1 *Full title: Key to identification of starch grains used as foods*

2 *Short title: Identification of starch grains*

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Abstract

The description of starch grains is important in different areas, such as bromatological, taxonomic, or archaeobotanic analyzes, among others. Although there are data on the microscopic description of starches used as foods, this work has proposed the development of a key to identify the starch grains in 13 raw materials obtained in specialized stores, markets and fairs to facilitate its identification. For optical microscopy, the material was deposited on a slide, being sealed with a coverslip in 50% glycerin. The samples were also mounted on double-sided tape and colloidal graphite on aluminum stubs and analyzed in scanning electron microscopy (SEM). Therefore, an unpublished starch identification key is proposed, together with the update of important data for its morphological description.

Introduction

Starches are products of the polymerization of glucose mainly in plants during the photosynthesis, responsible for the energy reserve, consisting of grains of different shapes, sizes and stratifications [5, 6, 15].

The description and identification of starch constitutes the basic knowledge to develop diverse investigative research, since its use for analysis of falsifications and adulterations in flours and foods [11, 12], even to the development of hypotheses about the cultivation and domestication of plants in populations of hominids as old as the Paleolithic [2,7].

Although there are data on the microscopic description of starches used as foods, sometimes the inconclusive description of the morphological patterns of very similar starch grains, as well as the lack of detail inherent in clear-chamber drawings, can lead to wrong identifications. However, there is still little research that has like objectives organizes this information in a practical way to the researcher [11, 12, 13]. The authors of these papers use illustrated schemes to characterize the starch grains found in flours. In turn, this illustrations was based on studies dating from the late nineteenth and early twentieth century [9]. According to them, the microscopic visualization of a starch grain may reveal a single or crossed central or eccentric point or fissure, these formations being called hilum. The authors consider important characteristics to identify starches: the shape, the structure, the state of aggregation and the type of hilum.

Other works [3, 8, 12, 14, 16] present the morphological characterization of starch, but this does not occur in such an embracing way, in the morphological aspect. In addition, there is a relevant study that classified families according to the morphology of the starch [4]. And it is

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 grains [17].

Therefore, it is necessary a work that organize this informations in a practical way for a secure identification of starch grains. It is relevant to use an identification key, which allows to inform which starch was found in a certain sample in a practical and simple way [17]. Thus, investigative work for starch grains identification can be developed either in bromatology, systematics or archaeobotany, with the use of this tool.

So this paper has proposed to develop a key to identify the starch grains in 13 raw materials obtained in specialized stores, markets and fairs: oats, rye, barley, corn, wheat, soybeans, beans, bananas, rice, sweet potatoes, false-arrowroot, cassava and potato. The materials was collected in the cities of Rio de Janeiro, Nilópolis and Nova Iguaçu, RJ, Brazil It were also accompanied by the review and updating of the descriptions and terms found in literature, as well as the registration through photomicrographs and electromicrographs of the material composing the key, to make it possible for any analyst to rapidly identify such materials.

Material and Methods

In this paper, 13 raw material were used, from which starch grains were obtained, called: oats (*Avena sativa* L. — Poaceae), rye (*Secale cereale* L. — Poaceae), barley (*Hordeum vulgare* L. — Poaceae), corn (*Zea mays* L. — Poaceae), wheat (*Triticum sp.* L. — Poaceae), soybean (*Glycine max* Merr. — Fabaceae), bean (*Phaseolus vulgaris* L. — Fabaceae), banana (*Musa × paradisiaca* L. — Musaceae), rice (*Oryza sativa* L. — Poaceae), sweet potato (*Ipomoea batatas* Lam. — Convolvulaceae), false-arrowroot (*Maranta ruiziana* Körn — Marantaceae), cassava (*Manihot esculenta* Crantz — Euphorbiaceae) and potato (*Solanum tuberosum* L. — Solanaceae) (Table 1).

Table 1. Characteristics of the samples used

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

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69 For analysis in light field and polarization microscopy, the samples were mounted between
70 microscope slide and cover slip, using 50% glycerin (with distilled water) as the mounting medium.
71 To obtain the seed starch, such as beans and soybeans, there was cold extraction in water, after
72 imbibition of the seed for 12 hours, followed by maceration in grain and pistil. In the banana, the
73 technique of direct crushing was made between the microscope slide and cover slip, and in sweet
74 potato and potato, a free-hand cross-section was made [10, 11].

75 The microscope slides were analyzed using the trinocular photonic microscope, with
76 planchromatic objective, model Alltion ABM103, containing PZO ocular micrometric ruler of 100
77 $\mu\text{m} \pm 1 \mu\text{m}$. The polarization microscopy was performed using filters for polarization PZO in the
78 same microscope [1].

79 Micrographs were made by means of an 8MP digital still camera coupled to the photonic
80 microscope and the computer.

81 For Scanning Electron Microscope (SEM) analysis, the samples were mounted on double
82 tape on 12.7 mm diameter aluminum stubs, both of the Ted Pella Inc., and then analyzed for SEM
83 Phenom World, Pro X model, with load-reducing sample port, no sample metallization required.
84 The electron coils was configured in Image and Point modes at 10 kV and 15 kV, respectively, for
85 generating electromicrographs, at varying microscopic magnifications. The measurements were
86 made by the software contained in the device [1].

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88 **Results and Discussion**

89 In this study, a high frequency of spherical, polyhedral, truncated and ovoid grains was
90 observed. However, the presence of campanulate and round-triangular shaped grains were specific
91 for identification of cassava and false-arrowroot, respectively.

92 Differences in some classifications were observed, comparing with the bibliography [6, 9,
93 11, 12]. In sweet potato, truncated grains are reported because their polygonal faces are visible
94 under light microscopy. This is because the authors preferred to name two types of grains: truncated
95 indefinitely and polyhedral. However, a classification that distinguishes indefinitely truncated and
96 polyhedral grains is difficult, which make the characterization become redundant. For this reason, it
97 is preferable was to denominate these grains as polyhedral. However, there is an important
98 subdivision for the truncated grains: the round-triangular grains, which are grains truncated on two
99 faces forming a triangular structure with a spherical/rounded base, being peculiar in false-arrowroot.

100 The authors also do not cite clearly the presence of spherical and polyhedral grains in
101 cassava, however much their illustrations represent their presence. In wheat, barley and rye, the

authors added the presence of ellipsoid grains to describe lenticular grains that revolve around their axis, making their thinner face visible. However, it is preferable to describe as ellipsoids only the predominantly biconvex grains, without the angle of rotation interfering significantly in the classification, since this two-dimensional view is unique to optical microscopy. Potato and banana starches are examples of true ellipsoid grains. In barley, are not cited the in-Y hilum, or any analogous characterization. Even if this characteristic being differential between rye and barley.

It is important to note that barley, rye and wheat have lenticular and spherical grains, which may indicate a peculiar characteristic of Triticeae, the tribe of these vegetables. The same happens with beans and soybean, two species of the tribe Phaseoleae that have spherical, ovoid and reniform grains.

Therefore, the granule descriptions were in accordance with the literature, updating dubious characteristics previously proposed:

- As to state of aggregation: simple or isolated and compound, the compounds subdivided into grouped, aggregated and homogeneously compound;
- As to shape: campanulate, ellipsoid, lenticular, ovoid, polyhedral, pyriform, reniform, round-triangular, spherical and truncated;
- As to the type of hilum: in-Y, linear, punctuated or dotted and star-shaped;
- And as to position of the hilum: concentric and eccentric.

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

Key to identification

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

135	Campanulate grains predominantly, extinction cross has a slightly sinuous contour in polarizing	
136	microscopy.....	<i>Ipomoea batatas</i> (Fig. 2A-C)
137	Polyhedral grains with punctuated hilum predominantly, absent truncated or campanulate	
138	grains.....	<i>Zea mays</i> (Fig. 2D-F)
139	6. Spherical grains predominantly, also found lenticular grains with punctuated hilum, lamellae	
140	visible or not.....	<i>Triticum sp.</i> (Fig. 2G-I)
141	Lenticular grains predominantly, also found some spherical grains.....	8
142	Reniform grains predominantly, also found some spherical grains.....	9
143	7. Single grains predominantly, also found grouped grains.....	<i>Manihot esculenta</i> (Fig. 2J-L)
144	Single round-triangular grains predominantly.....	<i>Maranta ruiziana</i> (Fig. 3A-C)
145	8. grains presenting a linear, in-Y, cross-shaped or star-shaped hilum with concentric lamellae	
146	predominantly.....	<i>Hordeum vulgare</i> (Fig. 3D-F, K-L)
147	grains presenting a cross-shaped or star-shaped hilum with the concentric lamellae not always	
148	visible.....	<i>Secale cereale</i> (Fig. 3G-J)
149	9. grains presenting a linear longitudinally elongated hilum with fissures or lateral branches	
150	predominantly.....	<i>Phaseolus vulgaris</i> (Fig. 4A-C)
151	grains presenting a linear longitudinally elongated hilum predominantly, no fissures or lateral	
152	branches are found.....	<i>Glycine max</i> (Fig. 4D-F)

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156 **Figure 1.** A-C. Rice starch (*O. sativa*). A. grains aggregated in the center of the image (arrow). Bar
157 = 40 μm . B. Aggregate grains in detail. Bar = 20 μm . C. Detail seen in SEM, where the multifaceted
158 aspect of the grains composing an aggregate (arrow) is observed. Bar = 20 μm . D-F. Oat starch (*A.*
159 *sativa*). D. Homogeneously compound grains (arrows). Bar = 80 μm . E. Characteristic birefringence
160 of homogeneously compound grains (arrows) in polarized light. Bar = 100 μm . F. Detail of the
161 grains (arrows). Bar = 20 μm . G-I. Banana starch (*Musa* $\times$ *paradisiaca*). G. Ellipsoid grains
162 (arrows) within the parthenocarpic fruit pulp parenchyma cells. Bar = 100 μm . H. Characteristic
163 birefringence of banana starch (arrows) in polarized light. Bar = 100 μm . I. Larger grains, allowing
164 the observation of the lamellae (arrows). Bar = 20 μm . J-L. Potato starch (*S. tuberosum*). J.
165 Characteristic pyriform grains (arrows). Bar = 100 μm . K. Extinction cross (well visible) on the
166 grains in polarized light (arrows). Bar = 100 μm . L. grains in detail, sometimes allowing the
167 observation of lamellae (arrow). Bar = 20 μm .
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173 **Figure 2.** A-C. Sweet potato starch (*I. potatoes*). A. Characteristic campanulate starch grains
 174 (arrows). Bar = 100 μ m. B. Extinction cross well visible in starches, with characteristic sinuosity
 175 (arrows) in polarized light. Bar = 40 μ m. C. Predominant polyhedral grains, with presence of
 176 campanulate grains (arrows). Bar = 100 μ m. D-F. Corn starch (*Z. mays*). D. Typical polyhedral
 177 grains (arrows). Bar = 100 μ m. E. Highly visible extinction cross (arrows) in polarized light. Bar =
 178 40 μ m. F. Polyhedral grains in detail (arrows), showing the geometric aspect of well-marked faces
 179 with punctuate hilum. Bar = 20 μ m. G-I. Wheat starch (*Triticum sp.*). G. Lenticular grains (arrows)
 180 characteristic of the species. Bar = 40 μ m. H. Characteristic birefringence of wheat (arrows) in
 181 polarized light. Bar = 40 μ m. I. Lenticular grains (black arrows) and spherical grains in detail (white
 182 arrows). Bar = 30 μ m. J-L. Cassava starch (*M. esculenta*). J. Predominant and other truncated
 183 polyhedral grains (arrows). Bar = 100 μ m. K. Extinction cross well visible in starches (arrows) in
 184 polarized light. Bar = 40 μ m. L. Grouped campanulate (white arrows) and truncated (black arrows)
 185 grains in detail. Bar = 20 μ m.

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192 **Figure 3.** A-C. False-arrowroot starch (*M. ruiziana*). A. Predominant polyhedral grains, others
193 truncated (black arrow) and round-triangular (white arrow). Bar = 100 μ m. B. Extinction cross
194 visible in starches (arrows) in polarized light. Bar = 40 μ m. Fig. C. Larger round-triangular grains
195 (arrows). Bar = 20 μ m. D-F. Barley starch (*H. vulgare*). D. Lenticular grains (black arrows) and
196 truncated grains (white arrow). Bar = 40 μ m. E. Characteristic birefringence of barley (arrows). Bar
197 = 40 μ m. F. Larger lenticular grains, with detail for the concentric lamellae (arrow). Bar = 20 μ m.
198 Fig. G-J. Rye starch (*S. cereale*). Fig. G-H. Lenticular grains, observe the cruciform hilum (arrow).
199 Bar = 40 μ m. I. Lenticular grains in greater microscopic magnification, with detail for in-Y grain
200 (arrow). Bar = 20 μ m. J. Predominant lenticular grains (arrows) to the SEM. Bar = 80 μ m. K-L.
201 Barley starch in SEM. K. Lenticular grains (black arrows) and polyhedral grains (white arrows).
202 Bar = 20 μ m. L. Starch grains in detail, highlighting the polyhedral grains (arrows). Bar = 20 μ m.
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206 **Figure 4.** A-C Bean starch (*P. vulgaris*). A. Predominant reniform grains, with characteristic hilum
 207 with fissures or lateral branches (arrows). Bar = 80 μ m. B. Typical birefringence of the bean, which
 208 evidenced its hilum (arrow) in polarized light. Bar = 100 μ m. C. Reniform grains in greatest
 209 increase. Observe the linear hilum that branches laterally (arrow). Bar = 20 μ m. Fig. D-F. Soy starch
 210 (*G. max*). D. Predominant reniform grains, with linear hilum (arrows). Bar = 100 μ m. E. Typical
 211 birefringence of soybean (arrows) in polarized light. Bar = 100 μ m. F. Detail where, in addition to
 212 the reniform granule (white arrow), spherical and ovoid grains (black arrows) are observed. Bar =
 213 30 μ m.

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215 Conclusions

216 In these paper it was possible to review the knowledge regarding the identification of the
 217 most common starches in foods. The identification key shows its relevance and application in
 218 several areas with interest in the subject, especially in research involving bromatology and
 219 taxonomy. With this, it was possible to update the information described in the literature. Important
 220 characteristics for the identification of starch grains, which were redundant or misunderstood in the
 221 literature, were updated, and light field microscopy images were confirmed by scanning electron
 222 microscopy.

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