Physicochemical and morphological characterization of potato starch (*Solanum tuberosum* L.) as raw material for the purpose of obtaining bioethanol

Caracterización fisicoquímica y morfológica del almidón de papa (*Solanum tuberosum* L.) como materia prima con propósito de obtención de bioetanol

Sonia Patricia Lizarazo H.[1], Germán Gonzalo Hurtado R.[2], and Luis Felipe Rodríguez C.[3]

ABSTRACT

In Colombia, there are geographic areas where the potato crop is the principal economic product. The diversity of potato varieties, has resulted in differences in sizes and in chemical and physical compositions. These variables are defined by genetic factors, agricultural practices, and climatic and soil conditions. The physical characteristics of the different potato varieties are directly related to aspects of production and performance, unlike the chemical composition and morphology of starch granules, which define the nutritional quality and industrial use. In this research, an analysis of the physicochemical and morphological properties of native starches from six potato varieties was carried out, forming a pilot study for the selection of promising varieties for ethanol production. For this purpose, the samples were dried to constant weight. The ash, amylose and amylopectin contents showed significant differences between the varieties. Similarly, differences were observed in the shape and size of the granules, variables that influenced the gelatinization temperature and viscosity of the final products. These variations in the physicochemical properties and morphology of the starches may affect the use of starch and in the production of ethanol.

Key words: starch granules, potato products, viscosity, morphology, amylose, amylopectin.

RESUMEN

En Colombia existen zonas geográficas donde el cultivo de papa es el principal producto de su economía. La diversidad de variedades de papa, hacen que posean diferencias en tamaño, composición química y físicas. Estas variables están definidas por el factor genético, prácticas de cultivo, condiciones climáticas y de suelo. Las características físicas de las diferentes variedades de papa están directamente relacionadas con los aspectos de producción y rendimiento, a diferencia de la composición química y la morfología de los gránulos del almidón, los cuales definen la calidad nutritiva y su uso en la industria. En esta investigación se realizó el análisis de las propiedades fisicoquímicas y morfológicas de los almidones nativos provenientes de seis variedades de papa, constituyéndose en un estudio previo para la selección de variedades promisorias para la obtención de etanol. Para lo cual, las muestras fueron secadas hasta alcanzar un peso constante. El contenido de cenizas, amilosa y amilopectina muestran diferencias significativas entre las variedades. Igualmente, se observaron diferencias en la forma y tamaño del gránulo, variables que incidieron en la temperatura de gelatinización y la viscosidad de los productos finales. Estas variaciones en las propiedades fisicoquímicas y morfología de los almidones, pueden incidir en el uso del almidón así como en la obtención de etanol.

Palabras clave: gránulos de almidón, productos de la papa, viscosidad, morfología, amilosa, amilopectina.

Introduction

The potato (*Solanum tuberosum* L.) is composed of components that provide energy to those who consume it, providing macro and micronutrients and substances such as water, cellulose, hemicellulose, pectin, glycoalkaloids, organic acids, and enzymes, among others. After a harvest, tubers contain on average 80% water and 20% dry matter (60% of which is starch (Pertuz, 2004)). The composition can be modified by factors such as variety, the area's soil type, climate and growing conditions. Diseases, pests and duration of production cycles also affect potatoes (Pertuz, 2004).

In Boyaca - Colombia, there are regions where different potato varieties are grown, characterized by their variability in size, density and chemical composition (Barrientos and Ñústez, 2014; Lizarazo *et al.*, 2015). These variables, if they are well defined by the genetic factor, also depend on agricultural practices, climatic conditions and soil type.

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[1] Grupo de Investigacion para el Desarrollo Empresarial Agroindustrial Sostenible (IDEAS), Universidad Pedagogica y Tecnologica de Colombia (UPTC). Tunja (Colombia). soniaplizarazo@gmail.com

[2] Corporacion Centro de Innovacion del Tropico Alto Sostenible (CEI3TAS), Universidad Pedagogica y Tecnologica de Colombia (UPTC). Tunja (Colombia)

[3] Department of Rural Development, Faculty of Agricultural Sciences, Universidad Nacional de Colombia. Bogota (Colombia)

The variables that affect the suitability of a product for the potato processing industry are defined by the starch content (amylose and amylopectin), which influences the quality and classification for different industrial processes, in which sugars as the most important elements, it is found in greater amounts in the flesh of the tuber (Singh *et al.*, 2005). With so many variations, it follows that a difference can be seen in the morphology of starch granules.

Starch possesses various applications: it is used for reinforcing fabrics, as an adhesive and binder, as a sizing agent (polishing, gloss and firmness) in the textile industry, paints, lacquers and varnishes and as a fermentation substrate, among others. Among the advances in research, the use of "surrogates", low calorie fat, thermoplastic and biodegradable materials has been reported. However, many of these uses, seen in the food industry and, others, depend on the properties of the colloids of two components, amylose and amylopectin, which behave as a molecular constituent determining to the size, structure and function that is intended to (Wang and White, 1994; Villacrés, 2004; Costas, 2009).

In general, native starches are used because they regulate and stabilize the texture of foodstuff and because of their thickening and gelling properties. However, when used under other conditions to produce other products, it is necessary to identify their performance against variables such as temperature, pressure and pH. It is important in determining its functionality, since it cannot infer the behavior against thermal decomposition, high retrogradation and syneresis (Amani *et al.*, 2005; Bello-Pérez *et al.*, 2009).

According to the above, the conditioning of potato starch is one of the more important phases of bioethanol production. Dry starch must be hydrated, adding hot water, in order to obtain a mixture with not more than 40% solids, in a stage called gelatinization; its purpose is to make it more suitable for the availability of sugars (Lucas *et al.*, 2013). Starch can be converted into glucose via hydrolysis. The main advantage of the enzymatic process, as compared to acid hydrolysis (Sassner *et al.*, 2006).

The enzymatic process has two consecutive phases, denominated liquefaction and saccharification, where amylases and glucoamylases and, enzymes are involved, respectively. Similarly, thermostable amylases act efficiently during the liquefaction process, producing molecules with a different number of glucose units (Castaño and Mejía, 2008). Subsequently, in the saccharification stage, glucose syrup is produced from dextrins and finally the alcoholic fermentation, with the main purpose of obtaining ethanol and carbon dioxide.

The overall objective of this study was to determine the physical, chemical and morphological characteristics of the starches from six potato varieties grown in Boyaca (Colombia), with the purpose of evaluating their viability as feedstock for the production of bioethanol under laboratory conditions.

Materials and methods

The experiment and measurements were carried out at the Biological Control Laboratory attached to the Faculty of Agricultural Sciences of the Universidad Pedagogica y Tecnologica de Colombia (UPTC) in Tunja (Boyaca, Colombia). Moreover, the morphological analysis of the starch granules with electronic scanning calorimetry and polarized light microscopy was carried out at the Universidad Nacional de Colombia (Bogota) in the laboratories of the Faculty of Sciences - Department of Chemistry.

This study used an experimental design with a bi-factorial arrangement completely random distribution. The first factor corresponded to the studied potato varieties and the second to the hydrolysis conditions for the production of bioethanol. The Laboratory tests were developed in duplicate.

We worked with six potato varieties, which came from the central province of Boyaca, Colombia. This area is characterized as being a potato production center, whose municipalities mostly have an altitude of around 2,843 m, a 12.9°C average temperature, 78% relative humidity and 700 and 2,000 rain of precipitation mm yr^{-1} (Porras and López, 2004).

Potato varieties were selected according to their productivity, agricultural supply commercial importance (Tab. 1). The tubers were chosen plots established by producers recognized that it was harvested and randomly took 2 kg of tubers for the different experimental tests.

TABLE 1. Origin of the studied potato varieties (*S. tuberosum*).

Variety	Hometown
Diacol Capiro	Ventaquemada
ICA Unica	Ventaquemada
ICA Purace	Ventaquemada
Tuquerreña	Siachoque
Parda Pastusa	Ventaquemada
Merengo o Marenga*	Siachoque

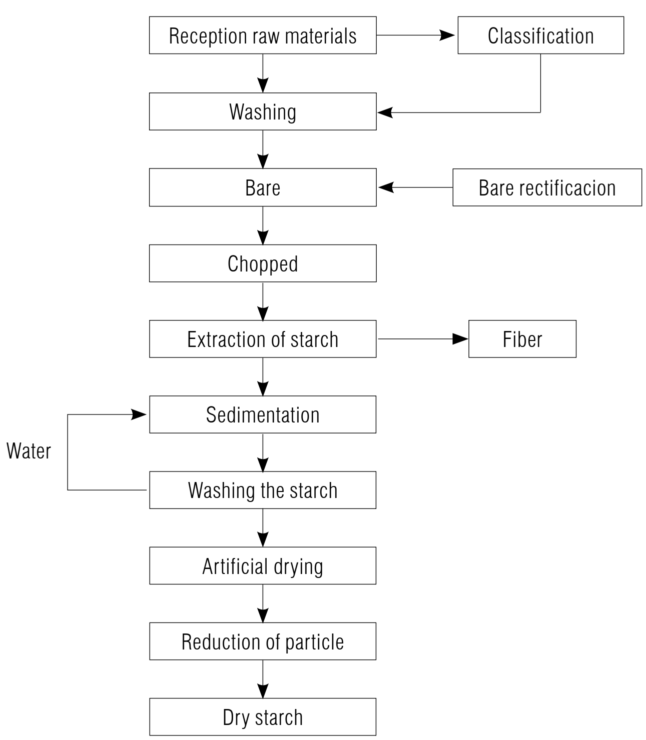
* Clone not is registered as variety (Ñústez, 2011).

Process for the extraction of native starch

The potato tubers were classified by health, cleaned and manually peeled. The starches were obtained according to the method described by Singh and Singh (2001), using 1 kg of each of the varieties, which was fractionated, and the product was placed in distilled water with sodium metabisulfite (35 g/100 L). Then we proceeded to the extraction of starch, washed several times with distilled water until the effluent was clear liquid (Singh and Singh, 2001).

The starch was allowed to settle, decanted and filtered. The resulting paste was placed in a hot air oven at 40°C for 24 h. Upon receiving the dry starch, it was passed through an attrition mill, Cemotec® 1090 sample mill (Höganäs, Sweden), to reduce the particle size, sieved using a 100 mesh and, finally, stored in sealable bags, sealable, as described in Fig. 1. The filtrate was allowed to stand and then it was decanted to recover the supernatant, the water was removed and filtered to remove the starch that may have been suspended in water. The resulting paste was spread out on trays that were placed in a hot air oven at 40 for 24 h. The obtained starch was transferred to a spray mill Cemotec® 1090 sample mill (Höganäs, Sweden), to reduce the particle size. Subsequently, the starch powder was sieved using 100 mesh to remove impurities. Finally, it was stored in zip lock bags. The operations constituting this method are seen in Fig. 1.

FIGURE 1. Flow chart of the process for producing the potato starch.



Physicochemical, compositional and morphological properties of the starches analysis

The compositional analysis of the starches from the different varieties was conducted at the Laboratory of Food Science, School of Veterinary of the UPTC using the AOAC method (Latimer, 2012).

- Ash: direct incineration (AOAC 942.05)
- Crude fiber: Weende (Based on 668 NTC)
- Fat: soxhlet extraction (Based on 668 NTC)
- Moisture and other volatile matter: thermogravimetry at 103°C (Based on ISO 6496).
- Crude protein: Kjeldahl (Based on NTC 4657)
- Carbohydrate: by difference.

The Bertrand volumetric method, which is based on precipitation separation after all reducing species different sugars (with saturated Pb $(CH_3\text{-}COO)_2$ solution or Hg $(CH_3\text{-}COO)_2$ was used for the determination of the total carbohydrates and Na_2CO_3).

Analysis of composition properties, morphological properties and gelatinization and retrogradation

The evaluation of the functional properties was carried out using the following methodological procedure:

Quantification of amylose and amylopectin. The quantification of the amylose/amylopectin potato starch was performed by the calorimetric technique. The amylose fraction had affinity to the complex with iodine. The colored complex was quantified colorimetrically.

Starch granule morphology. This test was performed using the technique of high-resolution light microscopy, when preparing the solid tablet samples to facilitate the observation.

Polarized light microscopy. A microscope with polarized light, a 40X magnification and a digital camera were used. The starch samples were sprinkled on a microscope slide, a drop of distilled water was added, the samples were mixed with a spatula and a coverslip was positioned on top. The images were captured with Pixel Mixer, ver. 3.0 (Pixela Corporation, Japan).

Gelatinization temperature. The Grace technique was used (Grace, 1977), 5 g of starch were weighed on a dry basis, which were dissolved in distilled water to a volume of 100 mL, the water was heated in a 250 mL beaker at

85°C and 50 mL of the suspension were placed in a 100 mL beaker, adding the sample in water at 85°C, and stirred constantly until the starch slurry until a paste is formed, continuously recording the temperature with a thermometer until it remained stable for several seconds, then the gelatinization temperature was read (Grace, 1977).

Results and discussion

The starch was obtained from the tubers in a state of optimum ripeness, which were harvested early in order to preserve the characteristics of the product. All of the starches were subjected to the same environment ensuring conditions of equilibrium in the laboratory.

Isolation of the native starch

The results of the starch extraction, expressed as a percentage, are shown in Tab. 2.

The potato starch extracted from the different varieties presented a white except the Merengo clone, which had a reddish color in solution, which was inhibited by the addition of metabisulfite at a concentration of 35 mg/100 mL. When drying the starch, it was ascertained that a temperature of 40°C is favored uniformly evaporating the water and higher temperatures formed a rigid surface (crust), which prevented uniform drying and enabled the growth of microorganisms that affect the lifespan of starches.

Table 2 shows that the yield of the native potato starch per 100 g of pulp on a dry basis was 8.79 g in ICA Unica variety and only 3.38 g for Marengo variety. These results are relatively low, attributable to the characteristics of the variety (Cáceres, 1991) and crop management (Arias and Arnaude de Chacón, 2010). By contrast, the results found for the Parda Pastusa, Diacol Capiro and Tuquerreña varieties were 14.04, 14.87 and 24.15 g, respectively, expressed as a high dry matter content, confirming a high correlation with the starch content (Gómez and Wong, 1998), which inferred its potential use in the production of bioethanol.

Analysis of composition properties, morphological properties and starch gelatinization

Physico-chemical properties. The Tab. 3 presents the values for the composition of the native starches from the different potato varieties.

The moisture content in the six potato starches ranged between 14.57 and 18.82% for the Tuquerreña and Marengo varieties, respectively. These results differ from information reported by other studies for native potato starches, ranging from 7.00 to 13.3% (Hoover, 2001). This variation may be caused by the production system, low seed quality and improper use of crop management practices, among others (Arias and Arnaude de Chacón, 2010; Porras and López, 2004).

The protein in the native starches showed no significant differences with values between 0.42 - 0.43%. The fat value was 0.35% for 'Tuquerreña' and 0.32% in other varieties. These results are consistent with those reported in studies conducted on potato starch contents, which are between 0 and 0.05% (Gunaratne and Hoover, 2002).

TABLE 2. Means, ranges, standard deviation and coefficient of variation of the starch extracted (g) from the five varieties and the potato clone.

Variety	Means	Minimum	Maximum	Standard deviation	Coefficient of variation*
Diacol Capiro	14.87	14.02	15.72	0.85	0.7225
ICA Unica	8.79	8.33	9.25	0.46	0.2116
ICA Purace	13.81	13.10	14.52	0.71	0.5041
Tuquerreña	24.15	23.26	25.05	0.89	0.8010
Parda Pastusa	14.04	13.03	15.05	1.01	1.0201
Merengo o Marenga	3.38	3.31	3.45	0.07	0.0049

* As we compare the dispersion between two or more variables together, or a single variable into two or more groups studied.

TABLE 3. Proximate analysis of the native starch potato varieties.

Variety	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Total carbohydrates	Standard deviation	Coefficient of variation*
Diacol Capiro	15.23	0.42	0.32	0.02	0.66	83.35	0.08	0.01
ICA Unica	17.20	0.43	0.32	0.02	0.87	81.15	0.33	0.11
ICA Purace	17.20	0.42	0.32	0.02	0.52	81.52	0.24	0.06
Tuquerreña	14.57	0.43	0.35	0.02	0.53	84.09	0.94	0.88
Parda Pastusa	17.20	0.43	0.32	0.02	0.87	81.22	0.33	0.11
Merengo o Marenga	18.82	0.43	0.32	0.04	0.38	80.02	0.20	0.04

* As we compare the dispersion between two or more variables together, or a single variable into two or more groups studied.

Values for the trace fiber of 0.015%, as reported by Correa and Giraldo (1995), were observed (Correa and Giraldo, 1995). The ash content in the starches showed highly significant differences, presenting a high content in ICA Unica and Parda Pastusa (0.87%). This increase in value, possibly related to the mineral content of the soil in which the plant material and product of the process of fertilization of the crop comes (Devlin, 1989). These results are similar to those obtained in other studies, which have reported that the contents of phosphorus and calcium influence the final content of the ash (Gunaratne and Hoover, 2002). The ash content directly influences the content, product quality and suitability for industrial use of the starch (Devlin, 1989). The reported ash values directly influenced the process of obtaining ethanol; some authors have indicated that interfere with the process of enzymatic hydrolysis; for this reason, it is essential to conduct an assessment of the gelatinization temperature. This ability is directly related to the process of enzymatic hydrolysis since it is difficult when there is a presence of amyl phosphoric ester (Murgas and Vásquez, 2013).

Functional properties. The proportion of amylose and amylopectin, affect the standing in the molecular structure of the granule, was closely related to the organization of these chains; the architecture of the granules and the presence of other substances that may interact and whose presence depends on the purity, efficiency extraction and affects its industrial uses (Aparicio-Trapala, 2003).

Table 4 presents the contents of amylose and amylopectin, highlighting the fact starch reached range of 9.95% for 'Diacol Capiro'. For the 'ICA Unica', this value was 9.47% and the 'ICA Purace' had a range of 9.34%, presenting significant variations when compared with the Tuquerreña, Parda Pastusa and Marengo varieties since the values obtained corresponded to 18.13, 18.64 and 10.27%, respectively.

TABLE 4. Contents of amylose and amylopectin in the starch of the potato variety tubers.

Variety	Amylose	Amylopectin
DiacolCapiro	9.95±0.26	90.15±0.42
ICA Unica	9.47±0.05	90.53±0.05
ICA Purace	9.34±0.07	90.66±0.06
Tuquerreña	18.13±0.20	81.87±0.20
Parda Pastusa	18.64±0.06	81.34±0.03
Merengo o Marenga	10.27±0.78	89.03±0.18

The differences found in the amylose content, due to the variability between one variety and another, and how they do cultural practices, aspect affected by weather conditions and soil type (Singh *et al*., 2003a). Also, the amylose content is important because this potato component is responsible for gelling processes and starch retrogradation when a product undergoes a baking process (Gunaratne and Hoover, 2002), so that tubers with a low starch content, if Diacol Capiro variety possess ideal characteristics for industry pre-fried. Therefore, it appears that the amylose/amylopectin ratio was higher for the 'Parda Pastusa' and 'Tuquerreña', and, therefore, stronger gels were obtained with a higher tendency for retrogradation, whereby the behavior of the starch paste in aqueous systems can depend on it, and other physical, chemical and mineral content features (Sassner *et al*., 2006).

The amylose and amylopectin chains of the potato starch differed not only in their physical properties but also in their proportions because the amylose was between 18.64 and 18.13% for the Parda Pastusa and Tuquerreña varieties, respectively, as seen in Tab. 4, while being compared with each other varieties showed that are mainly amylopectin sources. This indicates that glucose concentration is produced, as will hydrolyzing the starch to maltodextrins substrate necessary to be transformed by the yeast to ethanol in the fermentation stage.

Starch granule morphology. Starch is composed of granules of different sizes (diameters between 10 to 100 μm) and shapes (round, elliptical, oval, lenticular or polygonal) depending on the biological source from which they are partly semi-crystalline and insoluble in water at room atmosphere (Lideboom *et al*., 2004). Its content of amylose and amylopectin, gelatinization temperature, gel consistency and texture, viscous behavior and thermal properties, allowing their use in the food industry (Singh *et al*., 2005).

Figure 2 shows the images obtained through out the study of the micrographs for the native potato starches. As the regular forms of the native granules are round to ovoid and observed. These observations are consistent with those reported in the literature by various authors (Gunaratne and Hoover, 2002; Singh *et al*., 2003b). In general, various sizes were found in the tested samples (4-20 μm), where larger granules predominated. Several properties of starches are mainly related to the granule size, chemical composition, enzyme susceptibility, gelatinization crystallinity pulping properties, swelling and solubility (Lideboom *et al*., 2004).

Polarized light microscopy. The native starch granules varied in shape and size from one species to another, but all of them had a common characteristic. Thus, under the microscope and polarized light illumination, the starch

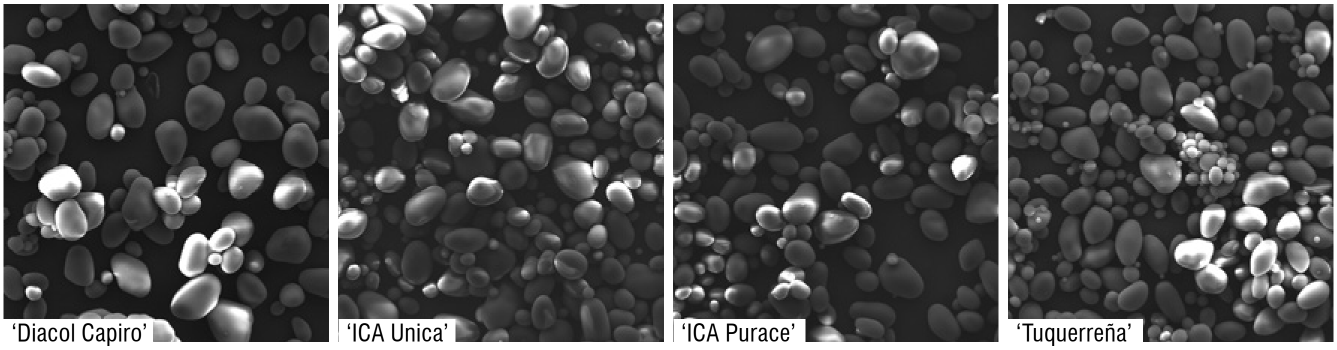


FIGURE 2. Morphology of the native starch granule of the potato varieties using the 500x high-resolution optical microscopy technique.

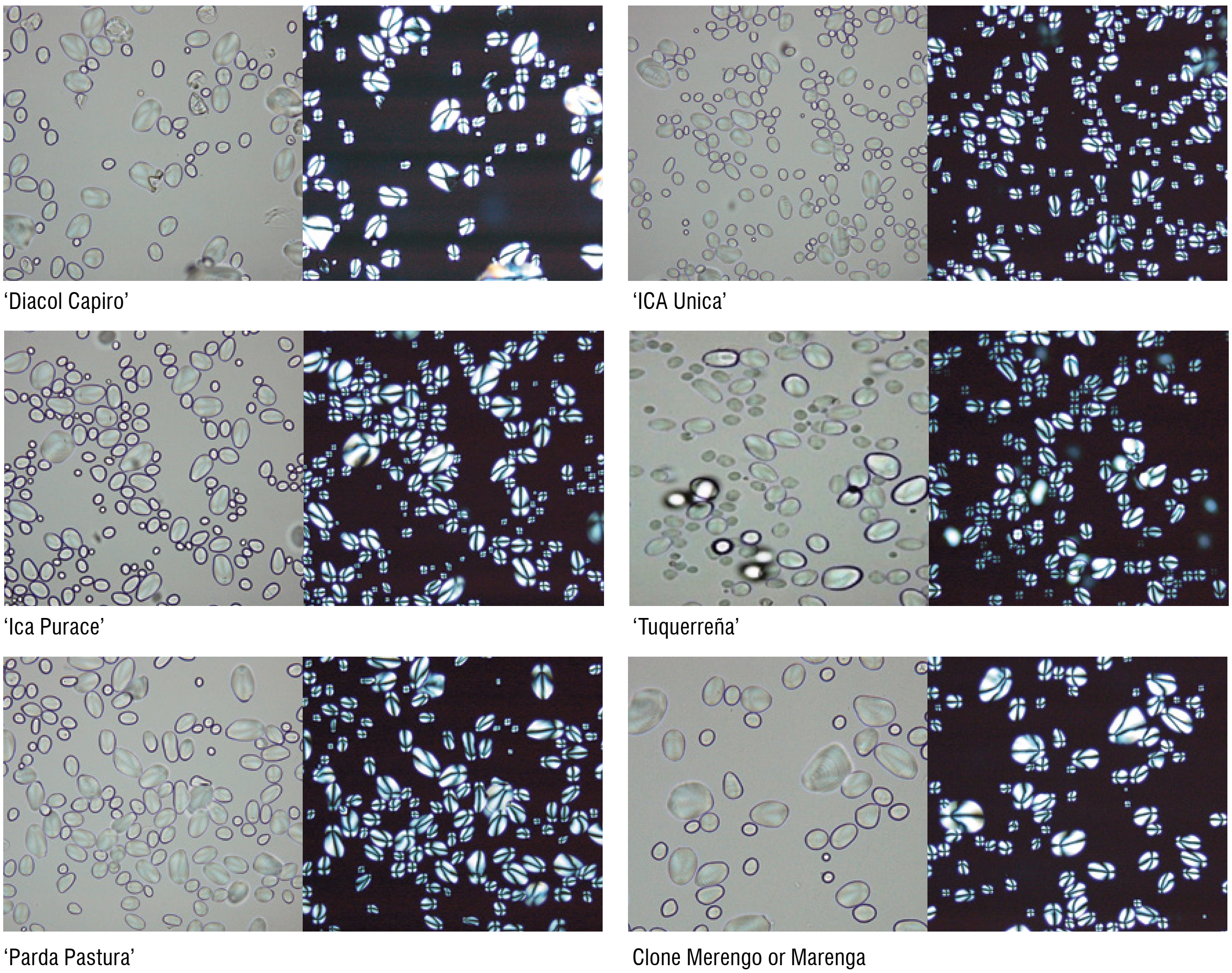


FIGURE 3 Granule morphology of the native potato starch using the technique of 40X polarized light microscopy.

grains stained with iodine presented a "maltese cross", as illustrated in Fig. 3, indicating the distinctive existence of a common internal order. When the granules were heated in excess water (pulping), the "cross" began to disappear, demonstrating that this molecular order is interrupted. The physical properties of starch -its stability and phase transformations, gels starch granules or raw, soft pasta- are directly linked to the molecular order.

Figure 3, the native potato starch shows a typical ovoid and spherical forms; the analysis coincides with that reported by Hoover (2001). Similarly, starches showed a birefringence, where the hilim was concentrically arranged and corresponded to the "Maltese cross" (Vásquez and Tham, 2007). As a result, it follows that there was a high degree of molecular organization with radially arranged chains of amylose and amylopectin. The 'Capiro Diacol' and 'ICA Purace' starches presented a granule size larger than the other varieties, which was directly related to the variety. From observing the granules under a polarized light, it follows that the analyzed starches from the six potato varieties had their own distinctive characteristics.

Gelatinization temperature of the starch from the potato varieties. According to the temperatures reported in Tab. 5, showed that inelastic pastes are still difficult to liquefy. This is due to the absence of the complexation phenomenon, aspects phenomenon is reported as occurring during cooking of starches from cereals, in which complexes are formed from fatty acid and amylose (Singh *et al*., 2005). Therefore, this phenomenon was evident in the obtained starches, with a high amylose content, when heated and carried gelatinization temperature, which gave more power to the gel form and this in turn required more energy to break (Svegmark *et al*., 2002).

TABLE 5. Gelatinization temperature for the potato starches according to the variety.

Variety	Initial gelatinization temperature (°C)
DiacolCapiro	60
ICA Unica	55
ICA Purace	55
Tuquerreña	50
Parda Pastusa	50
Merengo o Marenga	45

Likewise, it was observed that, as the temperature increased, the starch granules increased in size due to their ability to swell to absorb water from the environment. Notably, the initial size that is part differ from sample to sample as reported research Sánchez and Cardona (2005).

Therefore, it follows that the granule size inversely affects the gel point because, the greater the size of the smallest granule is the temperature, as in the Marengo clone, in which the gel point reached 45°C, while for the 'ICA Purace' and 'ICA Unica' it was 55°C. For the Parda Pastusa and Tuquerreña varieties showed a mean granule size among the six cultivars tested, as seen in Fig. 3.

Also, the amylopectin hydrolyzed by chemical and enzymatic methods showed a reduced swelling power; swelling is a primary property of this and is controlled by the crystallinity of starches according to reports from Tester and Karkalas (1999).

The slight difference in the solubility of the native potato starch with regard to cassava and maize, could be attributed to a lower amylopectin presence in the native corn and cassava starch. There is a possibility that the side branches (amylopectin) of the starch molecules and a lower grain size, facilitated the entry of water into the intermolecular spaces, increasing the solubility of the polymer, with a higher proportion of amylopectin the dissolution. This of course resulted in an increase of the solubility of the molecules in the water and in the stability of the viscosity.

When starch granules are broken by agitation, this creates a number of fractions that play different roles, as well as properties of gelatinization and swelling of the granules; these fractions are: native granules, fragmented granules (ordered or gel forming) and soluble low molecular weight material. Sorted fragments are derived from the broken walls of granules and have the highest level of integration in native structures; gel formers fragments behave as such in cold water and orderly lack integrity, while the produced fragments are broken, so it follows that branches formed from low molecular weight structures are produced. Usable and necessary to perform the process of hydrolysis by action of the enzyme α-amylase (Tester and Karkalas, 1999), process required to obtain bioethanol condition.

Conclusions

In general, the six analyzed potato varieties differed in terms of their shape, size, color and pulp, but the chemical composition did not change significantly, except for ash. The inferred a possible presence of amino phosphoric ester, which may interference with the action of the enzyme during the hydrolysis process, as reported by some authors.

The starch content presented differences in the behavior of the physicochemical properties, the amylose/amylopectin ratio and the gelatinization temperature. This was directly related to the granule morphology, evidencing differences in the shape and size, as observed by using the high-resolution optical microscopy.

From the results in potato varieties, it follows that the tested intrinsic and extrinsic properties of the potato varieties, behaved differently when exposed to temperatures, a prerequisite for obtaining ethanol. This is explained by the absorption capacity and the starch granule size in addition to the amylose, which can alter the structural order of resulting gels.

In particularly, the starch content present in the different varieties showed versatility due to the physicochemical properties found for this functional polysaccharide.

The Parda Pastusa and Tuquerreña varieties, possessed the most desirable characteristics for the production of bioethanol at the absolute laboratory level.

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