INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA RIO DE JANEIRO

Perspectivas da Ciência e Tecnologia, v.11 (2019)

MORPHOLOGICAL ANALYSIS OF STARCH GRANULES IN FLOURS AND ANOTHER FOODS IN THE RIO DE JANEIRO STATE, BRAZIL

Análise morfológica dos grânulos de amido em farinhas e outros alimentos no estado do Rio de Janeiro

Artur Manoel Leite Medeiros[1]*, Carlos Alexandre Marques[2]

[1] Laboratório de Morfologia Vegetal. Depto. de Botânica, Centro de Ciências da Saúde (CCS), Bloco A, Universidade Federal do Rio de Janeiro.

[2] Laboratório de Microscopia (B-127). Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), *campus* Nilópolis.

*Autor para correspondência: arturmelahel@gmail.com

Recebido em: 02/09/2019, Aceito em: 16/12/2019, Publicado em: 21/12/2019.

DOI: http://dx.doi.org/10.22407/1984-5693.2019.v11.p.128-140

ABSTRACT

The description of starch is an important aspect on several investigative studies: from the analysis of food adulteration and in the authenticity of common flours, to the development of hypotheses about the cultivation and domestication of plants in hominid populations as old as the Paleolithic. In Brazil, there's a few data available on the microscopic description of starch, so this work aimed to analyze morphologically the starch granules in popular sources of starch obtained in specialized stores, markets, and fairs of three municipalities of the state of Rio de Janeiro and develop an identification key of them. The samples was analyzed by optical and scanning electron microscopy (SEM). Falsifications were found in three arrowroot flours of different brands and origins. Consequently, it reflects the contribution of starch granules microscopy analysis to indicate the importance of legitimate cultivation of arrowroot and other traditional crops of the Brazilian flora. Spherical, polyhedral and ovoid granules are frequent in the species studied, with round-triangular and campanulate granules specific for identifying false-arrowroot and cassava, respectively. The descriptions and misunderstands found in literature were updated. For example the distinction of lenticular granules from ellipsoids in wheat, barley, and rye. Finally, a starches identification key is proposed, together with an update of important data for its morphological description.

Keywords: starch granules; raw materials authenticity; tradicional crops; microscopy.

RESUMO

A descrição do amido é um aspecto importante em vários estudos investigativos: desde a análise da adulteração de alimentos e na autenticidade de farinhas, até o desenvolvimento de hipóteses sobre o cultivo e a domesticação de plantas em populações de hominídeos tão antigas quanto as do Paleolítico. No Brasil, existem poucos dados disponíveis sobre a descrição microscópica de amidos, portanto este trabalho teve como objetivo analisar morfologicamente os grânulos de amido em fontes populares de amido obtidas em lojas, mercados e feiras especializadas de três municípios do estado do Rio de Janeiro e desenvolver uma chave de identificação destes. As amostras foram analisadas por microscopia fotônica e microscopia eletrônica de varredura (MEV). Falsificações foram encontradas em três farinhas de araruta de diferentes marcas e origens. Consequentemente, reflete a contribuição da análise microscópica dos grãos de amido para indicar a importância do cultivo legítimo de araruta e outras culturas tradicionais da flora brasileira. Grânulos esféricos, poliédricos e ovóides foram frequentes nas espécies estudadas, além de grânulos redondo-triangulares e campanulados serem específicos para identificação de araruta e mandioca, respectivamente. As descrições e os equívocos contidos na literatura foram atualizados. Por exemplo, a distinção entre grânulos lenticulares elipsoides de trigo, cevada e centeio. Por fim, é proposta uma chave de identificação de amidos, juntamente com uma atualização de dados importantes para sua descrição morfológica.

Palavras-chave: grânulos de amido. autenticidade de matérias-primas; culturas tradicionais; microscopia.

INTRODUCTION

Starches are products of the polymerization of glucose mainly in plants during photosynthesis, responsible for the energy reserve, consisting of granules of different shapes, sizes, and stratifications (FONTES e FONTES, 2005; TAIZ et al., 2015).

The description and identification of starch constitutes the basic knowledge to develop diverse investigative researches, since its use in the analysis of food adulteration and in the authenticity of common flours and foods (HARRINGTON, 1875; MENEZES JUNIOR, 1949), even to the development of hypotheses about the cultivation and domestication of plants in populations of hominids as old as the Paleolithic (FREITAS, 2002; CORNERO et al., 2015; KOVÁRNÍK e BENES, 2018).

Although there are few data available on the microscopic description of starches used as food, sometimes the inconclusive description of the morphological patterns of very similar starch granules, as well as the lack of detail inherent in clear chamber drawings, can lead to wrong identifications. However, there are just a few researches that aim to organize this information in a practical way to the researcher (e.g. MENEZES JUNIOR, 1949; OLIVEIRA e AKISUE, 2005; OLIVEIRA et al., 2015).

The authors of these papers use illustrated schemes to characterize the starch granules found in flours. In turn, these illustrations were based on studies dating from the late nineteenth and early twentieth century (KRAEMER, 1899, 1902; GALT, 1900; HÉRAIL, 1912; REICHERT, 1912). According to them, the important characteristics to identify starches are: the shape, the structure, the state of aggregation and the type of hilum.

Other works (CZAJA, 1978; CORTELLA e POCHETTINO, 1994; SOUZA et al., 1995; TORRENCE et al., 2004; GAMA et al., 2015) present the morphological characterization of starch, but this doesn't occur in such an embracing way, in the morphological aspect. In addition, there's a relevant study that classified families according to the morphology of the starch (DENARDIN e SILVA, 2009). And it's important to mention the identification key to the Mexican Starch Groups, where the most important characters for the classification were the diameter of the starch granules (UGENT e VERDUM, 1983).

Therefore, it's necessary a work that organizes those information in a practical way for a secure identification of starch granules. It's relevant to use an identification key, which allows informing which starch was found in a certain sample in a practical and simple way (e.g. UGENT e VERDUM, 1983).

Thus, an investigative work for starch granules identification can be developed either in bromatology, ethnobotany, systematics or archaeobotany, all of them with the use of this tool. So this paper aims to analyze morphologically the starch granules in popular sources of starch obtained in specialized stores, markets and fairs of three municipalities of the state of Rio de Janeiro and develop an identification key of them.

It was also accompanied by the review and updating of the descriptions and terms found in literature, as well as the registration through photomicrographs and electron micrographs of the material composing the key, to make it possible for any analyst to rapidly identify such materials.

MATERIAL AND METHODS

In this paper, samples of 14 popular sources of starch were obtained in specialized stores, markets and fairs in the cities of Rio de Janeiro, Nilópolis and Nova Iguaçu, all localized in the Rio de Janeiro state, Brazil. The samples was obtained in 2017 (Table 1). The biological material was deposited in the Laboratório de Microscopia of the Instituto Federal do Rio de Janeiro (IFRJ), *campus* Nilópolis.

Table 1. Characteristics of the samples used.

Common name	Flour	Plant organ	Scientific name	Family
Sweet potato	No	Tuberous root	*Ipomoea batatas* Lam.	Convolvulaceae
Cassava	Yes	Tuberous root	*Manihot esculenta* Crantz	Euphorbiaceae
Bean	No	Seed	*Phaseolus vulgaris* L.	Fabaceae
Soybean	No	Seed	*Glycine max* Merr.	Fabaceae
arrowroot	Yes	Rhizome	*Maranta arundinacea* L.	Marantaceae
False-arrowroot	Yes	Rhizome	*Maranta ruiziana* Körn	Marantaceae
Banana	No	Fruit	*Musa × paradisiaca* L.	Musaceae
Barley	Yes	Caryopsis	*Hordeum vulgare* L.	Poaceae
Corn	Yes	Caryopsis	*Zea mays* L.	Poaceae
Oats	Yes	Caryopsis	*Avena sativa* L.	Poaceae
Rice	No	Caryopsis	*Oryza sativa* L.	Poaceae
Rye	Yes	Caryopsis	*Secale cereale* L.	Poaceae
Wheat	Yes	Caryopsis	*Triticum sp.*	Poaceae
Potato	No	Tuber	*Solanum tuberosum* L.	Solanaceae

For analysis in light field and polarization microscopy, the samples were mounted between microscope slide and coverslip, using 50% glycerin (with distilled water) as the mounting medium. To obtain the seed starch, such as beans and soybeans, there was cold extraction in water, after imbibition of the seed for 12 hours, followed by maceration in grail and pistil. On the banana, the technique of direct crushing was made between the microscope slide and coverslip, and in sweet potato and potato, a free-hand cross-section was made (JOHANSEN, 1940).

The microscope slides were analyzed using the trinocular photonic microscope, with planachromatic objectives, model Alltion ABM103, containing PZO ocular micrometric ruler of 100 µm ± 1 µm. The polarization microscopy was performed using filters for polarization PZO in the same microscope (BENCHIMOL et al., 1996). Micrographs were made through an 8MP digital camera coupled to the photonic microscope and the computer.

For Scanning Electron Microscope (SEM) analysis, the samples were mounted on double tape on 12.7 mm diameter aluminum stubs, both of the Ted Pella Inc., and then analyzed for SEM Phenom World, Pro X model, with load-reducing sample port, no sample metallization required. The electron coils was configured in Image and Point modes at 10 kV and 15 kV, respectively, for generating electron micrographs, at varying microscopic magnifications. The measurements were made by the software contained in the device.

RESULTS AND DISCUSSION

This study found falsifications of arrowroot flour of different brands and origins, which reflects the importance of rescuing traditional crops and their impact on the food industry. One sample showed sweet potato, while the other showed cassava and the last showed false-arrowroot starch granules.

Arrowroot is a herbaceous plant originating in South America, occurring from the Guyana to Rio de Janeiro, with evidence of cultivation by native people for over 7,000 years (SHARMA et al., 2016).

Arrowroot flour was gradually replaced by cassava because it’s easier to process. The difficulty in obtaining pure starch made the food industry abandons the product and then the arrowroot practically disappeared from the market. In the late 1990s, the Embrapa

Agrobiologia worked on re-introducing the arrowroot in southern Bahia, Brazil, along with pataxós from the tribe Coroa Vermelha (PINSKY, 1999). Since the 2000s, the rescue of arrowroot crop in Rio de Janeiro had been carried out at the "Fazendinha Agroecológica", an agreement between Embrapa Agrobiologia and the Universidade Federal Rural Rio de Janeiro (NEVES et al., 2005).

Campanulate and round-triangular granules were specific for the identification of cassava and false-arrowroot, respectively. Equally, the in-Y hilum differentiates barley of rye. These differences are updates to the literature (e.g. MENEZES JUNIOR, 1949; FONTES e FONTES, 2005; OLIVEIRA e AKISUE, 2005; OLIVEIRA, 2015).

The mentioned literature relates the presence of ellipsoid granules in wheat, barley, and rye. However, it's necessary to clarify that the ellipsoid form derives from a particular perspective of observation: the lenticular granules, when analyzed under optical microscopy, may rotate on their axes, thus revealing an incorrect ellipsoid appearance. Notwithstanding, it's preferable to describe as ellipsoids the granules without the interference of the angle of rotation, since this two-dimensional view is unique to optical microscopy. Potato and banana starches are examples of true ellipsoid granules (Figure 1G-L).

After observing the morphology of the granules already mentioned, it was made the following identification key:

1. Single granules..2
Compound granules…...…..3
2. Ellipsoid granules..…...4
Polyhedral granules...5
Lenticular, reniform or spherical granules..6
3. Aggregate granules...*Oryza sativa* (Fig. 1A-C)
Homogeneously composed granules...*Avena sativa* (Fig. 1D-F)
4. Ellipsoid starch granules with characteristic longitudinal elongation, rounded ends and eccentric lamellae..*Musa × paradisiaca* (Fig. 1G-I)
Ovoid or pyriform granules, eccentric lamellae..........................*Solanum tuberosum* (Fig.1J-L)
5. Truncated granules predominantly, also found some round-triangular granules....................7

Campanulate granules predominantly, extinction cross has a slightly sinuous contour in polarizing microscopy...*Ipomoea batatas* (Fig. 2A-C)

Polyhedral granules with punctuated hilum predominantly, absent truncated or campanulate granules..*Zea mays* (Fig. 2D-F)

6. Spherical granules predominantly, also found lenticular granules with punctuated hilum, lamellae visible or not..*Triticum sp.* (Fig. 2G-I)

Lenticular granules predominantly, also found some spherical granules..................................8

Reniform granules predominantly, also found some spherical granules...................................9

7. Single granules predominantly, also found grouped granules...*Manihot esculenta* (Fig. 2J-L)

Single round-triangular granules predominantly..........................*Maranta ruiziana* (Fig. 3A-C)

8. Granules presenting a linear, in-Y, cross-shaped or star-shaped hilum with concentric lamellae predominantly...*Hordeum vulgare* (Fig. 3D-F, K-L)

Granules presenting a cross-shaped or star-shaped hilum with the concentric lamellae not always visible...*Secale cereale* (Fig. 3G-J)

9. Granules presenting a linear longitudinally elongated hilum with fissures or lateral branches predominantly...*Phaseolus vulgaris* (Fig. 4A-C)

Granules presenting a linear longitudinally elongated hilum predominantly, no fissures or lateral branches are found...*Glycine max* (Fig. 4D-F)

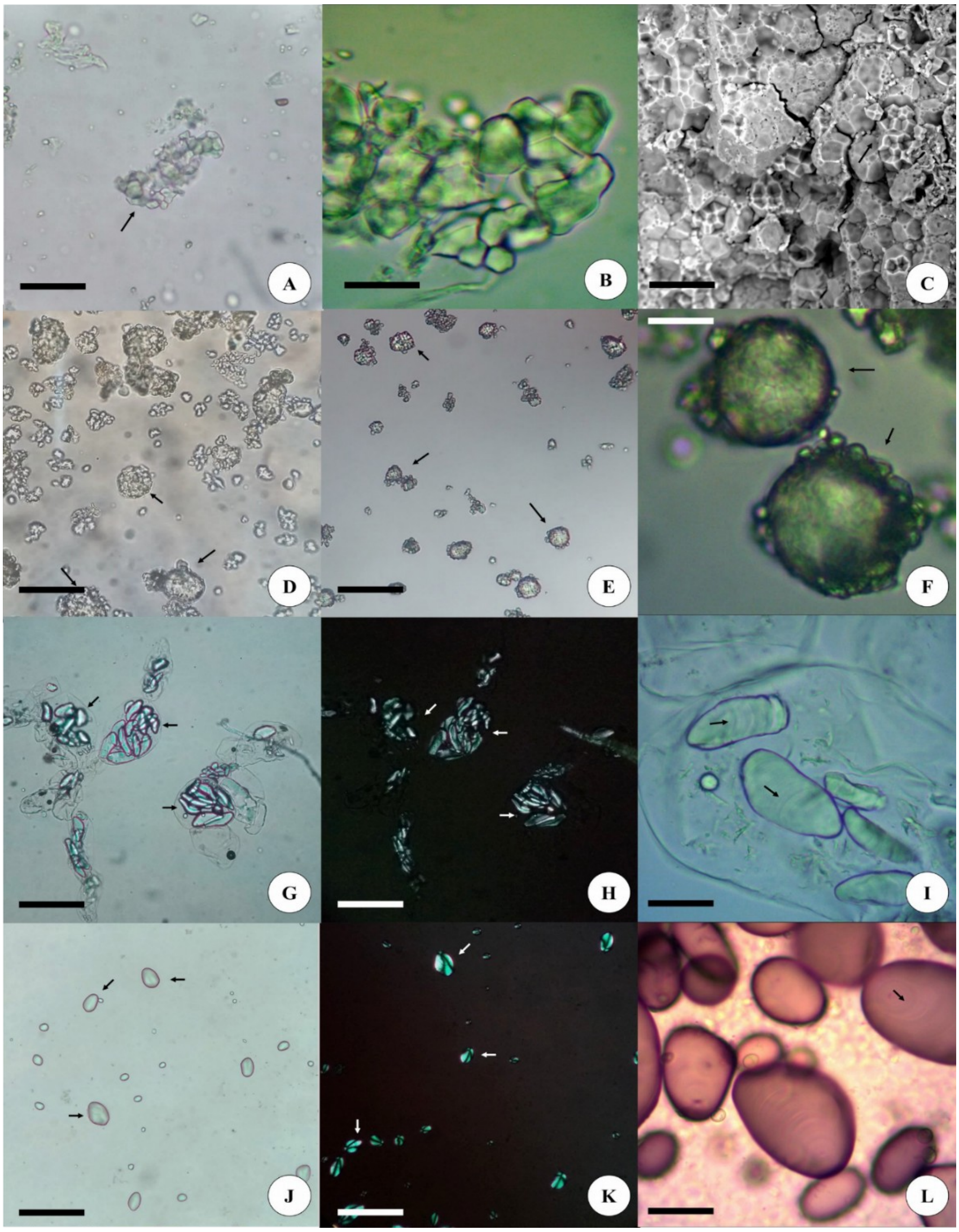

Figure 1. A-C. Rice starch (*O. sativa*). A. Granules aggregated in the center of the image (arrow). Bar = 40 µm. B. Aggregate granules in detail. Bar = 20 µm. C. Detail is seen in SEM, where the multifaceted aspect of the granules composing an aggregate (arrow) is observed. Bar = 20 µm. D-F. Oat starch (*A. sativa*). D. Homogeneously compound granules (arrows). Bar = 80 µm. E. Characteristic birefringence of homogeneously compound granules (arrows) in polarized light. Bar = 100 µm. F. Detail of the granules (arrows). Bar = 20 µm. G - I. Banana starch (*Musa × paradisiaca*). G. Ellipsoid granules (arrows) within the parthenocarpic fruit pulp parenchyma cells. Bar = 100 µm. H. Characteristic birefringence of banana starch (arrows) in polarized light. Bar = 100 µm. I. Larger granules, allowing the observation of the lamellae (arrows). Bar = 20 µm.

Figure 1 (previous page). J – L. Potato starch (*S. tuberosum*). J. Characteristic pyriform granules (arrows). Bar = 100 µm. K. Extinction cross (well visible) on the granules in polarized light (arrows). Bar = 100 µm. L. granules in detail, sometimes allowing the observation of lamellae (arrow). Bar = 20 µm.

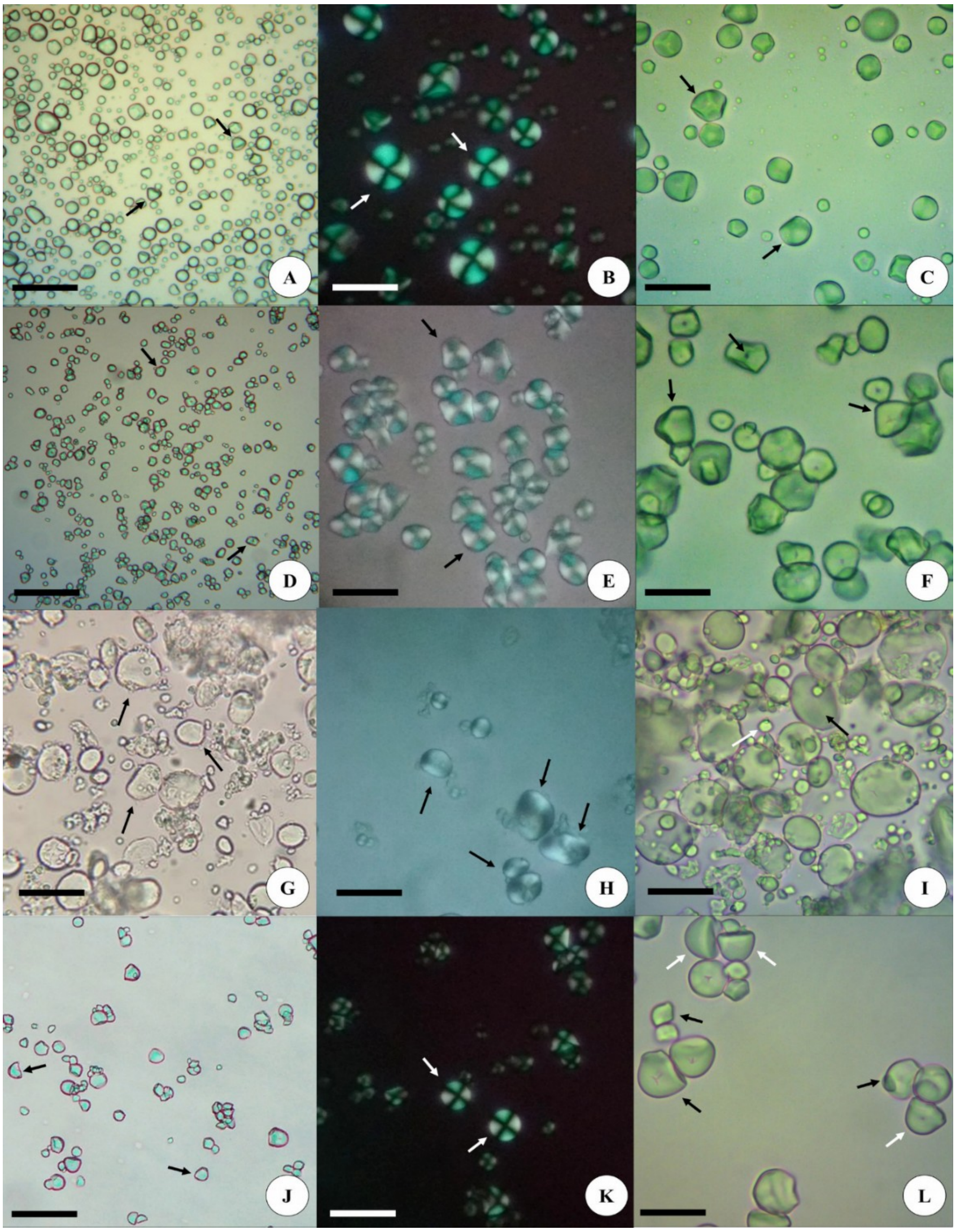

Figure 2. A - C. Sweet potato starch (I. batatas). A. Characteristic campanulate starch granules (arrows). Bar = 100 µm. B. Extinction cross well visible in starches, with characteristic sinuosity (arrows) in polarized light. Bar = 40 µm. C. Predominant polyhedral granules, with the presence of campanulate granules (arrows). Bar = 100 µm. D-F. Corn starch (Z. mays). D. Typical polyhedral granules (arrows). Bar = 100 µm.

Figura 2 (previous page). E. Highly visible extinction cross (arrows) in polarized light. Bar = 40 µm. F. Polyhedral granules in detail (arrows), showing the geometric aspect of well-marked faces with punctuate hilum. Bar = 20 µm. G-I. Wheat starch (Triticum sp.). G. Lenticular granules (arrows) characteristic of the species. Bar = 40 µm. H. Characteristic birefringence of wheat (arrows) in polarized light. Bar = 40 µm. I. Lenticular granules (black arrow) and spherical granules in detail (white arrow). Bar = 30 µm. J-L. Cassava starch (M. esculenta). J. Predominant and other truncated polyhedral granules (arrows). Bar = 100 µm. K. Extinction crosses well visible in starches (arrows) in polarized light. Bar = 40 µm. L. Grouped campanulate (white arrows) and truncated (black arrows) granules in detail. Bar = 20 µm.

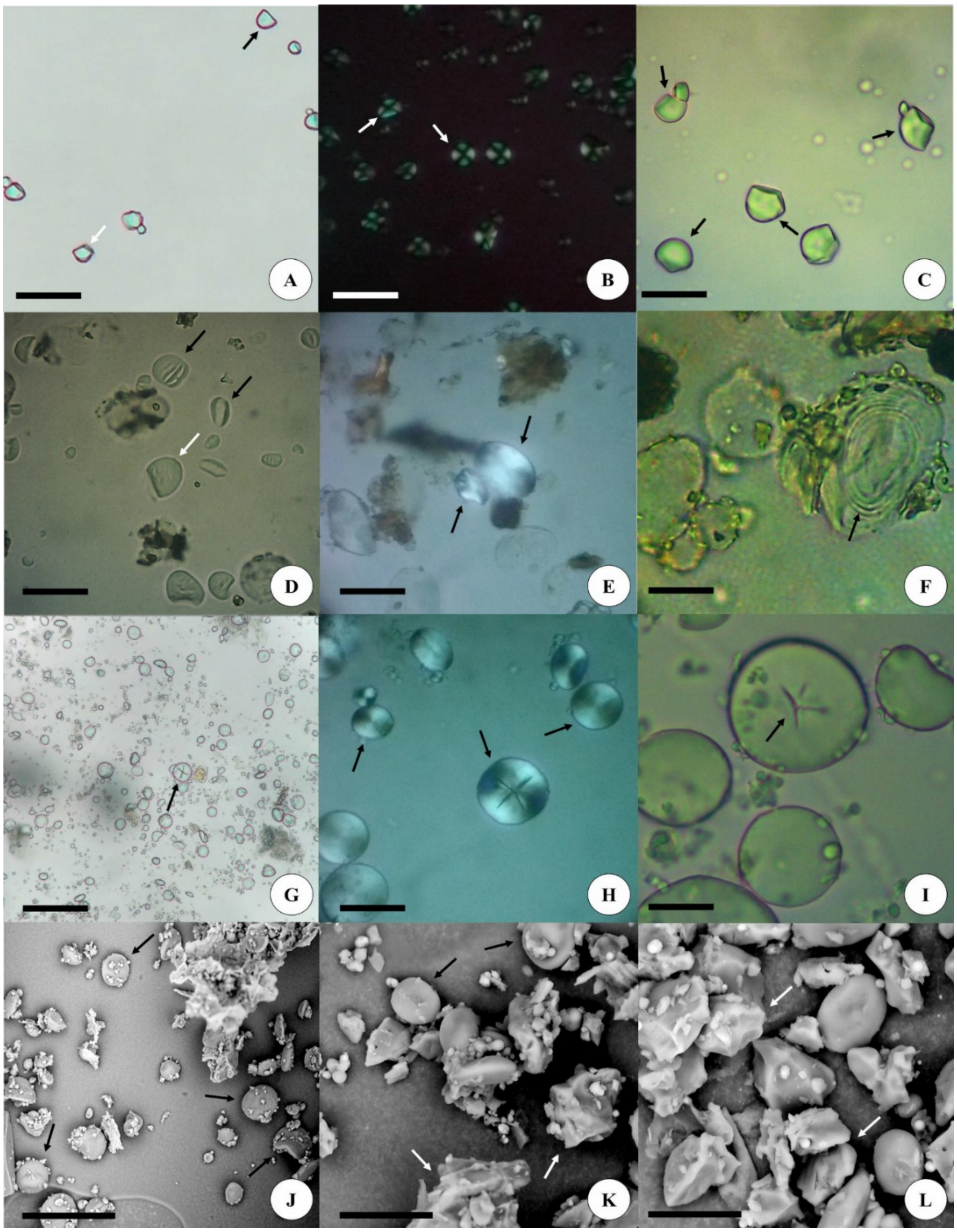

Figure 3 (previous page). A – C. False-arrowroot starch (*M. ruiziana*). A. Predominant polyhedral granules, others truncated (black arrow) and round-triangular (white arrow). Bar = 100 µm. B. Extinction cross visible in starches (arrows) in polarized light. Bar = 40 µm. Fig. C. Larger round-triangular granules (arrows). Bar = 20 µm. D-F. Barley starch (*H. vulgare*). D. Lenticular granules (black arrows) and truncated granules (white arrow). Bar = 40 µm. E. Characteristic birefringence of barley (arrows). Bar = 40 µm. F. Larger lenticular granules, with detail for the concentric lamellae (arrow). Bar = 20 µm. Fig. G-J. Rye starch (*S. cereale*). Fig. G-H. Lenticular granules, observe the cruciform hilum (arrow). Bar = 40 µm. I. Lenticular granules in greater microscopic magnification, with detail for in-Y granule (arrow). Bar = 20 µm. J. Predominant lenticular granules (arrows) to the SEM. Bar = 80 µm. K-L. Barley starch in SEM. K. Lenticular granules (black arrows) and polyhedral granules (white arrows). Bar = 20 µm. L. Starch granules in detail, highlighting the polyhedral granules (arrows). Bar = 20 µm.

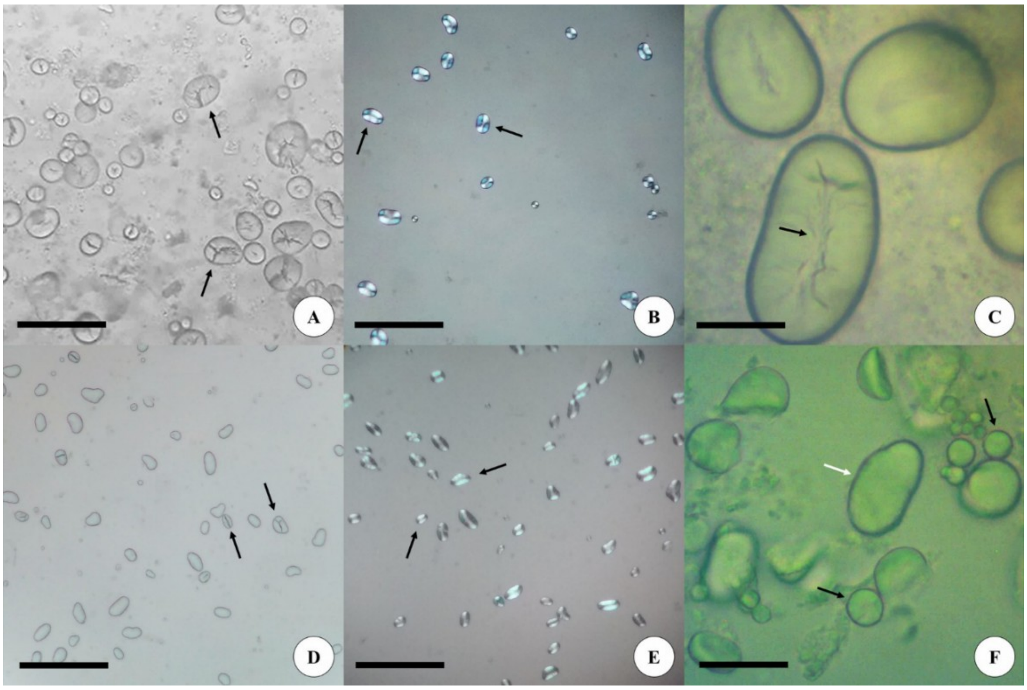

Figure 4. A-C Bean starch (*P. vulgaris*). A. Predominant reniform granules, with characteristic hilum with fissures or lateral branches (arrows). Bar = 80 µm. B. Typical birefringence of the bean, which evidenced its hilum (arrow) in polarized light. Bar = 100 µm. C. Reniform granules in the greatest increase. Observe the linear hilum that branches laterally (arrow). Bar = 20 µm. Fig. D-F. Soy starch (*G. max*). D. Predominant reniform granules, with linear hilum (arrows). Bar = 100 µm. E. Typical birefringence of soybean (arrows) in polarized light. Bar = 100 µm. F. Detail where, in addition to the reniform granule (white arrow), spherical and ovoid granules (black arrows) are observed. Bar = 30 µm.

CONCLUSIONS

In this paper, it was possible to review the knowledge regarding the identification of the most common starches in foods. It was also possible to verify that starch identification may be an alternative to examine the authenticity of arrowroot flours. Consequently, it reflects the contribution of starch granules microscopy analysis to indicate the great importance of the legitimate cultivation of arrowroot and other traditional crops of the Brazilian flora. Without traditional agriculture, products such as arrowroot can easily be replaced by those with more industrial exploitation such as cassava. Thus, the identification key shows its relevance and application in several areas with interest in the subject, especially in research involving bromatology, ethnobotany, systematics or archaeobotany. Important characteristics for the starch granules identification were updated, and the light field microscopy morphological descriptions were confirmed by scanning electron microscopy.

REFERENCES

BENCHIMOL, M *et al.* **Métodos de estudo da célula**. Rio de Janeiro: FENORTE; UENF, 1996.

CORNERO S. *et al.* Identificación de microestructuras y análisis arqueobotánico en sitios del Holoceno tardío de la llanura aluvial del Paraná medio. **Dominguezia 31**(1), 15-21, 2015. Disponível em: <http://www.dominguezia.org/volumen/articulos/3112.pdf>. Acesso em: 15 set. 2019.

CORTELLA, AR; POCHETTINO, ML. Starch granule analysis as a microscopic diagnostic feature in the identification of plant material. **Economic Botany**, **48**, 171-181, 1994.

CZAJA, AT. Structure of starch granules and the classification of vascular plant families. **Taxon 27**, 463-470, 1978.

DENARDIN, CC; SILVA, LP. Estrutura dos grânulos de amido e sua relação com propriedades físico-químicas. **Ciência Rural 39**(3), 945-954, 2009. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782009000300052>. Acesso em: 15 set. 2019.

FONTES, EAF; FONTES, PR. **Microscopia de alimentos**: fundamentos teóricos. Viçosa: UFV, 2005.

FREITAS, FO. Uso de grãos de amido na identificação e análise de materiais arqueológicos vegetais. **Boletim de Pesquisa e Desenvolvimento Embrapa 23**, 5-26, 2002. Disponível em: <https://ainfo.cnptia.embrapa.br/digital/bitstream/CENARGEN/23196/1/bp023.pdf>. Acesso em: 15 set. 2019.

GALT, HM. **The microscopy of the more commonly occurring starches**. Ballière: Tindall ad Cox, 1900.

GAMA, TSS. *et al.* Morfologia dos grãos de amido de cultivares de mandioca (*Manihot esculenta* Crantz) em Caxiuanã, Pará, Brasil. **Scientia Amazonia 4**(3), 63-68, 2015. Disponível em: <http://scientia-amazonia.org/wp-content/uploads/2016/06/v4-n3-63-68-2015.pdf>. Acesso em: 15 set. 2019.

HARRINGTON, MW. About starch. **The American Naturalist 9**(6), 339-346, 1875. Disponível em: <https://www.journals.uchicago.edu/doi/pdfplus/10.1086/271500>. Acesso em: 15 set. 2019.

HÉRAIL, J. **Traité de matière médicale**. Paris: Baillière, 1912.

JOHANSEN, DA. **Plant microtechnique**. New York: McGraw-Hill, 1940.

KOVÁRNÍK, J; BENES, J. Microscopic analysis of starch grains and its applications in the archaeology of the Stone Age. **Interdisciplinaria Archaeologica Natural Sciences in Archaeology 9**(1), 83-93, 2018. Disponível em: <https://www.iansa.eu/papers/IANSA-2018-01-kovarnik.pdf>. Acesso em: 15 set. 2019.

KRAEMER, H. An examination of commercial flour. **Journal of the American Chemical Society**, **21**(8), 650-663, 1899.

KRAEMER, H. The structure of the starch grain. **Botanical Gazette 34**, 341-355, 1902. Disponível em: <https://www.journals.uchicago.edu/doi/pdfplus/10.1086/328297>. Acesso em: 15 set. 2019.

MENEZES JUNIOR, JBF. Investigações sobre o exame microscópico de algumas substâncias alimentícias. **Revista do Instituto Adolfo Lutz**, **9**, 19-77, 1949.

NEVES, MCP. *et al.* Araruta: resgate de um cultivo tradicional. **Comunicado Técnico Embrapa**, Seropédica, n. 79, p. 1-4, 2005. Disponível em: <https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAB-2010/33053/1/cot079.pdf>. Acesso em: 15 set. 2019.

OLIVEIRA, F; AKISUE, G. **Fundamentos de farmacobotânica**. São Paulo: Atheneu, 2005.

OLIVEIRA, F *et al.* **Microscopia de alimentos**. Rio de Janeiro: Atheneu, 2015.

PINSKY, L. De volta à mesa pataxó: redescoberta planta do receituário indígena. **Revista Época**, Rio de Janeiro, 13 dez. 2010. Ciência e tecnologia. Disponível em: <http://revistaepoca.globo.com/Revista/Epoca/0,,EMI166170-15224,00-CAPITAL+DO+TRANSPLANTE.html>. Acesso em: 15 set. 2019.

REICHERT, ET. The differentiation and specificity of starches in relation to genera, species, etc.: stereochemistry applied to protoplasmic processes and products, and as a strictly scientific basis for the classification of plants and animals. **Proceedings of the Society for Experimental Biology and Medicine 10**(2), 45-45, 1912.

SHARMA, HK. *et al.* (Ed.). **Tropical roots and tubers**: production, processing and technology. Nova Jersey: John Wiley & Sons, 2016.

SOUZA, JSI *et al*. **Enciclopédia agrícola brasileira**: A-B. São Paulo: EdUSP, 1995.

TAIZ, L. *et al.* **Plant physiology and development**. Sunderland, Massachusetts: Sinauer Associates, 2015.

TORRENCE, R. Identification of starch granules using image analysis and multivariate techniques. **Journal of Archaeological Science 31**(5), 519-532, 2004.

UGENT, D.; VERDUN, M. Starch granules of the wild and cultivated mexican species of *Solanum*, subsection Potatoe. **Phytologia 53**, 351-363, 1983.