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Characterisation, in vitro digestibility and expected glycemic index of commercial starches as uncooked ingredients

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Abstract

When selecting a native starch for specific food purposes, it is important to consider the differences between starches of varying botanical sources (cereal, tubers and roots). In this study uncooked starches as ingredients (corn, rice, wheat, tapioca and potato) were characterized according to microstructure, some (physicochemical, functional and thermal) properties, in vitro digestibility and expected glycemic index.

There was a significant variation in the granule shape and size distribution of the starches, when studied using Scanning Electron Microscopy (SEM) and particle size analysis. Particle size results distinguished among starches in monomodal (corn, tapioca, potato) and bimodal (rice, wheat) populations. Among all the samples, the potato showed the biggest size distribution granules while the rice showed the lowest. The examined properties and nutritional characteristics of starches were significantly different. Thermal properties were studied by means of Differential Scanning Calorimeter (DSC). DSC studies found that the transition temperatures (58.8 °C - 78.7 °C) and enthalpies of gelatinization (2.3 J/g - 8.2 J/g) of the starches appeared to be greatly influenced by microstructure and chemical composition (e.g. resistant starch). Potato and corn starches were hydrolyzed more slowly and to a lesser extent than ones. In particular, the highest resistant starch was recorded for potato. Nutritional properties such as slowly digestible starch and expected glycemic index values followed the order: rice > wheat > tapioca > corn > potato.

In general, these results provide the baseline information on the development of novel foods or native starch blends with tailored functional properties such as slow digestibility.

Keywords: starch, food structure, functional properties, physicochemical properties, resistant starches, expected glycemic index

1. Introduction

Commercial native starches are mainly obtained from cereals, such as corn, wheat and rice in most developed countries, and from tubers or roots, such as potato and tapioca (or cassava) in countries as India, Brazil, Thailand, Indonesia, Philippines and China (Moorthy 2002). In particular corn is the main raw material for starch production, accounting for about 80% of the world market. Native starch and its derivatives are widely used in food production and other branches of industry. The widespread application of starches as a group is due to their availability, cost, specific physico-chemical properties (Leszczyński 2004) and, recently, to their nutritional characteristics as slow digestibility (Rake et al. 2002). For nutritional purposes, starch has been in fact classified according to their rate of digestion into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al. 1992). Nowadays much attention is being given to SDS of

1 starches for the development of foods low in rapidly digestible starch and hence of low glycemic
2 index (GI). SDS is in fact considered to be beneficial for the slow and prolonged release of glucose
3 in metabolic disorders such as diabetes or glycogen storage diseases (GSDs, in particular type I)
4 (Bjorck and Asp 1994; Zhang and Hamaker 2009), and satiety (Lehmann and Robin 2007). The rate
5 and extent to which starch is digested and absorbed (in vitro and in vivo hydrolysis) vary
6 considerably depending on the structure, the conditions and the degree of food processing (i. e.
7 cooking or baking starch under hydrating conditions), the physico - chemical characteristics of the
8 starch (Englyst et al. 1999; Kaur et al. 2010). Progress towards correlating structural and physical
9 properties of the starch granule to its digestibility, is almost evident (Tester et al. 2006; Dona et al.
10 2010). Furthermore, there is still a deficiency of information on in vitro starch digestibility
11 including nutritionally important starch fractions (rapidly digestible, slowly digestible, and resistant
12 starches and GI) and properties of different botanical sources. Thus, the aim of this study was to
13 investigate the microstructure, the some physical and functional properties of commercial starches
14 obtained from cereals (corn, rice and wheat) and from roots and tubers (tapioca and potato), with
15 the intent to provide the baseline information for their potential use as uncooked ingredients for
16 starchy tailored foods. Moreover, the in vitro digestibility and expected GI of selected starches were
17 evaluated.

18 **Materials and methods**

1 **Materials**

2 Commercial starches from corn (Giglio 2TBS, Roquette Italia S.p.A., Italy), rice (Le Farine
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4 3 Magiche Lo Conte, IPAFOOD s.r.l., Italy), wheat (Frumina Paneangeli, Cameo S.p.A., Italy),
5
6 4 tapioca (Novation® 3300, Ingredion™, United Kingdom) and potato (Roquette Italia S.p.A., Italy)
7
8
9 5 were provided by the CAISIAL - Centre for Food Innovation and Development in the Food
10
11 6 Industry, University of Naples Federico II, Italy.

13
14 7 The main nutritional characteristics (% w/w) indicated by the producers can be reassumed as
15
16 8 follows: corn starch - proteins 0.3, fiber 0, fat <0.1, minerals <0.1; wheat starch - proteins 0.35,
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18 9 fiber 0, fat 0.1, minerals <0.1; rice starch - proteins 1.0, fiber 0, fat <0.1, minerals 0.6; tapioca
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21 10 starch: proteins <0.5, fiber <1.0, fat <0.15, minerals <0.15; potato starch - proteins 0, fiber 0, fat
22
23 11 <0.1, minerals 0.19.

27 13 **Scanning Electron Microscopy analysis**

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30 14 Starches were dried at the critical point and coated with gold particles in an automated critical point
31
32 15 drier (model SCD 050, Leica Vienna). Microstructure of starches was examined by means of
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34 16 Scanning Electron Microscopy (SEM) (LEO EVO 40, Zeiss, Germany) with a 20 kV acceleration
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36
37 17 voltage and a magnification of $\times 4000$.

42 19 **Particle size analysis**

43
44 20 Particle size distributions of the starches were measured by light scattering (Mastersizer 3000,
45
46 21 Malvern Instruments, UK) in deionized water. The measurement range of the equipment was 0.01–
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48
49 22 3500 μm . The Fraunhofer diffraction model, assuming a standardized spherical shape, was used to
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51 23 analyse all samples. The results obtained were diameters of equivalent spheres expressed in volume.
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54 24 Each average value represents the mean of 3-7 independent measurements.

59 26 **Physicochemical properties**

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1 The moisture content of each sample was determined by the AACC method (number 44-15.02,
2 1999). Three samples, weighing approximately 3 g, were dried for 24 h at 105 °C. Samples were
3 removed from the oven and immediately placed in a desiccator prior to weighing after cooling and
4 within 30 min. The dried samples weight was subtracted to the respective initial weight. The results
5 were calculated as percentage of water per sample weight (%).

6 The pH of starches was measured by using a digital pHmeter (MP220, Mettler, Toledo) according
7 to the AACC method (number 02-52.01, 1999).

8 **Functional properties**

9 The solubility of starches was determined at 20°C and 70°C by the method of Mishra and Rai
10 (2006).

11 Water holding capacity (WHC) was determined at 20°C by the method of Xu et al. (2013). WHC
12 was calculated as:

$$13 \text{WHC} = 100 \times (\text{weight of wet sample} - \text{weight of dry sample}) / \text{weight of dry sample (dry basis)}$$

14 **Gelatinization thermal properties**

15 The gelatinization properties of the starches were assessed by means of a Differential Scanning
16 Calorimeter (DSC) (Q200, TA Instruments, Milan, Italy). Samples of approximately 6 mg were
17 hydrated in the Tzero aluminium hermetic pans at about 70% moisture. The pan was closed with a
18 lid and weighed. All samples were heated from 30 °C to 100 °C at 10 °C min⁻¹ using an empty pan
19 as the reference. The gelatinisation properties, as enthalpy of gelatinization (ΔH), onset (T_o) and
20 peak (T_p) temperatures, were measured. In addition, Peak Height Index (PHI) was calculated as
21 described by Krueger et al. (1987):

$$22 \text{PHI} = \Delta H / (T_p - T_o)$$

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Average values of four measurements were calculated for each sample.

In vitro starch digestibility and expected glycemic index

Measurement of resistant starch (RS) and non-resistant starch (solubilised, Non-RS) were determined using an enzymatic assay kit (Resistant Starch Assay Kit, Megazyme International Ireland) by AACC method (number 32-40.01, 2009). The total starch (TS) was expressed as the sum of RS and Non-RS. All these results were expressed as percentage weight / weight on dry basis.

In vitro kinetic of starch digestion was determined using AACC (2000) method 32-40.01 with minor modifications. Each starch sample (25 ± 0.1 mg) was incubated with pancreatic α -amylase (0.1 mg) and amyloglucosidase (3U) in 1 mL of 100 mM sodium maleate buffer (pH 6.0) in a shaking water bath (200 strokes/min, horizontal agitation) at 37°C for 30, 60, 90, 120, 150 and 180 minutes, so six incubation times in duplicate were employed for each starch. The enzymatic mixture added to each tube was set to $34 \pm 3^\circ\text{C}$. After incubation at each time, 1 mL of ethanol (99% v/v) was added to inactivate the enzymes, prior to centrifugation at 3500 rpm for 10 minutes. The supernatant was carefully decanted and the pellet was re-suspended with 2 mL of aqueous ethanol (50% v/v) and centrifuged again at 3500 rpm for 10 minutes. This ethanol washing was repeated one more time. The three supernatant solutions were combined together and the volume was adjusted to 25 mL with 100 mM sodium acetate buffer (pH 4.5). Aliquots (0.1 mL) of each solutions (in duplicate) were incubated with 10 μL of dilute amyloglucosidase solution (300 U/mL) in 100 mM sodium maleate buffer (pH 6.0) to hydrolyse the digested starch into glucose after 20 minutes at 50°C. The glucose content of the supernatant was measured using a glucose oxidase/peroxidase (GODOP) kit. The rate of starch digestion was expressed as the percentage of TS hydrolysed at each time (30, 60, 90, 120, 150 and 180 minutes).

Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation for 30 minutes and a further 120 minutes, respectively.

1 The digestion kinetics were described by means of a non- linear model following the equation
2 found by Goñi et al. (1997):

$$C = C_{\infty} (1 - e^{-kt}) \quad (3)$$

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11 where C was the hydrolysis degree at each time, C_{∞} was the maximum hydrolysis extent and k was
12 the kinetic constant. The hydrolysis index (HI) was calculated as the relation (as percentage)
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where C was the hydrolysis degree at each time, C_{∞} was the maximum hydrolysis extent and k was the kinetic constant. The hydrolysis index (HI) was calculated as the relation (as percentage) between the area under the hydrolysis curve (AUC, 0 - 180 min) of each sample and the AUC of white bread as reference food. Previous research has shown HI to be a good predictor of glycemcic response (Goni et al. 1997). Lastly, expected glycemcic index (eGI) was calculated using the equation proposed by Goñi et al.(1997):

$$eGI = 39.71 + 0.549HI \quad (4)$$

Statistical analysis

All experimental results are reported as means and standard deviation of at least three independent experiments. One way ANOVA with Duncan's multiple comparison test at the 95 % confidence level ($P \leq 0.05$) were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) on all experimental data.

Results and Discussion

Microstructural and particle size analyses

In order to study differences among starches at microstructural level, the characterisation of the microstructure by means of SEM and of light scattering was performed. The SEM images and particle size distribution of corn, rice, wheat, tapioca and potato starches are shown in Figure 1.

1 Qualitative differences concerning shape and size of starch granules were observed among the
2 different starches. Microstructural results of corn starch (Fig. 1 a) showed polyhedral, roundish
3 granules with small (~5 μm) and large (~18 μm) elements. Rice starch (Fig. 1 b) presented
4 characteristic clusters of very small granules, with an angular and polygonal shape and well-
5 defined edges; the size varied in a limited range (~3-8 μm). Wheat starch (Fig. 1 c) contained small
6 (< 10 μm , B- type) as well as large (> 10 μm , A- type) granules of roundish, spherical or polygonal
7 shapes. Tapioca starch (Fig. 1 d) seemed similar in size to corn starch, as also observed by Mishra
8 and Rai (2006), with small (~ 7 μm) and large (~ 21 μm) elements; the shape was spherical, with
9 some roundish or truncated granules.
10 Potato starch (Fig. 1 e) showed large oval with flattened and ellipsoid granules, in the range
11 between 15 and 50 μm . The surface analysis revealed a slight roughness with the presence of holes
12 and cracks for corn starch; sufficiently smooth surface with the presence of some fractures for rice
13 starch; considerable roughness with the presence of holes and protuberances for wheat starch; little
14 wrinkled granules and often characterized by the presence of holes and small grooves for tapioca
15 starch; a very smooth surface for potato starch.
16 The size distributions of starch granules (Fig. 1 f) were monomodal for corn, tapioca and potato
17 starches and bimodal for rice and wheat. In particular, the granule size distribution of corn, tapioca
18 and potato starches (determined using the Malvern Mastersizer) displayed only one peak around 15,
19 20 and 50 μm , respectively. The wheat starch instead exhibited a bimodal distribution of small (1 –
20 2.8 μm) and large (19 μm) granules, likely due to small B granules and large A granules,
21 respectively, while the bimodal distribution of rice samples showed a first population around 1.5
22 μm diameter and a second population around 10 μm size, due to the small granules clustered
23 together to minimize the surface area. Among all the starches, the potato starch showed the biggest
24 size distribution granules while the rice showed the lowest. These observations agree with the
25 review of Lindeboom et al. (2004) on the distribution of starch granule size in different biological
26 sources. The differences in granular size and proportion of starch granules are in fact related to the

1 botanical source from which the starch is isolated and even it could be associated with differences
2 in genotypes as reported by Shevkani et al. (2011) that studied the granule size distribution of
3 starches from Indian wheat lines.
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6 7 8 9 **Chemical composition and properties** 10

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The resistant (RS) and non-resistant starch (non-RS) compositions of starches were analysed and compared with corn (Table 1). ANOVA indicates that significant differences ($P < 0.05$) existed for different fractions of starches, RS (w / w d. b.) and non-RS (w / w d. b.) for each of the samples tested. As shown in Table 1, potato starch contained the highest RS content (69.6%) followed by tapioca (1.8%), corn (1.4%), and finally by wheat (1.1%) and rice (0.3%). The differences of structural characteristics of different starches may account for the differences of RS. RS is considered a dietary fibre because of its indigestibility by body enzymes. Raw potato starch is an enzyme resistant starch which is in fact associated with the large granule size (fig. 1e), B-type crystallinity, as well as different molecular weight and weight distribution (Liu et al. 2006), as compared to cereals and other starches. The low RS content of cereal samples is in line with what could be expected from their polymorph starch type: cereal starches are A-type starches, always having a low RS fraction of total starch, while most of the tuber and root starches exhibit the B-type diffractometric pattern; tapioca can show A, C or a mixed pattern (Moorthy 2004; Themeier et al. 2005). Therefore, RS results appeared in agreement with dimensional characteristics of samples (Fig. 1 a - e). The granule size distribution and shape are considered important for the functional and nutritional properties of the starches (Tester et al. 2006). When developing slowly digestible or resistant starches for human nutrition, the morphology and the particle size of the starch granules, as the relationship between surface area and starch volume, has to be evaluated. Although the method could be not suitable for samples with less than 2% w/w RS, these values agreed with the reported

1 results by McCleary et al. (2002) for corn and potato starches, by Themeier et al. (2005) for corn
2 and wheat samples and by Liu et al. (2006) for potato one.

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5 The levels of non-RS varied in a limited range for cereal starches and tapioca starch (90.1 - 92.1%)
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7 whilst the Non-RS in potato starch was only 22.2%. These results showed similar behaviour to that
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9 reported for RS results, as expected.

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12 As physicochemical properties, the pH and moisture content were investigated. The examined
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14 parameters are shown in Table 2. The pH analysis of starches showed significant differences ($P <$
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16 0.05) among samples, discriminating into five statistical groups ($P < 0.05$). pH is an important
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18 property in starch industrial applications, being used generally to indicate the acidic or alkaline
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20 properties of liquid media (Ashogbon and Akintayo 2012). The pH of the potato starch (7.26) was
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22 observed to be significantly the highest ($P < 0.05$), this shows that it has low acid content, while
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24 tapioca starch (4.86) was the lowest ($P < 0.05$). Significant differences ($P < 0.05$) were detected for
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26 moisture content of starches. The moisture content ranged between 9.3% (tapioca starch) to 18.1%
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28 (potato starch). Rice starch showed slightly lower moisture content (10.0%) than corn starch
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30 (10.1%). The trend of moisture content results was similar with pH results. Overall, the average pH
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32 and moisture content results of starches can be considered consistent with previous literature
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34 (Muazu et al. 2011; Ashogbon and Akintayo 2012).

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37 As functional properties, solubility at 20°C (cold) and at 70°C (hot) and water holding capacity
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39 (WHC) were investigated. The examined parameters are shown in Table 2. The solubility of
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41 starches showed significant differences ($P \leq 0.05$) among samples. It was observed that all starches
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43 exhibited very low solubility at 20°C and formed only a temporary suspension when stirred in
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45 water: this phenomenon can depend on the starch source (presence of hydroxyl groups in the starch
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47 molecules able to form hydrogen bonds with the aqueous medium) and the granule structure (semi-
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49 crystalline at low temperatures). Tapioca starch showed the highest cold solubility value compared
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51 to the other starches ($P < 0.05$), in dependence of botanical properties of the starch molecules such
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53 as amylose/amilopectin ratio, chain length and molecular weight distribution, degree/length of
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1 branching and conformation (Moorthy 2004). The solubility results for all starches showed that
2 their solubility increases with increase in temperature. The order of hot solubility was tapioca >
3 potato > corn, rice, wheat ($P < 0.05$). The results derived from the solubility at 70°C confirmed the
4 trend of the cold solubility values, obviously with higher values. The phenomenon is due to the fact
5 that the crystalline structure is disrupted when starch molecules are heated in excess water, and
6 water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose
7 and amylopectin (Singh et al. 2003). Cereal starches (corn, rice and wheat) did not differ
8 significantly each other ($P < 0.05$) and exhibited a lower solubility than the tuber and root starches
9 (potato and tapioca) at 70°C, which differed significantly each other (11.2% and 16.5%,
10 respectively). This behaviour could be due firstly to the fact that cereal starches have a more
11 compact structure and different crystallinity than tuber starches; secondarily the temperature used
12 (70°C) was higher than the gelatinization temperature of tubers but lower than the required for
13 cereal starches (Mishra and Rai 2006). The differences in the physical state of the amylose
14 component in native wheat and potato starches observed in the past by Kulp and Lorenz (1981)
15 could justify the significant difference ($P < 0.05$) among the values of their hot solubilities: in wheat
16 starch amylose is present in a helical form whilst it is in an amorphous form in potato granule.
17 Singh et al. (2003) also reported that the higher value of potato starch than corn, rice and wheat
18 starch could be probably due to a higher content of phosphate groups on amylopectin, responsible
19 of a repulsion effect and of a consequent hydration increase by weakening the extent of bonding
20 within the crystalline domain.

21 In order to use uncooked starches as ingredient for novel food, tapioca starch improved techno-
22 functional properties in terms of cold solubility; therefore it could be used for shaken liquid or semi-
23 liquid products.

24 Results of WHC ranged from 69.2% for corn starch to 100% for rice starch and discriminated
25 starches into four statistical groups ($P < 0.05$). WHC is a room temperature measurement and does
26 not indicate the behaviour of the starches when heated, but is an important parameter for food

1 processing applications, such as texture of semi- liquid products, custards and doughs, because this
2 is supposed to imbibe water thereby attaining body thickening and viscosity. The differences ($P <$
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4 0.05) in WHC (Table 2) of the starches among the different species, similarly among the different
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6 cultivars, could be attributed to the starch structure (differences in amylose /amylopectin content
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8 and size) and extent of interactions between starch chains and water. It can be deduced from WHC
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10 results that rice starch has more proportion of amorphous regions within its small granules than corn
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12 starch. Results appeared in agreement with those reported in literature (Mishra and Rai 2006;
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14 Olayemi et al. 2008; Xu et al. 2013).
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22 **Gelatinization thermal properties**

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27 Starch gelatinization is the collapse (disruption) of molecular orders within the starch granule
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29 manifested in irreversible changes in properties such as granular swelling, native crystalline
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31 melting, loss of birefringence, and starch solubilization (Atwell et al. 1988). It is an important factor
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33 contributing to starch functionality and is widely exploited in the food industry (Ratnayake and
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35 Jackson 2007). The thermal properties of starches were studied by means of DSC, and related DSC
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37 characteristics (ΔH - transition enthalpy, T_o - transition onset temperature, T_p - transition peak
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39 temperature and PHI - Peak height index) are summarized in Table 3.
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44 A single endothermic transition, corresponded mainly to the gelatinization transition of the starch,
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46 was observed in the DSC profiles of all tested starches (data not shown) as the high level of
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48 moisture (70%) at which samples were scanned. The transition temperatures (T_o and T_p) varied
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50 significantly ($P < 0.05$) among the examined starches. These fall within the range of gelatinization
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52 temperatures commonly observed for starches: T_o from 58.8 to 69.2 °C and T_p from 65.4 to 78.7
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54 °C (Table 3). In particular, wheat samples exhibited the lowest T_o (58.8 °C) and T_p (65.4 °C)
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56 values as compared to other starches. Differences in the transition temperature range were also
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58 highlighted by the values of the PHI, which indicate the shape and degree of symmetry of
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1 endotherms. A low PHI indicates a broad transition range and suggests structural heterogeneity in
2 starch granules. The PHI values of potato starch were the highest (0.9), followed by corn and
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5 3 tapioca, wheat and finally by rice, indicative of a narrow transition range and of a resistance to
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7 4 gelatinization process. According to Kruerger et al. (1987), gelatinization of starch is a cooperative
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10 5 process, such that structural relations between amorphous and crystalline regions within the starch
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12 6 granules are responsible for the sharpness of thermal transition and the temperature at which it
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14 7 occurs. Moreover, the gelatinization temperature provides an indication of the minimum
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17 8 temperature required to cook a given sample and it has implications on the stability of other
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19 9 components in a formula and also indicates energy cost. Thus, potato starch, with the major T_o , T_p
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21 and PHI values ($P < 0.05$), will require the most energy for it to be cooked than other starches.
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24 1 Tapioca and corn starches showed a similar thermal behavior about T_p and PHI parameters, while
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27 12 the transition temperatures of rice and wheat starches were lower as compared to others, and the
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29 13 endothermic peak of rice was the broadest ($PHI = 0.2$).
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32 14 The ΔH values obtained in this study (Table 3) ranged between 2.3 to 8.2 J/g and discriminated
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34 15 significantly starches into five statistical groups ($P < 0.05$). The order of enthalpy was potato > corn
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37 16 > tapioca > wheat > rice ($P < 0.05$). The ΔH primarily reflects the loss of molecular (double-helical)
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39 17 order within the starch granules and it refers to the energy required to disrupt the native structure of
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42 18 the starch granules (Cooke and Gidley 1992). In fact starches with higher ΔH show higher
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44 19 crystallinity and vice versa (Singh et al. 2010). Furthermore the highest ΔH of potato starch
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47 20 suggests that the starch granules that melt during gelatinization are resistant and strongly associated
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49 21 within its native structure, in agreement with granule size results of potato (Fig. 1) and RS values
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52 22 (Table 1). Likewise the low value of transition temperatures and the lowest ΔH of rice starch may
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54 23 be attributed to the reduced thermal granule stability of this cereal characterized by small size
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57 24 granules (Fig. 1f) and the lowest RS content (Table 1). Tapioca showed a middle thermal behavior
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59 25 that may be due to some degree of starch modification, which occurred during processing and
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62 26 storage. ΔH values obtained for tapioca starch were in fact less than values reported for tapioca by
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1 Sun and Yoo (2015) (15.4 J /g) and less than corn starch results (3.8 J /g). From these observations,
2 it seems that the higher RS content of starch, the higher the ΔH of the sample.
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5 **In vitro starch digestibility and expected glycemic index**

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11 The effects of botanical origin on in vitro starch digestion were investigated by measuring the
12 released glucose content during starch digestion. Fig. 2 shows the hydrolysis curves of starches that
13 were compared with those of the reference food (white bread).The hydrolysis kinetics of starches
14 showed similar hydrolysis trend, where the percentage of digested starch increased gradually. As
15 expected, all of the starches selected in this study were found to depress starch hydrolysis by
16 digestive enzymes, compared with the control bread. The rate and extent of starch digestion were
17 the highest in the rice, followed by wheat, tapioca, corn and potato (Fig. 2).
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23 Starch and starchy food can be classified according to their digestibility. Rapidly digestible starch
24 (RDS) and slowly digestible starch (SDS) of different starches are shown in Table 4. RDS is rapidly
25 and completely digested in the small intestine and is associated with more rapid elevation of
26 postprandial plasma glucose whereas SDS is more slowly digested in the small intestine and is
27 generally the most desirable form of dietary starch (Jenkins et al. 1981). The RDS and SDS
28 fractions of starches differed significantly ($P < 0.05$) with botanical source. The RDS content was in
29 the range of 2.8 - 16.9%, while the SDS contents ranged from $5.4 \pm 0.4\%$ of potato starch to $12.8 \pm$
30 0.6% of rice one. Rice starch showed significantly higher RDS, SDS (Table 4) in comparison to
31 other starches. Rice had the highest digestibility because of its small granules, as described in
32 microstructural and particle results (Fig. 1 b, f). In fact the digestibility properties are directly
33 related to the particle size, the smaller the particle the higher the digestibility. Since enzyme
34 hydrolysis took place first on the surface of starch granules, the particle size of the starch and the
35 surface area to starch ratio play in fact an important role for the digestibility: a smaller granule size
36 as rice (Fig. 1 b, f) shows higher enzymatic susceptibility, while a large, compact and smooth
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1 surface as for example potato granules (Fig. 1e,f) can explain significantly the lowest results of
2 nutritional fractions: 2.8% (RDS) and 5.4% (SDS) and the resistance of potato granules against
3 enzymatic digestion. The SDS present in raw starch agree with the ΔH results (Table 3) too.
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5 Samples with the lowest SDS contents, as potato and corn, had in fact higher enthalpy values than
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7 other starches. The relationship between thermal properties and digestibility of starches seems
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9 concrete for all the selected starches in this study.
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14 The expected glycemic index (eGI) for different starches are shown in Table 4. eGI results differed
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16 significantly varying between 61.3 of rice to 43.5% of potato (Table 4). The order of eGI starch was
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18 rice > wheat > tapioca > corn > potato. The eGI was affected by the amount of RS (Table 1) and
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20 RDS present. In particular, the RS content had an inverse relationship with eGI, while RDS had
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22 positive and significant influence on eGI. Higher percentages of RDS in starch generally relate to a
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24 higher degree of starch digestion and consequently with a higher degree of eGI (Englyst et al. 1999;
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26 Rosin et al. 2002). eGI is related to nutritional quality of food and a product with a low GI is
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28 preferable not only in individuals with diabetes, but also in healthy individuals (Björck and Asp
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30 1994). Considering the in vitro digestibility results of tapioca, it might be a potential alternative to
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32 corn starch in the formulation of products for diabetics and weight management and could lead to
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34 the formulation of novel foods characterized by the slow release of glucose, that is to say low
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36 glycemic index and prevention of fasting hypoglycemia. Moreover, the bland taste of the tapioca
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38 could represent an advantage over the cereal starches as uncooked ingredient because of the absence
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40 of the specific cereal flavour due to the lipids present in these starches (Moorthy 2004).
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51 **4. Conclusions**

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53 Significant differences in microstructure, properties, in vitro digestibility and expected glycemic
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55 index were observed among starches of different botanical sources (cereal, tubers and roots).
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58 Structural characterization of starches by means of SEM and particle size analyses showed
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60 characteristic differences concerning shape and size of granules attributed to the botanical origin,
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1 which determines morphology and crystalline organization. The examined (physicochemical,
2 functional, thermal and nutritional) properties of starches were significantly different and correlated
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4 to the internal structure of starches. In particular, gelatinization thermal properties might be an
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6 important parameter for the indication of starch digestibility in processed foods. The in vitro
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8 digestibility and expected glycemic index of potato, corn, tapioca were significantly lower than
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10 wheat and rice. Among the studied starches, tapioca was well comparable to corn starch in
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12 physicochemical and thermal properties and in nutritional potentials (resistant starch, slowly
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14 digestible starch and expected glycemic index), therefore it could serve as an alternative to
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16 uncooked corn starch.
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21 The information gathered through this investigation may help the food industry to choose suitable
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23 starches for the development of novel foods or native starch blends with altered digestibility (slow)
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25 and tailored functional properties.
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Captions to Figures

Fig 1 SEM images of starch granules at magnification 4000x of: a) corn, b) rice, c) wheat, d) tapioca, e) potato and f) Particle size distribution of starches: (■) corn, (●) rice, (▲) wheat, (□) tapioca and (Δ) potato.

Fig 2 Total starch hydrolysis rate of (◆) white bread and of starches: (■) corn, (●) rice, (▲) wheat, (□) tapioca, (Δ) potato.

Table 1 Nutritional fractions of starches, expressed as means \pm S.D.

Starch source	RS (%)	Non RS (%)
Corn	1.44 ^b \pm 0.03	91.11 ^b \pm 1.2
Rice	0.31 ^a \pm 0.05	92.05 ^c \pm 0.7
Wheat	1.07 ^a \pm 0.07	90.07 ^b \pm 1.2
Tapioca	1.77 ^b \pm 0.05	91.61 ^b \pm 1.1
Potato	69.59 ^c \pm 1.77	22.21 ^a \pm 1.3

^{a-e} Means within the same column with different letters are significantly different ($P < 0.05$; Duncan test)

1 **Table 2** Properties of starches, expressed as means \pm S.D.

Starch source	pH	moisture content (%)	Solubility (%)		WHC (%)
			20°C	70°C	
Corn	6.43 ^b \pm 0.1	10.09 ^b \pm 0.8	0.50 ^a \pm 0.2	3.81 ^a \pm 0.2	69.24 ^a \pm 1.2
Rice	6.83 ^d \pm 0.0	10.03 ^b \pm 0.2	0.75 ^a \pm 0.1	3.53 ^a \pm 0.4	99.99 ^d \pm 1.3
Wheat	6.62 ^c \pm 0.3	12.68 ^c \pm 0.9	1.28 ^b \pm 0.4	3.27 ^a \pm 0.1	82.75 ^b \pm 1.5
Tapioca	4.86 ^a \pm 0.1	9.29 ^a \pm 0.8	2.43 ^c \pm 0.2	16.46 ^c \pm 0.1	91.42 ^c \pm 1.2
Potato	7.26 ^e \pm 0.5	18.06 ^d \pm 1.4	1.18 ^b \pm 0.1	11.21 ^b \pm 0.9	83.60 ^b \pm 1.6

2 ^{a-e} Means within the same column with different letters are significantly different ($P < 0.05$; Duncan test)

Table 3 Gelatinization thermal parameters of the analyzed starches, expressed as means \pm S.D.: To, onset temperature; Tp, peak temperature; Δ H, transition enthalpy; PHI, peak height index.

Starch source	To (°C)	Tp (°C)	ΔH (J / g)	PHI
Corn	67.15 ^d \pm 0.9	73.26 ^d \pm 0.8	3.75 ^d \pm 0.2	0.64 ^c \pm 0.2
Rice	60.28 ^b \pm 0.9	67.85 ^b \pm 0.6	2.29 ^a \pm 0.1	0.17 ^a \pm 0.0
Wheat	58.77 ^a \pm 0.7	65.41 ^a \pm 0.4	2.64 ^b \pm 0.1	0.41 ^{a,b} \pm 0.1
Tapioca	65.14 ^c \pm 0.1	72.14 ^{c,d} \pm 0.7	3.11 ^c \pm 0.0	0.45 ^{b,c} \pm 0.0
Potato	69.18 ^e \pm 0.6	78.71 ^e \pm 0.4	8.23 ^e \pm 0.4	0.86 ^d \pm 0.0

^{a-e} Means within the same column with different letters are significantly different ($P < 0.05$; Duncan test)

Table 4 Effect of botanical origin on starch nutritional fraction (RDS, rapidly digestible starch, and SDS, slowly digestible starch) and expected glycemic index (eGI) of the analyzed starches. Each value is expressed as means \pm S.D.

Starch source	RDS (%)	SDS (%)	eGI
Corn	2.95 ^a \pm 0.9	12.84 ^b \pm 0.8	47.9 ^b \pm 0.1
Rice	16.90 ^d \pm 0.9	31.95 ^e \pm 0.6	61.3 ^e \pm 0.2
Wheat	14.85 ^c \pm 0.7	29.41 ^d \pm 0.4	58.8 ^d \pm 0.1
Tapioca	9.55 ^b \pm 0.1	23.49 ^c \pm 0.7	55.2 ^c \pm 0.0
Potato	2.83 ^a \pm 0.6	5.35 ^a \pm 0.4	43.5 ^a \pm 0.1

^{a-e} Means within the same column with different letters are significantly different ($P < 0.05$; Duncan test)

Fig 1

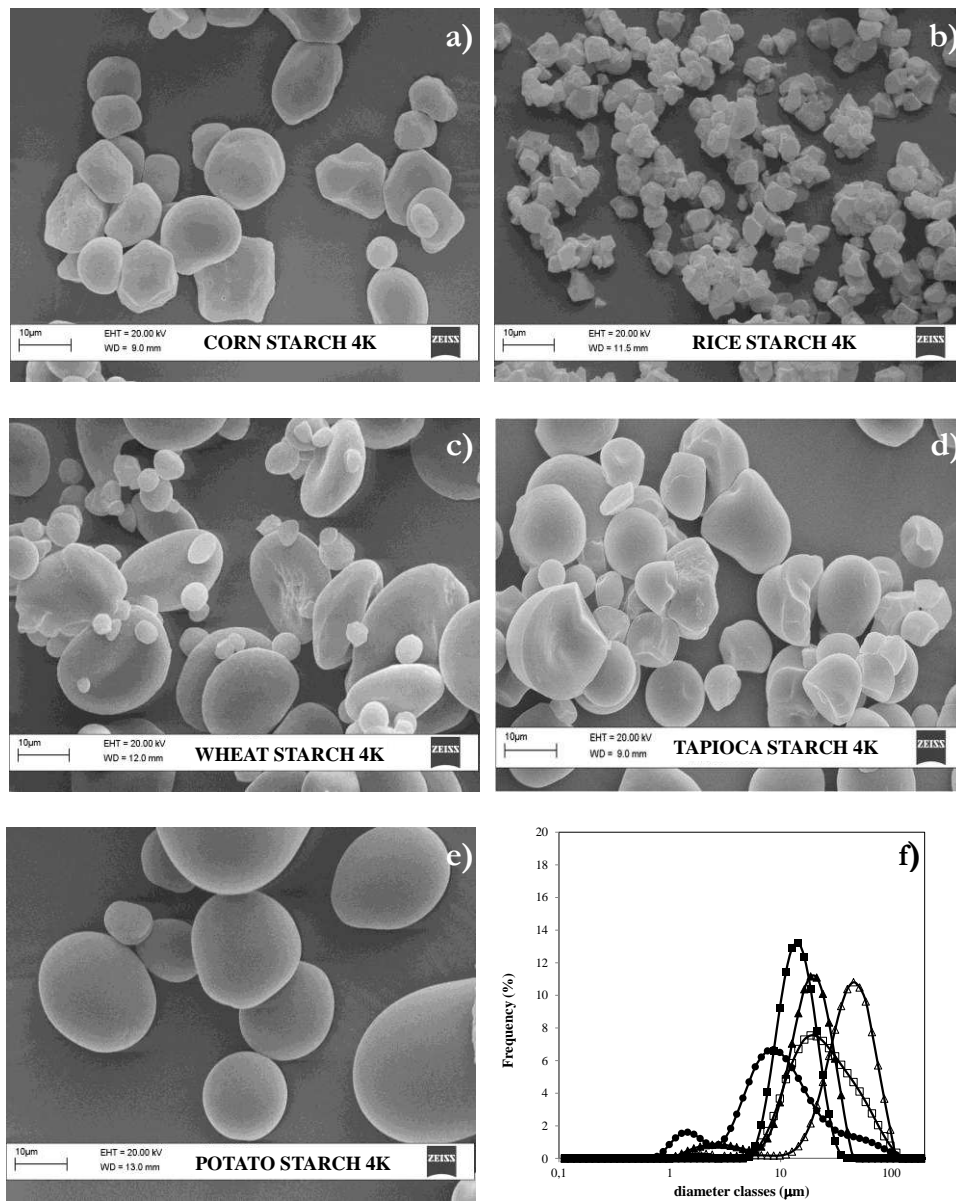


Fig. 2

