temperatures, the binding forces within the granule are progressively attenuated, which implies that the swelling power increases [22] [39]. The SP of the ANCh was 8.13 g of water / g of starch at a temperature of 60ºC and 28.64 g of water / g of starch when the solution reached 90 ° C (Fig. 4). However, in contrast to other sources of native starch as pallar (*Phaseolus lunatus L.*), the swelling power increases between 70 ° and 80 °C, showing a decrease at temperatures above 80 °C [6]. For starches from other Annonaceae such as sugar apple (*Annona squamosa*) and guanabana (*Annona muricata*), SW values higher than those obtained for ANCh are observed, at temperatures of 80 ° C, 42.37 g of water / g of starch and 27.40 g of water / g of starch respectively [30]. While for native tuber starches such as oca, ulluco and mashua, the swelling power increases slightly between temperatures of 60 °C and 90 °C [22]. The differences between the reports and the studies may be due to the availability and quantity of amylose within the starch granules. Likewise, the variation in the swelling power within the same species may be due to the phosphorus content and the state of maturity of these [31] [40]. In this way, starches with higher SP can be used in meat products, bakery products, dressings, jellies, candies, that is, products that require water retention. [21] [41] [15] [6]

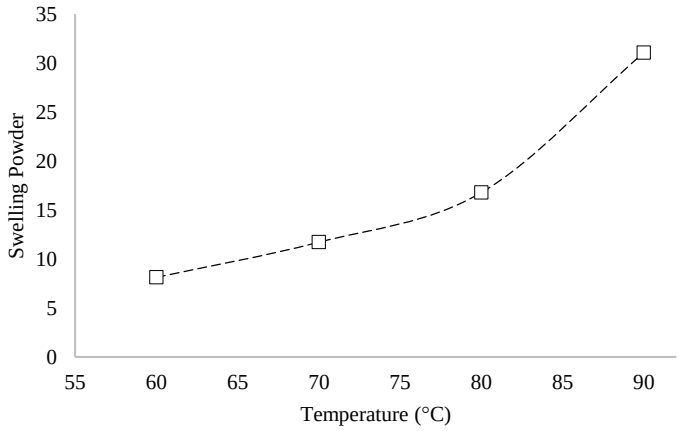

Fig. 4 Swelling power of native Annona cherimola Miller starch.

The water absorption capacity (WAC) of ANCh increases with increasing temperature because the starch granules hydrate and undergo granule swelling. The maximum WAC value reached was 23.618 g water / g of starch at a temperature of 90 °C, close to 25.0 g water / g of starch from the avocado seed [43]. This difference may be due to the size of the starch granule and botanical sources, from which its composition, morphology, molecular structure, amylose and amylopectin content vary. [37] [44]

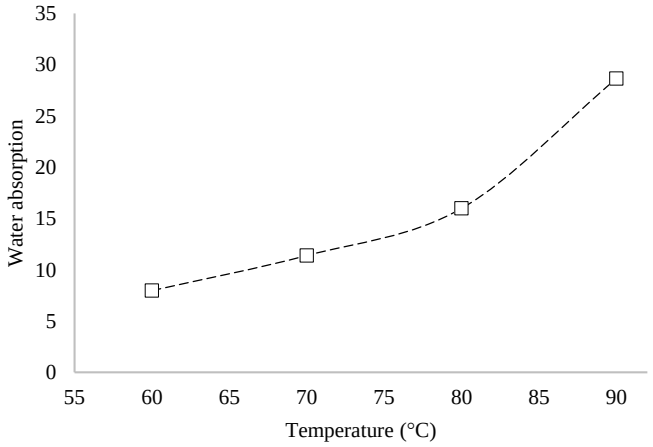

Fig. 5 Water absorption capacity of native Annona cherimola Miller starch.

V. CONCLUSIONS

The post-harvest low-quality cherimoya fruits allowed the extraction of native starch, presented an extraction yield of 10% concerning the pulp and a purity of 72%. The native *Annona cherimola Miller* starch obtained its maximum values of solubility index, swelling power and water absorption capacity when the solution reached 90 °C. For that, native starch could improve rheological properties as water retention capacity and solubility, especially in products that require being subjected to temperatures around 90°C.

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