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Full Length Research Paper

Influence of starch source in the required hydrolysis time for the production of maltodextrins with different dextrose equivalent

José Luis Montañez Soto[1]*, Luis Medina García[1], José Venegas González[1], Aurea Bernardino Nicanor[2] and Leopoldo González Cruz[2]

[1]Centro Interdisciplinary Research for Regional Integral Development, National Polytechnic Institute, Unit Michoacan. Justo Sierra 28, Jiquilpan, Mich. C. P. Mexico 59510, Mexico.
[2]Technological Institute of Celaya, Technology and Garcia Cubas Av s/n. C.P. 38010, Celaya, Guanajuato, Mexico.

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The starches were statistically different ($p < 0.05$) in their protein, ash, fat, phosphorus, amylose and amylopectin content. The amylose/amylopectin ratios in corn, potato and rice starches were 0.389, 0.282 and 0.220, respectively. The phosphorus content in the same order of starches was: 0.15 ± 0.01; 0.80 ± 0.02 and 0.95 ± 0.02 g/kg, respectively. The chemical composition of the different starches, and specifically its amylose and amylopectin content, its phosphorus content and the way it is bound to the starch molecule, affect the functional properties like the viscosity of gels and the enzymatic hydrolysis rate of these molecules. The rice starch is easily hydrolyzed by the α-amylase enzyme from *Aspergillus oryzae* and therefore, it required less time to obtain maltodextrin by enzymatic hydrolysis from rice starch compared with those from corn or potato starch. Under saturation conditions of the enzyme, the dextrose equivalent content was proportional to the hydrolysis time, regardless of the starting starch source.

Key words: Starch, maltodextrins, dextrose equivalent, amylose, amylopectin.

INTRODUCTION

The native starch has an industrial restrictive use due to its few functional properties, and for increasing them, it is necessary to make some physical, chemical or enzymatic changes in its molecule, increasing the range of products with specific characteristics which could cover a wide range of applications in various fields of food and pharmaceutical industries (Rocha et al., 2005). An easy and quick way to obtain carbohydrates with specific functional properties is through the hydrolysis of starch.

*Corresponding author. E-mail: montasoto@yahoo.com.mx. Tel: 353-53-302-18.

Abbreviations: ADP, Average degree of polymerization; **GRAS,** generally recognized as safe; **FDA**, food and drug administration; **DRS**, direct reducing sugars; **TRS,** total reducing sugars**; DE**, dextrose equivalents; **DB**, dry basis; **min,** minutes.

The dextrose equivalent (DE) is a measure of the degree of hydrolysis of the starch molecule which is defined as the direct reducing sugar content (ARD) expressed in percent glucose on a dry basis. Depending on the degree of hydrolysis of the starch molecule is obtained a range of products that, according to its DE content, they are classified on maltodextrins and syrups; maltodextrins has a DE < 20, whereas syrups has DE ≥ 20 (McPherson and Seib, 1997).

The maltodextrins are defined by Food and Drug Administration (FDA) as a mixture of nutritive carbohydrate, non-sweet, with different degree of polymerization, consisting of D-glucose units joined by glucosidic bounds α(1,4) and α(1,6), which together have a DE < 20, are presented as a white powder or concentrated solutions and they are classified as ingredients generally recognized as safe (GRAS) (Marchal et al., 1999; Storz and Steffens, 2004). In the food industry, maltodextrins are used to supply nutritional value, to give

consistency and texture, control sweetness and hygroscopicity, control the freezing point, to prevent the crystallization and the non-enzymatic browning, to regulate osmolality, spray-drying aid, are excellent fat replacers, film formers and not mask flavors (Chronakis, 1998; Herrera et al., 2000; Wang and Wang, 2000).

The main starch commercial sources for maltodextrins industrial production are: corn, potatoes and rice, but they can also be obtained from a variety of starchy materials such as tapioca, wheat, sorghum, etc., which depend on the availability and price of the raw materials produced in each country (Jimenez et al., 2007; Antonio et al., 2009; Jing et al., 2011). Maltodextrins are obtained industrially by controlled hydrolysis of starch, either by use of acids, enzymes, or by combination of both (Lumdubwong and Seib, 2001). The carbohydrate profile of the maltodextrins obtained, that is, its average degree of polymerization (GPP), the linearity and the degree of branching of carbohydrates which constitute them, is influenced by the source and concentration of the starting starch, the method and the hydrolysis conditions (temperature and time), as well as the type and concentration of the used enzyme in this process, which means that there may be maltodextrins with the same DE, but different molecular composition, linearity and branching of the carbohydrates integrate them, giving them different physicochemical and functional properties to each of them (Chronakis, 1998; Marchal et al., 1999). Based on the differences in chemical composition and structure of the starting starch, the enzymatic hydrolysis time required to obtain desired maltodextrin with DE will be different for each type of starting starch. The objective of this research was to determine how it affects the composition of the starch source, the required enzymatic hydrolysis time for the production of maltodextrins with different DE in a batch reactor.

MATERIALS AND METHODS

The starches of corn, potato, rice and the soluble were obtained from (SIGMA, Mexico). The α-amylase enzyme [α(1,4)-D-glucan-glucanohydrolase, EC 3.2.1.1.] from *Aspergillus oryzae* was obtained from (SIGMA, United States of America). It contains 33.30% protein and presents 31 units of activity per milligram of solid. One unit of activity is defined as the amount of enzyme required to release one milligram of maltose from starch in a time of three minutes at pH 6.9 and 20°C. Kit K-TSTA for the determination of total starch was obtained from (MEGAZYME, Ireland). All other reagents were analytical grade.

Starches partial characterization

The moisture content, ash, protein and fat in the different starches was determined according to the methodology described in the AOAC (1997), total carbohydrates were obtained by difference. The total starch was quantified by the method described by Rose et al. (1991), which uses a digestion mixture integrated by the enzymes α-amylase (EC.3.2.1.1) and amyloglucosidase (EC.3.2.1.3) (K-TSTA Megazyme, Ireland) in a 200:1 w/w ratio, respectively.

Amylose content was determined colorimetrically (Farhat et al., 1999) and the amylopectin content was obtained as the difference between the total starch and amylose. Finally, the phosphorus content was obtained according to the method described by Smith and Caruso (1964).

Temperature effect on enzymatic activity

The effect of temperature on enzyme activity α-amylase from *A. oryzae* was carried out by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959), which is based on quantification of the reducing groups caused by the action of the enzyme on the starch molecule and it is adequate because glucose is the unique reducing sugar (López et al., 2004). The hydrolysis conditions were: substrate concentration, 1%; pH, 6.9; enzyme concentration, 2 µg/mL; reaction time, 10 min and temperatures, from 20 to 70°C. Soluble starch dissolved in 0.02 M phosphate buffer and 0.07 M NaCl, pH 6.9 was used as substrate. The starch was gelatinized previously by heating to boiling for 5 min and continuous stirring. The developed color was read at 540 nm and it was interpolated in a maltose standard curve (100 to 1000 µg/mL) to determine the specific activity of the enzyme at each temperature.

Changes in the starch gel viscosity with the hydrolysis time

The changes of gels viscosity of corn, rice and potato starches caused by the action of α-amylase from *A. oryzae* were monitored using a Brookfield viscometer LVT model, serial number 59073 (Anton Paar, USA). To this effect, 500 g of the dispersions with 10% w/w of each of the starches were prepared in beakers of 600 mL initially, using 0.02 M phosphate buffer plus 0.07 M NaCl and pH 6.9 as solvent. To achieve complete dissolution and gelatinization of starch, the dispersions were heated to boiling with constant stirring for 5 min, and then they were cooled and placed in a water bath at 50°C. Subsequently were added 1000 µg of the α-amylase enzyme, which is equivalent to (2 µg/g). The mixture was kept under constant stirring and its apparent viscosity was determined every 5 min for 2 h.

Dextrose equivalent variation with the hydrolysis time

Using the procedure described above, in 2 L beakers, 1000 g of gels with 10% w/w of each of the starches were prepared and studied. Then the beakers were placed in a water bath at 50°C and agitated slightly once, after which 2000 µg of α-amilase enzyme was added (2 µg/g). The mixtures were kept under constant stirring and every 15 min, samples of 50 g were taken, which were boiled for 3 min immediately in order to inactivate the enzyme. Subsequently the direct reducing sugars (DRS) were determined in the samples and with these; the DE content was finally obtained (Delheye and Moreels, 1988).

Production of maltodextrins with 5, 10, 15 and 20 DE

Once the relationship between the DE and the hydrolysis time for each starch source under the hydrolysis conditions was established (substrate concentration, 10% w/w; enzyme concentration, 2 µg/g; pH, 6.9 and temperature, 50°C), we proceeded to validate these relationship. To this effect, 2 kg of gels 10% w/w were prepared initially, pH 6.9 of each of the starches used as substrate, according to the procedure described above. The containers with the substrates were placed in a water bath at 50°C and shaken once, then α-amylase enzyme was added to give a final concentration of 2 µg/g. The reaction mixture was homogenized and kept on constant

Table 1. Chemical composition of starches.

Component (g/kg)	Corn starch	Potato starch	Rice starch
Moisture	81.5±1.5[a]	85.2±2.0[a]	83.5±1.8[a]
Ash	2.4±0.1[a]	4.5±0.2[b]	5.8±0.2[c]
Protein	4.5±0.1[a]	1.5±0.1[b]	5.9±0.2[c]
Lipid	6.8±0.2[a]	1.3±0.1[b]	8.6±0.2[c]
Carbohydrates	904.8±2.8[a]	907.5±3.0[a]	896.2±2.5[a]
Total starch	912.6±2.5[a]	906.2±2.2[a]	897.8±1.8[a]
Amylose	255.5±2.0[c]	199.5±1.5[b]	161.6±2.0[a]
Amylopectin	657.1±0.4[a]	706.7±0.6[b]	736.2±0.2[c]
Amylose/amylopectin	0.389±0.002[a]	0.282±0.002[b]	0.220±0.002[c]
Phosphorus	0.15±0.01[a]	0.80±0.02[b]	0.95±0.02[c]

Average values of three measurements ± standard deviation. Different letters in the same row indicate statistically significant differences (Tukey $p < 0.05$) for each determination.

agitation and to each of the theoretical required hydrolysis times on each substrate were separated 500 g of sample to obtain maltodextrins with 5, 10, 15 and 20 DE, respectively. Immediately after, the samples were heated to boiling for 3 min to inactivate the enzyme and then cooled and dried in cold by using a Labconco lyophilizer (Supplier Scientific, Mexico). The obtained powders were analyzed to determine its moisture content, DRA and the DE, according to the methodology described above.

Statistical analysis

All analyses were done in triplicate and data corresponds to mean values ± standard deviation of the series. An analysis of variance and the Tukey test was applied to a confidence level of 95% to determine statistically significant differences ($p < 0.05$) between samples.

RESULTS AND DISCUSSION

Starches partial characterization

There were no statistically significant differences ($p < 0.05$) in moisture content, carbohydrates and total starch between the different starches, but they were differences in the content of the rest of its components like ash, proteins, lipids, amylose, amylopectin and phosphorus (Table 1). The ash and phosphorus content were directly related, both were higher in the rice starch and lower in the corn starch. The ashes of the starches are composed of inorganic ions such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} and P^{5+} mainly, many of which came from the process water (Absar et al., 2009; Dona et al., 2010). Even after the extraction and purification process, the proteins and lipids are present in minute quantities in each starch, because they form complexes with the components that integrate the cover of the native starch granules and these minor constituents provide specific characteristics to each starch type (Lovedeep et al., 2002). It has been reported that lipids present on starches negatively affects their functional properties such as: water absorption capacity, solubility and clarity, by preventing binding to water molecules, also they are responsible for rancidity during storage of the starch (Wolfgang et al., 1999). Additionally, starches with high protein content are prone to Maillard reactions during its liquefaction; reactions in which undesirable colorations are generated (Bello et al., 2002).

On the other hand, the amylose content was higher in the potato starch, followed by the corn starch and finally by the rice starch (Table 1). In all starches, the content of amylose was lower than the amylopectin, and therefore, the relationship between both was always less than unity. It is reported that the amylose is found in greater proportion in starch from cereals than in starch from tubers or roots (Beynum and Roels, 1985). The amylose and amylopectin ratio provide distinctive characteristics specific to each type of starch; it is critical to obtain gels with good mechanical properties because it affects its solubility and degradability (Aboubakar et al., 2007). In general, the amylose and amylopectin contents depend on the starch source and affects the degree of branching and the average degree of polymerization (ADP) of the maltodextrins obtained, and hence affects their functional properties too (Chronakis, 1998; Rocha et al., 2005).

Temperature effect on enzymatic activity

In most study on maltodextrins production, the temperature at which the starch enzymatic hydrolysis is performed is selected from the specifications of the supplier of the enzyme, and it has now made a study of the effect of temperature on enzyme activity with respect to its temperature of maximum activity and stability possible (Delgado et al., 2009). Based on the fact that temperature directly affects the enzyme activity by increasing the kinetic energy of molecules that favor the intermolecular collisions, it therefore increases the effective number of collisions that lead to the breakup of enzyme-substrate complex, given rise to product formation and release of the enzyme to initiate a new cycle of activity. The study of

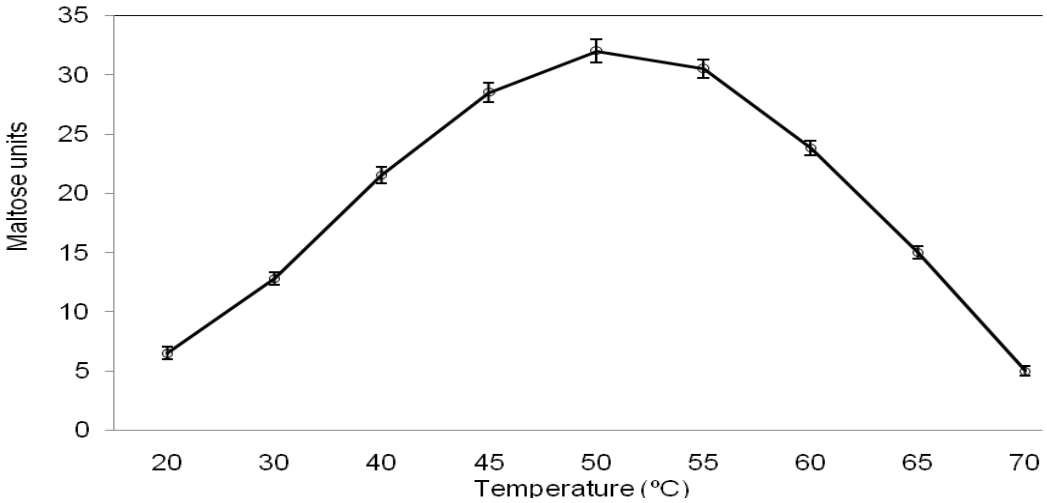

Figure 1. Effect of temperature on enzymatic activity of α-amylase from *Aspergillus oryzae*. Conditions: t = 10 min, [S] = 1.0% and [E] = 2 µg/ml, pH 6.9.

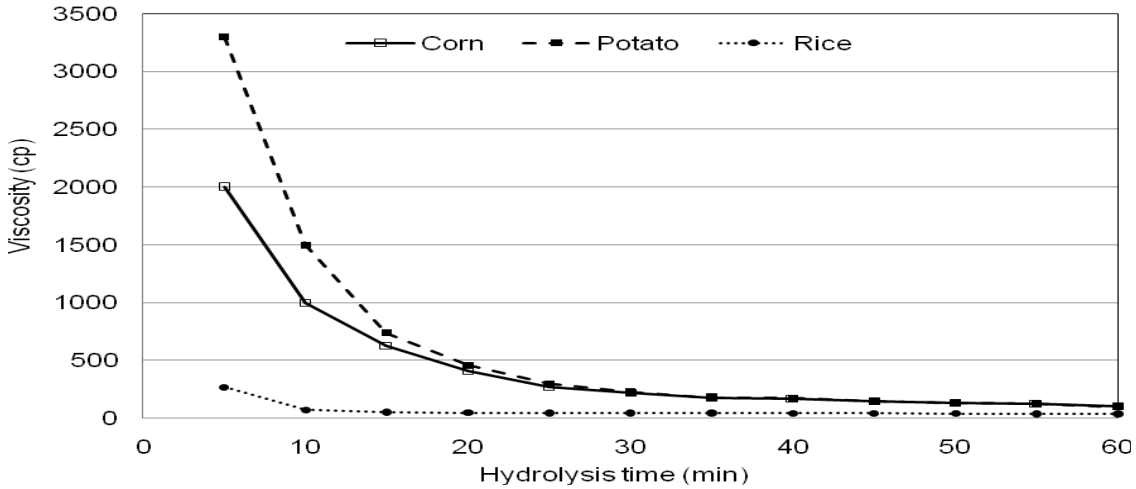

Figure 2. Effect of decreasing the viscosity of the starch gels due to the action of α-amylase from *Aspergillus oryzae*. Conditions: T = 50°C, [S] = 10% at pH 6.9 and [E] = 2 µg/ml.

the effect of temperature on the enzymatic reaction rate allows us to determine the temperature of maximum activity of the enzyme (temperature above which it usually starts its inactivation), as well as optimization of the enzymatic process, which must operate at a temperature of maximum activity and stability of the enzyme (Baks et al., 2008). In the range of 20 to 50°C, the rate of starch hydrolysis catalyzed by α-amylase enzyme from *A. oryzae*, was directly proportional to the temperature of the reaction, reaching the maximum rate at 50°C. Temperatures above 50°C cause the enzyme inactivation (Figure 1). It has been reported that maltodextrins prepared at different temperatures also have different physicochemical and functional properties, because high temperature of digestion give rise to polymers with a heterogeneous distribution of molecular weights (Marchal et al., 1999).

Changes in the starch gel viscosity with the hydrolysis time

The potato starch gels developed higher viscosity than corn starch gels and this in turn gave greater viscosity than rice starch gels (Figure 2). The viscosity of the gels decreases sharply during the first minutes of enzyme activity on the constituents of the starch, which is indicative tha the α-amylase from *A. oryzae* is an enzyme with endo-hydrolytic activity that is, broken internal bonds of the molecules and generates polymers with lower

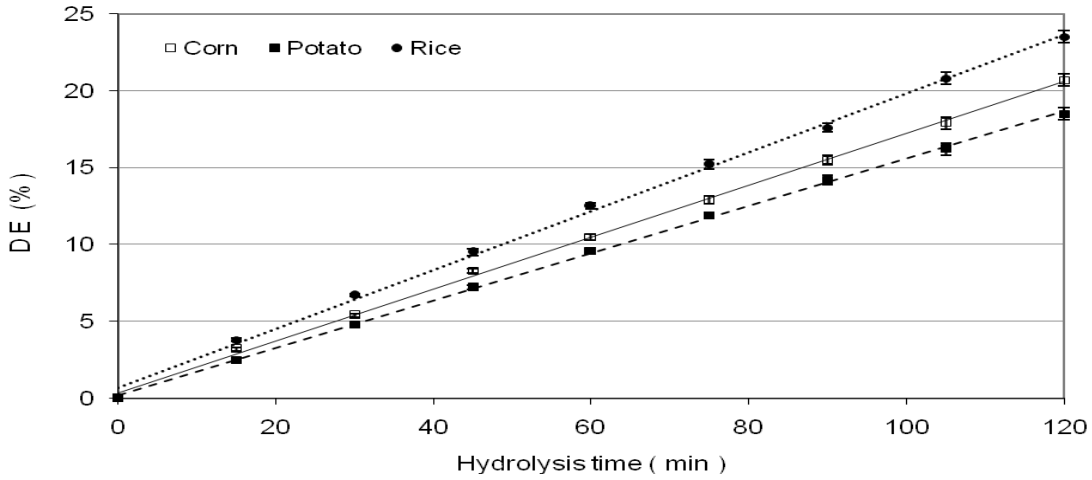

Figure 3. Variation of dextrose equivalent with hydrolysis time of the different starch gels. Conditions: T = 50°C, [S] = 10% at pH 6.9 and [E] = 2 µg/ml.

molecular weight. This decrease on gels viscosity is reflected in a decrease of the overall resistance of the sample to flow (Khatoon et al., 2009). The viscosity of potato starch gel is equal to the viscosity of corn starch gel at 30 min of hydrolysis ($p < 0.05$). At higher hydrolysis time, a minimal decrease of the viscosity of gels was observed.

It has been reported that under the same preparation conditions (pH, temperature and concentration), the starches from tuber and root such as potatoes and tapioca produce gels with higher viscosity than cereal starches, which is due to different power swelling presenting each of the starches, owing to their different chemical composition, amylose/amylopectin ratio, ADP of its constitutes, as well as its lipid and phosphorus content (Absar et al., 2009). Beynum and Roels (1985) reported that the phosphorus content in starches and the chemical form in which it is in the starches molecule has a significant influence on the functional properties of these materials. In cereal starches, phosphorus is present mainly as phospholipids, while the potato starch phosphorus is as phosphate groups which are esterified to carbon 6 of the glucopyranosyl residues of the amylopectin molecule. The presence of these phosphate groups gives the starch granule a negative surface charge which imparts properties of polyelectrolyte when the potato starch is dispersed in water, by this, the granules of potato starch presents a fast granule swelling and a sharp increase in the viscosity of the dispersions, compared with cereal starches (Absar et al., 2009; Khatoon et al., 2009).

Starches with high amylose form gels with good mechanical properties and high resistance to chemical or enzymatic degradation, but have the disadvantage of being poorly soluble, are opaque and systems have a tendency of retrogradation, process which involves the insolubilization and spontaneous precipitation of amylose molecules, mainly because their linear chains are oriented in parallel and interacting through its multiple hydroxyl groups, through hydrogen bonds (Dona et al., 2010). According to our results, the initial viscosity developed by the gels of the different starches cannot be attributed only to its content ratio of amylose and amylopectin or its phosphorus content, but seems to be due more to the manner in which the phosphorus is bonded to the starch molecule, since due to their higher content of phosphorus, the rice starch gels had to develop higher viscosity than the potato starch gel, which was not so.

Dextrose equivalent variation with the hydrolysis time

Under conditions of saturation of the enzyme, the DE content was directly proportional to the hydrolysis time, regardless of the starting starch source (Figure 3). The hydrolysis rate (slope of the line) was greater with the rice starch (0.19 DE/min), less in the corn starch (0.17 DE/min) and even less in the potato starch (0.15 DE/min), which can be attributed to the different affinity of the enzyme by each starting starches, given the chemicals and structural differences of the starch molecules.

Dona et al. (2010) mentioned that the susceptibility of starch granules to its enzymatic hydrolysis depends on a set of factors that include its solubility, granular structure, crystallinity, the method of preparation and the nature of the starch, and those molecules bound to it for example, fiber, fat and protein. It has been found that starches with higher amylose content are more resistant to enzymatic hydrolysis (Polakovic and Bryjak, 2004; Rocha et al., 2005). Absar et al. (2009) found that the enzymatic hydrolysis rate of tuber and root starches like potatoes and tapioca was not greatly influenced by their amylose content and median granule size, but it is influenced by

Table 2. Dextrose equivalent (DE) and hydrolysis time (t) ratio for each starch.

Starch source	DE with (t)* ratio
Rice	DE=0.1917(t)+0.68
Corn	DE=0.1686(t)+0.38
Potato	DE=0.1540(t)+0.19

*t, Hydrolysis time (min).

Table 3. Deviation between the DE-expected and the DE-real of the maltodextrins obtained from the different starch sources.

Starch source	DE-expected (%)	Time (min)	DE-real (%)	Deviation (%)
Rice	5.00	22.5	5.79±0.05	15.80±1.0
	10.00	48.6	9.76±0.08	2.40±0.8
	15.00	74.7	14.54±0.10	3.07±0.7
	20.00	100.8	20.18±0.15	0.90±0.4
Corn	5.00	27.4	5.93±0.05	18.60±1.0
	10.00	57.1	10.49±0.08	4.90±0.8
	15.00	86.7	15.37±0.10	2.53±0.7
	20.00	116.4	20.19±0.16	0.95±0.8
Potato	5.00	31.2	6.10±0.06	22.00±1.2
	10.00	63.7	10.13±0.08	1.30±0.8
	15.00	96.2	15.01±0.10	0.07±0.3
	20.00	128.6	20.1±0.12	0.50±0.5

their content of phosphorus which negatively affects the enzymatic hydrolysis of starch molecules. It has been reported that the rice starch has an average diameter of 5 microns, while the average diameters of the corn starch and potato are 15 and 33 microns, respectively (Beynum and Roels, 1985). According to this, the average diameter of the granules seems to be the dominant factor affecting the rate of enzymatic hydrolysis of different starches. Another important factor affecting the rate of enzymatic hydrolysis of starch is its content of phosphorus and more than this, the manner in which the phosphorus is bonded to the starch molecule.

Table 2 shows the relation between DE with the hydrolysis time (t) for the different starches and under the working conditions established. These linear relationships between DE and the hydrolysis time under conditions of saturation of the enzyme have been observed already by other investigators (Jimenez et al., 2007). It should be understood that these mathematical expressions were obtained for a given concentration of enzyme used in the hydrolysis process, so that in practice, according to the provisions and needs of the business, it is possible to decrease or increase the required hydrolysis times to obtain the various maltodextrins, with only increase or decrease, respectively in the enzyme concentration in the hydrolysis process (Baks et al., 2008).

Production of maltodextrins with 5, 10, 15 and 20 DE

The relationships between DE and hydrolysis time, predicted satisfactorily the required hydrolysis time to produce maltodextrins with 5, 10, 15 and 20 DE from respective starch source, except for maltodextrins with 5 DE, since in this case, there was a difference of 15.8, 18.6 and 22.0% between the theoretical DE with real DE of the maltodextrins obtained from rice starch, corn and potato, respectively (Table 3). These differences are due to the manner in which the experiment was carried out, since during the first minutes of hydrolysis, the viscosity of the gels is very high and difficult to sample at the end of the required hydrolysis time, which hinders the rapid inactivation of the enzyme and therefore, the obtained maltodextrins had a DE greater than expected in all cases. It is convenient to perform the hydrolysis separately for each one of searched maltodextrins, since in this way, at the end of the required hydrolysis time, it is easier for the inactivation of the enzyme immediately and thus, the DE of the obtained maltodextrin would be closest to the DE of the expected maltodextrin.

Conclusions

There were significant statistical differences ($p < 0.05$) in the chemical composition of the different starches studied as to their content of ash, fat, protein, phosphorus, amylose and amylopectin. It is very important to know the temperature effect on the enzymatic reaction rate to determine its temperature of activity maximum and thus establish the conditions of the enzymatic process at temperature of maximum activity and enzyme stability.

The viscosity of the gels is influenced by the content of phosphorus, and more specifically, by the way in which the phosphorus is bound in the starch molecule. The average diameter of the granules seems to be the dominant factor affecting the rate of enzymatic hydrolysis of different starches. The chemical composition of the different starches, and specifically its amylose and amylopectin content, its phosphorus content and the way it is bound to the starch molecule, affect the functional properties like the viscosity of gels and the enzymatic hydrolysis rate of these molecules by the α-alpha-amylase enzyme from *A. oryzae* and therefore, the required hydrolysis time to obtain maltodextrins from each one of them.

The dextrose equivalent was directly proportional to the hydrolysis time of the different starches under the enzymatic hydrolysis conditions established in the process of obtaining maltodextrins. The rice starch is easily hydrolyzed by the enzyme α-amylase from *A. orizae* compared with corn or potato starch and therefore, it required less time to obtain maltodextrin by enzymatic hydrolysis of starch from rice and higher times from corn or potato starch.

The gels of potato starch developed an initial viscosity greater than corn or rice starch gels. The starch chemical composition and specifically its lipids, proteins, phosphorus, amylose and amylopectin content, have great influence on the starch susceptibility to its enzymatic hydrolysis, as well as its physicochemical and functional properties.

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