Determination of amylose content in rice

Consideration on the norm
ISO 6647:2015
(part 1 and part 2)

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Determination of amylose content...a little bit of history

• Since the first measurements in 1943 (McCready and Hassid), the technique used was the spectrophotometer (UV-VIS);
• Till with the ISO 6647: 2007 we talk about amylose, but in reality is apparent amylose as iodine also binds with linear chains in amylopectin ramifications;
• All the bibliography until today take into consideration the amylose determined by methods (ISO, AOAC ...) that returns the apparent amylose;
• All limits (European PDO, PGI, variety registrations for Italian Rice Varieties) are established on the basis of apparent amylose data.
ISO 6647:2007, how does it work?

• Part 1: reference method. Milling, delipidization, gelatinization of the sample, treatment with iodine and colour development. The analysis is performed in the UV-VIS spectrophotometer.

• The calibration curve is made by mixing with established ratios:
  - Potato amylose;
  - Amylopectin from waxy rice.
ISO 6647:2007, how does it work?

• Part 2: routine method. Milling, (NO delipidization), gelatinization of the sample, treatment with iodine and colour development. The analysis is performed in the UV-VIS spectrophotometer;

• The calibration curve is constructed with rice samples with known amylose content, constituted by the laboratory;

• The analysis is faster, but less accurate
ISO 6647:2015, what changes?

• Part 1: reference method. The suitably treated sample is analyzed with SEC technology.
• This part of the method is only used to provide SEC standards with which to construct the calibration curve, in Part 2 (as specified in the Scope).
ISO 6647:2015, what changes?

• Part 2: routine method. Milling, (NO delipidization), gelatinization of the sample, treatment with iodine and colour development. The analysis is performed in the UV-VIS spectrophotometer;

• The calibration curve is constructed with rice samples with known amylose content. Using the STD SEC obtained with Part 1 (or provided by IRRI) to constitute the calibration set in the routine;

• The Italian lab has chosen specifically the following varieties Italian rice varieties: Castelmochi, Puma, Selenio, Carnaroli, CRLB1
ISO 6647:2015: problems

• Applicative: not available at IRRI SEC standards with which to build the calibration curve, as indicated in section 8.4.1 and its footnote;

• Interpretive: the results found by applying the new norm are not consistent with those found with the old standard, with problematic interpretative implications (Regulations and Ministerial Decrees Italian (and European) that regulate such PDO and PGI or registrations of new rice varieties on the National Register)
ISO 6647:2015: problems

<table>
<thead>
<tr>
<th>Norm ref.</th>
<th>Type of rice</th>
<th>Country</th>
<th>Amylose content % LIMIT IN NORM Note 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg. UE 1151/2012</td>
<td>Arroz Carolino do Baixo Mondego</td>
<td>Portugal</td>
<td>17,5 - 22,5</td>
</tr>
<tr>
<td>C. 188/50 27/05/2016</td>
<td>Khao Sangyod Muang Phatthalung</td>
<td>Thailand</td>
<td>13 - 17</td>
</tr>
<tr>
<td>C. 277/6 22/08/2014</td>
<td>Bahia</td>
<td>Spain</td>
<td>19,1</td>
</tr>
<tr>
<td></td>
<td>Senia</td>
<td>Spain</td>
<td>16,3</td>
</tr>
<tr>
<td></td>
<td>Bomba</td>
<td>Spain</td>
<td>24,9</td>
</tr>
<tr>
<td></td>
<td>J. Sendra</td>
<td>Spain</td>
<td>17,5</td>
</tr>
<tr>
<td></td>
<td>Montsianell</td>
<td>Spain</td>
<td>18,1</td>
</tr>
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<td></td>
<td>Gleva</td>
<td>Spain</td>
<td>17,7</td>
</tr>
<tr>
<td></td>
<td>Sarcet</td>
<td>Spain</td>
<td>16,3</td>
</tr>
<tr>
<td></td>
<td>Albufera</td>
<td>Spain</td>
<td>25,6</td>
</tr>
<tr>
<td>Reg. UE 510/2006</td>
<td>Arroz del Delta del Ebro</td>
<td>Spain</td>
<td>19,41</td>
</tr>
<tr>
<td>Reg. UE 120/2013</td>
<td>Khao Hom mali Thung Kula Rong-Hai</td>
<td>Thailand</td>
<td>14 - 16</td>
</tr>
<tr>
<td>Provv. 09/09/2010</td>
<td>Vialone Nano</td>
<td>Italy</td>
<td>&gt; 21%</td>
</tr>
</tbody>
</table>

Note 1: limit values of amylose content in the regulations, intended as determined as apparent amylose (source: www.enterisi.it; 16/09/2016)
ISO 6647 2007 vs 2015

• The Italian lab has carried out (and continues to run) comparison tests. Some results:

<table>
<thead>
<tr>
<th>Italian rice varieties</th>
<th>6647-1:2007</th>
<th>6647-2:2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roma</td>
<td>14,92</td>
<td>11,12</td>
</tr>
<tr>
<td>Baldo</td>
<td>17,50</td>
<td>14,30</td>
</tr>
<tr>
<td>Carnaroli</td>
<td>22,10</td>
<td>16,89</td>
</tr>
<tr>
<td>Carnaroli II</td>
<td>23,00</td>
<td>17,97</td>
</tr>
</tbody>
</table>
ISO 6647:2015: what to do now?!

- The problem is of all laboratory which could be enable to applicate the normative because there isn’t the possibility to have SEC standards.
- The problem is of the customer (breeder, consumer industry, rice farmer..) : with the application of ISO 6647-2: 2015, obtain an analytical result that he is not able to use or compare with the previous.
- The norm ISO 6647:2015 as it is, it’s not ok
ISO 6647:2015: what to do now?!

Very good work of IRRI for the ISO 6647: 2015 which optimizes the determination of amylose, up to now determined as "apparent amylose" (even if not so explicitly named, but conventionally referred to as "amylose"), approaching the concept of "real amylose" or absolute.

Was it correct to attribute a different numbering to the standard?

Has the impact of this standard been underestimated with consolidated historical data?

if you want to make a data historian to adapt PDO and PGI, there are some application difficulties
ISO 6647:2015: what to do now?!

The RICE working group in UNI has worked as follow:

- Takes note of the alerts issued by the Laboratory of Rice Research Centre (Ente Nazionale Risi – Italy) in relation to the ISO 6647: 2015
- Planned an *ad hoc* meeting between members of the GL Rice at UNI (September 15, 2016)
- Related to ISO the applicative and interpretative difficulties of ISO 6647: 2015
- Has formulated a standard proposal that could solve the problems
ISO 6647:2015 vs new ISO/CD

ISO/CD 6647-1
Rice – Amylose content – Part 1: Determination of amylose
It is constituted by the norm ISO 6647:2007
ISO 6647:2015 vs new ISO/CD

ISO/CD 6647-2
Rice – Amylose content – Part 2: Determination of absolute amylose
It is constituted by the norm ISO 6647:2015-2 and in Annex B is reported the ISO 6647:2015-1 (for the preparation of SEC standard)
ISO/CD 6647

In this way it would escape interpretative and application problems!
Thank you for the attention!

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