Carbohydrate Polymers 101 (2014) 1176–1183

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Production of resistant starch by enzymatic debranching in legume flours

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ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form 9 October 2013
Accepted 9 October 2013
Available online 26 October 2013

Keywords:
Resistant starch
Legume
Pullulanase
Hydrolysis
Enzyme

ABSTRACT

Resistant starch (RS) was produced by enzymatic hydrolysis of flours from five different legumes: lentil, chickpea, faba bean, kidney bean and red kidney bean. Each legume was firstly treated thermally, then hydrolyzed with pullulanase for 24 h at 50 °C and pH 5 and lyophilized. At the end of each hydrolysis reaction, the RS amount ranged from 4.7% for red kidney beans to 7.5% for chickpeas. With respect to the curves of RS against hydrolysis time, a linear increase was observed initially and a plateau was generally achieved by the end of reaction. These curves were successfully modeled by a kinetic equation including three parameters: initial RS, RS at long operation time and a kinetic constant (k). Furthermore, the relative increase in hydrolysis, calculated using the kinetic parameters, was successfully correlated to the percentage of amylose.

1. Introduction

Legumes are a good source of slow release carbohydrates, possess a high level of proteins (18–25%) and contain minerals and vitamins. All these characteristics provide legumes beneficial physiological effects such as preventing and controlling metabolic diseases: diabetes mellitus, colon cancer and coronary heart disease (Tantamango, Knutsen, Beeson, Fraser, & Sabate, 2011; Wilson, 2013; Zhu, Jiang, & Thompson, 2012). When comparing legumes starches to cereals ones, the main difference is that the former have poor digestibility and lower glycemix index (GI) which causes slow promotion of postprandial glucose and insulin responses (Jenkins, Wolever, & Taylor, 1980). Current opinion on healthy eating habits may result in an increase the proportion of legume starches as an alternative to cereals (Tharanathan & Mahadevamma, 2003).

Starch is mainly composed of two different types of anhydroglucosepolymers (amylopectin and amylose) linked by α-(1,4) bonds in linear segments. In addition the former has branching points connected to the main chain by α-(1,6) bond. Amylose molecules possess a double helical structure which confers a considerable degree of crystallinity to the molecules (Dona, Pages, Gilbert, & Kuchel, 2010; Pérez & Bertoft, 2010). The structure of crystals is classified into three types: A, B and C, which differs in the degree of compacting. The type C is the most common in legume starches and the most resistant to digestion while type A is the typical one for cereals (Tharanathan & Mahadevamma, 2003).

The term resistant starch (RS) refers to the sum of starch and products of starch degradation which have not been absorbed in the small intestine of healthy humans (Englyst, Kingman, & Cummings, 1992). Moreover, this fraction passes on to the colon where it is fermented by the microflora producing mainly short chain fatty acids (SCFA). Due to this fact, RS has functional properties and positive effects in diabetes, some kinds of cancer, cardiovascular diseases, colonic health, obesity and osteoporosis (Lunn & Buttriss, 2007; Nugent, 2005; Sajilata, Singhal, & Kulkarni, 2006; Tomlin & Read, 1990). The amount of RS mainly depends on its botanical origin, this characteristic being the one that determines the most important variables in order to obtain RS: the amylose to amylopectin ratio, the type and arrangement of the partially crystallized structures of amylose, the average molecular weights and length-chain distribution of the component, and the particle size and enzyme inhibitors present in the medium (Alsaffar, 2011; Eerlingen and Delcour, 1993).

There are four different groups of resistant starch: (i) RS1 has a physically inaccessible form to enzymes, for instance: intact cells, partly milled grains and seeds, (ii) RS2 represents starch which is in a certain granular structure with crystalline regions and retrograded amylopectin, (iii) RS3 is mainly retrograded amylose and (iv) RS4 which is chemically modified starch (Hoover & Zhou, 2003; Sajilata, Singhal, & Kulkarni, 2006). RS1 and RS2 are residues of starch forms with a very slow and incompletely digestion in the small intestine; they become non-resistant starch by the appropriate treatment (milling, boiling, heating or merely chewing).

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http://dx.doi.org/10.1016/j.carbpol.2013.10.027

In the case of legumes, since the content of amylose is higher than cereals, they are thought to be a better raw material so as to produce RS (Yadav, Sharma, & Yadav, 2010). Furthermore, as their protein content is high there is an interaction protein–starch which provides a decrease in the glycemic responses (Geervani & Theophilus, 1981).

RS3 production can be accomplished by thermal or enzymatic processes, as well as with a combination of both. There are two main steps involved in the production of RS3 by thermal treatments: gelatinization and retrogradation (González-Soto, Agama-Acevedo, Solorza-Feria, Rendón-Villalobos, & Bello-Pérez, 2004; Lehmann, Rössler, Schmiedl, & Jacobasch, 2003; Miles, Morris, & Ring, 1985; Morris, 1990; Mutungi, Rost, Onyango, Jaros, & Rohm, 2009). In the former step the granule is totally hydrated and amylose loses its crystalline structure. By retrogradation, which is a cooling process, the amylose chains are reassociated by creating new double helices which are stabilized by hydrogen bonds. Thus, a highly packed crystal formation is achieved creating resistance to digestion by preventing α-amylase access to the glycosidic bonds.

Regarding the enzymatic treatments the main objective is to produce partially debranched amylopectin by using pullulanases. Several studies have been done in order to optimize hydrolysis conditions such as pH, temperature, time of reaction and enzyme to substrate ratio (Hizukuri, Takeda, Yasuda, & Suzuki, 1981; Leong, Karim, & Norziah, 2007; Milašinović, Radosavljević, & Dokić, 2010; Zhang & Jin, 2011). However, as the production depends strongly on the substrate, this optimization is highly specific. This treatment produces free branches which can act as amylose and create highly crystalline structures. Regardless of the method, the product is a modified starch with a higher crystallinity percentage which involves a higher resistance to temperature and hydrolysis by amylases.

The aim of the study was to evaluate the effects of thermal treatment, hydrolysis with pullulanase and lyophilization on the RS content of five legume flours, as well as studying the kinetic behavior during hydrolysis. In addition, the influence of the amylose content in the production of RS by means of enzymatic treatment was studied.

2. Materials and methods

2.1. Substrate

Five kinds of different legume seeds were acquired local supplier lentils (*Lens culinaris medicus*), chickpeas (*Cicer arietinum*), faba beans (*Vicia faba*) and red and white kidney beans (*Phaseolus vulgaris*). Legume flour was obtained by milling raw legume seeds until they could pass through a 500 μm screen.

2.2. Production of resistant starch

Legume samples were prepared by suspending 20% (w/w) of flour in sodium acetate buffer (pH 5.0). Then, the suspensions were submitted to consecutive thermal and enzymatic treatments. First, the samples were heated up to 100 °C for 15 min, diluted to 10% (w/w) with sodium acetate buffer (pH 5.0) and homogenized. In order to carry out the debranching process, it was employed the enzyme Promozyme 2D, a pullulanase (pululan 6-glucanohidrolase, E.C.3.2.1.41) donated by Novozymes (Bagsvaerd, Denmark). The enzyme was added to the homogenized suspension with a concentration of 5% (w/w) and the hydrolysis reaction was maintained at 60 °C for 24 h. During this time interval, samples were taken from the reaction mixture and deactivated by heating at 100 °C for 15 min. Finally, the samples were lyophilized in a Labconco freeze drying system (Kansas City, MO, USA) and stored until analysis for their RS content.

2.3. Determination of resistant, non-resistant and total starch

The RS, non-resistant starch (NRS) and total starch (TS) amount of all the samples was determined in three replicates using the method accepted by the AOAC and the AACC, AOAC Official Method 2002.02 and AACC Method 32-40, respectively. The necessary reagents, enzymes and standards were taken from the Resistant Starch Assay Kit purchased from Megazyme (Wicklow, Ireland).

Briefly, the samples were first conducted to an in vitro digestion with pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37 °C aiming at NRS fraction reduction to glucose. Afterward, the reaction was stopped by adding an equal volume of ethanol (99%). Then, it was washed twice with an ethanol dilution (50%) followed by centrifugation (1000 g, 10 min). RS fraction was contained in pellets while the NRS one remained in the supernatant. Once isolated, RS fraction was dissolved in 2 M KOH (20 min, ice-water bath, vigorous stirring), subsequently the solution was neutralized with acetate buffer (1.2 M). The RS fraction was quantitatively hydrolyzed to glucose by usage of AMG (3300 U/mL, 30 min, 50 °C, vol Substrate:vol Enzyme ratio – 8:0.1). As regards the NRS fraction, supernatant obtained during centrifugation step was diluted to 100 mL and then hydrolyzed with AMG (300 U/mL, 20 min, 50 °C). Finally the amount of NRS and RS was quantified by measuring the absorbance (510 nm) of the product obtained in the enzymatic reaction of the glucose amount with glucose oxidase/peroxidase reagent (GOPOD). The total amount was calculated by adding RS and NRS fractions.

2.4. Determination of amylose content

The amylose content was measured by the usage of the assay procedure K-AMYL 07/11 (Megazyme, Wicklow, Ireland).

Since a purification treatment was required so as to remove the lipid content, the first step consists of the dispersion of the flours in dimethyl sulphoxide by heating and mixing. Consequently, by the addition of ethanol (95% v/v), the starch was precipitated and recovered by centrifugation (2000 g, 5 min). Then, the pellets were dissolved in an acetate/salt solution (600 mM, pH 6.4) and two aliquots were taken in order to determine the amylose and the starch content. The former was mixed with concanavalin A aiming at precipitating amylopectin content which was removed by centrifugation (14 000 g, 10 min). The amylose content of the supernatant was enzymatically hydrolyzed to D-glucose by means of amyloglucosidase, β-maltoside and α-amylase. In the case of total starch determination, an aliquot of the acetate/salt solution was similarly hydrolyzed by the usage of the previously mentioned enzymes. In both cases the amount of D-glucose was measured colorimetrically by glucose oxidase/peroxidase. The absorbance of the samples was measured at 510 nm and the amylose content of the starch was calculated.

3. Results and discussion

3.1. Content of amylose, resistant and total starch in raw legume flours

The percentage of amylose in raw legume flours does not vary widely (Fig. 1). The highest value (27.9 ± 1.1%) corresponds to lentils followed by kidney beans (26.1 ± 0.7%). The lowest one (21 ± 0.6%) to red kidney beans, whereas chickpeas (23.0 ± 1.0%) and faba bean (23.73 ± 0.4%) amylose content had an intermediate value.

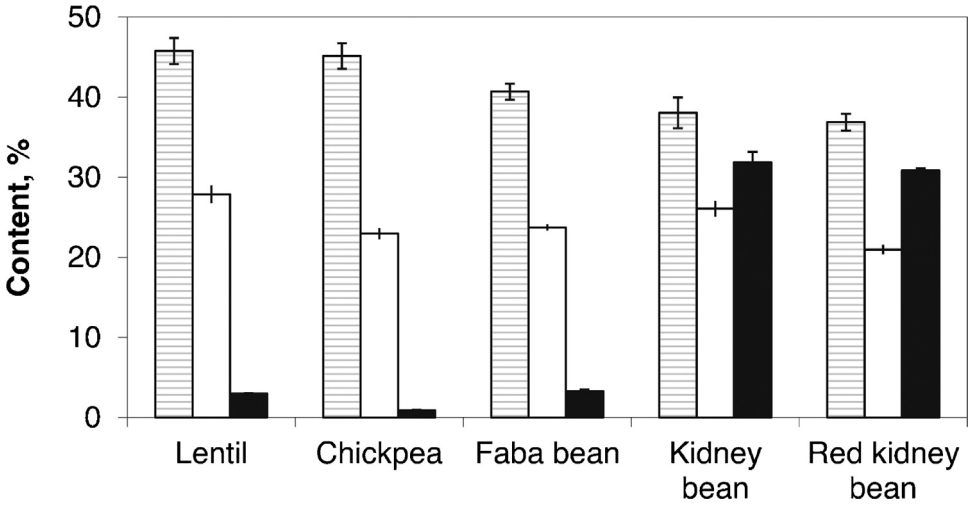

Fig. 1. Total starch (striped), amylose (white) and resistant starch (black) content (dry basis) in the raw legume flours assayed.

RS, NRS and TS percentages in flour from the raw legumes assayed in this study are shown in Fig. 1. Kidney and red kidney bean flour had the highest percentage of RS (31.84 ± 1.34% and 30.84 ± 0.28%, respectively) and lowest TS contents: 36.88 ± 1.05% and 38.04 ± 1.91%.

In contrast, chickpea flour showed the lowest RS amount (0.88 ± 0.07%) and one of the highest percentages of TS (45.13 ± 1.58%) along with lentils.

The analytical measurements for RS, NRS and TS obtained in this work can be compared to those reported previously by other authors, as shown in Table 1. When the RS values for lentils were compared to the present results (2.99 ± 0.05%), a generally higher content was reported with values ranged between 3.2 and 25.4%. Regarding TS, the percentage measured in this study (45.75 ± 1.65%) was contained in the range described in the literature (41.8–59.83%).

With respect to chickpeas, a similar behavior was observed. When comparing the RS content obtained in this research (0.88 ± 0.07%) to published results, higher values were reported (2.57–16.43%). On the other hand, the TS percentage measured in this study (45.13 ± 1.58%) was in the range obtained by other authors (41.36–52.17%).

Regarding the faba bean, present values (3.29 ± 0.20% for RS and 40.68 ± 0.99% for TS) were significantly lower after comparison to Table 1.

For kidney beans, the present value of RS was 31.84 ± 1.34%, considerably higher than the value shown in Table 1.

Table 1
Resistant (RS%) and total (TS%) starch content (dry basis) in raw legume flours found in the literature.

Legume	RS, %	TS, %	Analytical methods	References
Lentil	4.89 ± 0.27	–	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	Yadav, Sharma, and Yadav (2009)
	16.11 ± 1.2	46.72 ± 2.1	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	García-Alonso, Goñi, and Saura-Calixto (1998)
	7.4 ± 0.3	59.83 ± 1.55	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	Silva-Cristobal, Osorio-Díaz, Tovar, and Bello-Pérez (2010)
	3.2	41.8 ± 0.8	Holm et al. (1986)	Tovar, Björck, and Asp (1990)
	3.4	–	Adaptation of the Megazyme International Ltd., Ireland, UK method	Vasanthan and Bhatty (1998)
	25.4	53.3	Englyst, Kingman, and Cummings (1992)	Bednar, Patil, Murray, Grieshop, Merchen, and Fahey Jr (2001)
	14.4–14.9	46–47.1	AACC 32.40 2000	Chung et al. (2008)
	–	51.47 ± 0.87	Dahlqvist (1984)	Sotomayor, Frias, Fornal, Sadowska, Urbano, and Vidal-Valverde, (1999)
Chickpea	2.57 ± 0.09	–	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	Yadav, Sharma, and Yadav (2009)
	16.43 ± 1.6	41.36 ± 1.0	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	García-Alonso, Goñi, and Saura-Calixto (1998)
	3.1 ± 6.4	42.9 ± 46.3	AACC 32.40 2000	Chung, Liu, Hoover, Warkentin, and Vandenberg, (2008)
	7.8 ± 0.2	52.17 ± 0.93	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	Silva-Cristobal, Osorio-Díaz, Tovar, and Bello-Pérez (2010)
Faba bean	11.03 ± 2.94	88.93 ± 5.08	AACC 32.40 2000	Ambigaipalan et al. (2011)
Red kidney bean	3.2	33.2 ± 0.7	Holm et al. (1986)	Tovar, Björck, and Asp, (1990)
	45.36 ± 6.05	88.94 ± 12.3	AACC 32.40 2000	Ambigaipalan et al. (2011)
	24.6	42.6	Englyst, Kingman, and Cummings (1992)	Bednar, Patil, Murray, Grieshop, Merchen, and Fahey Jr (2001)
	35.0 ± 0.3	41.6 ± 1.2	Englyst, Kingman, and Cummings (1992)	Eyaru, Shrestha, and Arcot (2009)
Kidney bean	4.89 ± 0.27	–	Goñi, García-Diz, Mañas, and Saura-Calixto (1996)	Yadav, Sharma, and Yadav (2009)
	–	51.47 ± 0.87	Dahlqvist (1984)	Sotomayor, Frias, Fornal, Sadowska, Urbano, and Vidal-Valverde (1999)

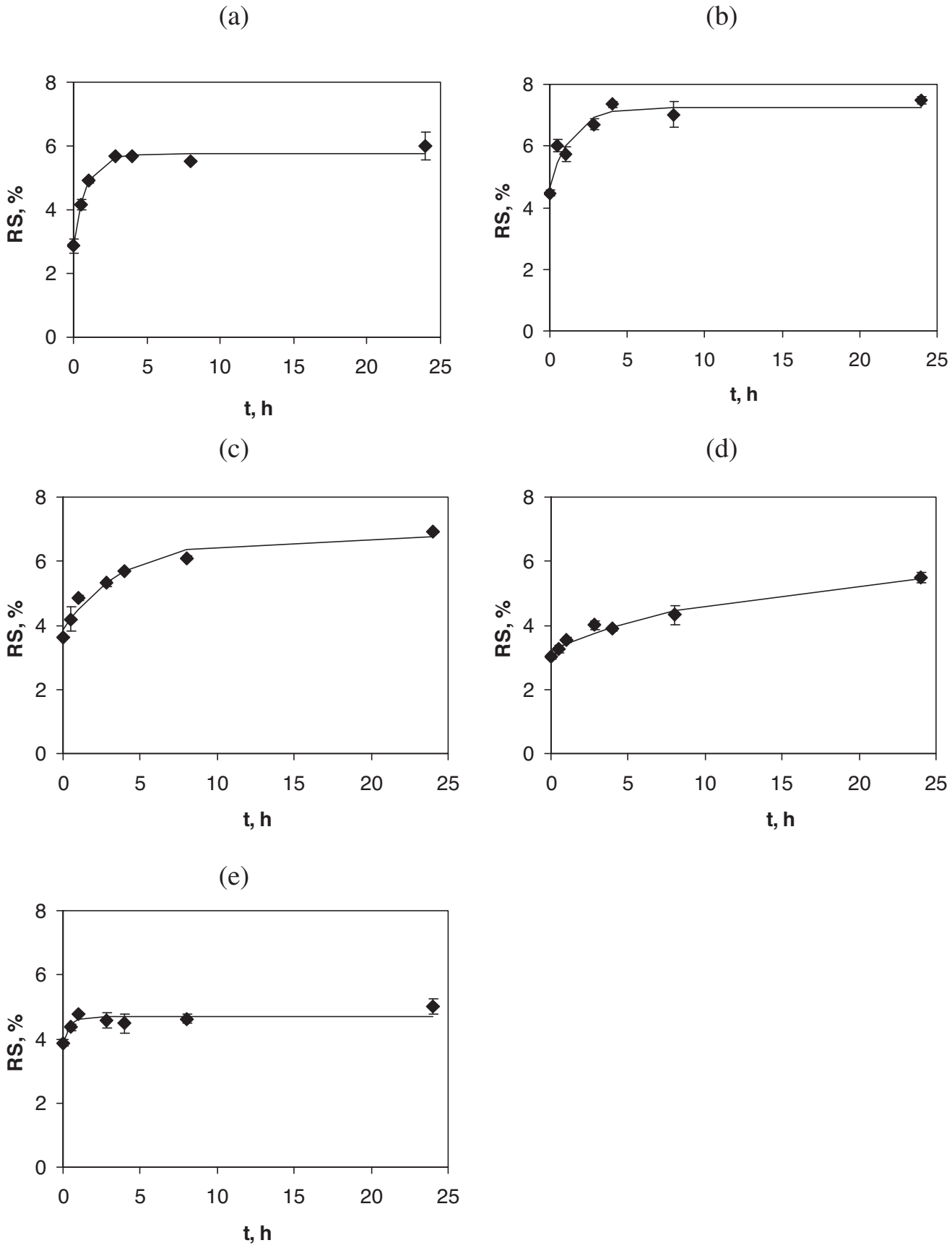

Fig. 2. Resistant starch content evolution during the hydrolysis of (a) lentil, (b) chickpea, (c) faba bean, (d) kidney bean and (e) red kidney bean flour with pullulanase at 60 °C and pH 5. The points in correspond to experimental values, while the solid line was calculated using the kinetic model (Eq. (2)).

Table 2
Kinetic model (Eq. (2)) parameters estimated for the hydrolysis of legume flour with pullulanase at 60 °C and pH 5.

Legume	k, h^{-1}	RS, %	RS_∞, %	$t_{90\%}$, h	$(RS_\infty - RS_0)/RS_0$	R^2
Lentil	1.23	2.86	5.75	1.87	1.01	0.983
Chickpea	0.74	4.67	7.26	3.11	0.55	0.911
Faba bean	0.24	3.87	6.78	9.46	0.75	0.964
Kidney bean	0.08	3.20	5.81	27.49	0.81	0.964
Red kidney bean	2.49	3.86	4.70	0.92	0.22	0.742

Finally, in the case of the red kidney beans, the present value for RS of raw legume was 30.84 ± 0.28%, amount which was contained in the broad interval from 3.2% to 45.4% that can be seen in Table 1. The total starch amounts previously measured were between 33.2% and 88.94%, interval which included the present value of 36.88 ± 1.05%.

As it has been described in this comparison, the RS amount reported for legumes varied over a very wide range. This fact could be related to the employment of different analytical methods in terms of discrepancy in variables such as substrate pretreatment, enzyme types and concentrations, pH and time of incubation, recovering of RS contained in pellets as well as the effect of shaking and stirring (McCleary et al., 2002; McCleary & Monaghan, 2002). Other factors that should be taken into account are the different varieties inside the same species or even the season of harvesting and other environmental conditions (Tester & Karkalas, 2001).

3.2. Production of resistant starch

3.2.1. Thermal treatment and lyophilization

It is noticeable the difference between the values of RS content for raw legumes and samples submitted to hydrolysis, which were heated up to 100 °C for 15 min and lyophilized. The RS amount decreased in the cases of lentil, kidney bean and red kidney bean by 4%, 90% and 87%, respectively. On the contrary, an increment of 431% and 18% was observed for chickpea and faba bean, respectively.

These variations can be explained because during the thermal treatment of the raw flours RS1 and RS2 are destroyed, whereas RS3 is produced by retrogradation (Berry, 1986). In the literature, all the legumes which were studied in the present work have similar values of gelatinization temperature (Ambigaipalan et al., 2011; Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008; Kaur & Singh, 2007; Su, Lu, & Chang, 1998), type C crystalline structure (Pérez & Bertoft, 2010) and a similar granule size (Hoover & Manuel, 1995; Yañez-Farias, Moreno-Valencia, Del Refugio Falcón-Villa, & Barrón-Hoyos, 1997). However, recent studies (Ambigaipalan, Hoover, Donner, & Liu, 2013a) have shown that the retrogradation is mainly governed by the amount of amylose leaching, the interactions between amylopectin and amylose and their mobility. Furthermore, a type C crystalline structure involves a mixture of types A and B, nevertheless the percentage of each one can vary depending on the source (Ambigaipalan, Hoover, Donner, & Liu, 2013b) and consequently, the amount of RS3 produced while retrogradation.

3.2.2. Thermal treatment, enzymatic hydrolysis and lyophilization

The evolution with time of the RS content generated during the 24-h debranching enzyme reaction of the thermal treated legumes flours can be seen in Fig. 2. Regarding lentils (Fig. 2a), it can be observed that the RS production increased linearly during the first hour, where RS increased from 2.87 ± 0.22% to 4.92 ± 0.07%, which represents an increase of 101%. Then, the curve takes a concave form, with a decreasing slope, until reaching a plateau after 2 h of hydrolysis, which was maintained at a RS value of 6.0 ± 0.44% until the end of the operation.

Concerning the hydrolysis of chickpeas (Fig. 2b), this substrate exhibited a slower production with a linear phase which lasted approximately 4 h. In this time interval, the RS% was increased from 4.47 ± 0.09% to 7.35 ± 0.11%. After 24 h, RS content reached a limit value of 7.47 ± 0.11%, achieving a 55% increase.

With reference to RS production from faba bean (Fig. 2c), there was again a rather clear linearity between RS amount and time during the first 4 h; interval of time in which the RS amount varied from 3.64 ± 0.02% to 5.69 ± 0.02%. For this legume, the constant value of RS contained attained for long reaction times was 6.91 ± 0.04%, the value which represents an increase of 75%.

As regards kidney bean debranching, in Fig. 2d, the linear relationship between RS production and time during the first 8 h can be noticeable, with an increase from 3.03 ± 0.08 to 4.33 ± 0.29% (an increment of 81%). This was the longest time for linear behavior observed in this study. Furthermore, it seems that for kidney bean, a plateau is not fully reached even after 24 h.

Finally, as regard to red kidney bean, Fig. 2e illustrates a linear behavior which lasted for 1 h and which increased the RS % from 3.88 ± 0.11% to 4.79 ± 0.25%. Afterwards, a constant value around 5% was maintained for the rest of the hydrolysis. In this case, the RS of the flour underwent an increase of 22%.

Curves with similar shapes can be found in the literature for different substrates such as maize (Milašinović, Radosavljević, & Dokić, 2010; Zhang & Jin, 2011), banana (Onyango & Mutungi, 2008) or cassava (González-Soto, Agama-Acevedo, Solorza-Feria, Rendón-Villalobos, & Bello-Pérez, 2004).

The experimental data represented in Fig. 2 led to the proposal of the following first-order kinetic equation to model the time evolution of the resistant starch content during the debranching process:

$$\frac{dRS}{dt} = k \cdot (RS_\infty - RS) \quad (1)$$

where RS_∞ is the percentage achieved at long operation times and k is a kinetic constant. As shown in Eq. (1), the product rate is governed by a driving force that corresponds to the percentage of remaining starch which is susceptible of being transformed into resistant starch. RS_∞ may be related to the amylopectin chain-length distribution since the development of partially crystalline structures which are resistant to digestion is due to the release of linear structures. The existence of this theoretical maximum may also be related to the inaccessibility of α-(1,6) bonds caused by any structure which is not susceptible to debranching.

By integrating Eq. (1) between (t = 0, RS = RS_0) and (t, RS), it is obtained:

$$RS = RS_0 + (RS_\infty - RS_0) \cdot (1 - \exp(-k \cdot t)) \quad (2)$$

where RS_0 is the amount of resistant starch of the unhydrolysed substrate. This parameter depends mainly on the species and variety of the substrate (which defines the amount of natural type 1 and 2 RS) as well as on the pretreatment to which the legume is submitted before enzymatic debranching (i.e. degree of milling in

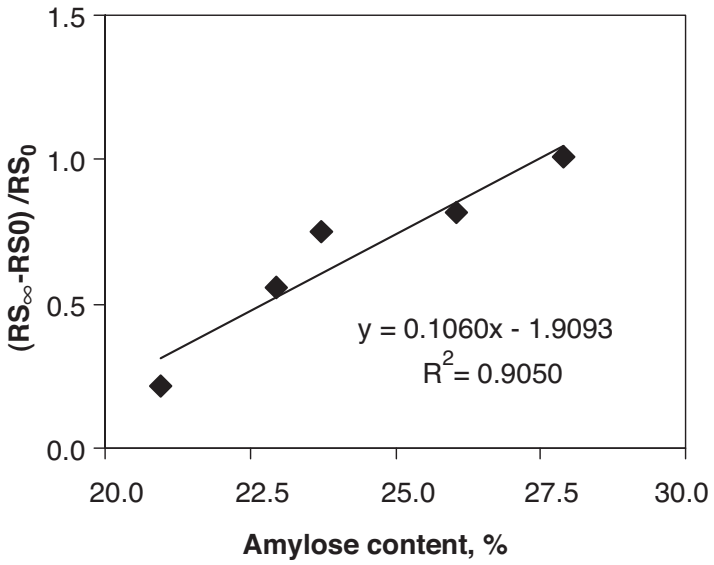

Fig. 3. Correlation between the relative increment of RS and the amylose content of raw legume flours.

order to obtain the flour and thermal treatment, which modify the natural RS amount and potentially produce RS3). Note that these values of RS_0 are conceptually different from the values measured for the raw legumes flours, since here the effect of the thermal pretreatment has to be considered.

As well as explaining the plateau achieved in the curves of RS against hydrolysis time at long operation times ($\lim RS_{t\to\infty} = RS_\infty$), Eq. (2) also predicts the linearity between RS and time at the first moments of the reaction. Effectively, considering the first order approximation of the Taylor series of Eq. (2) at $t = 0$:

$$RS = RS_0 + (RS_\infty - RS_0) \cdot k \cdot t \quad (3)$$

For each legume, the parameters involved in Eq. (2) (RS_∞, RS_0 and k) were estimated by non-linear regression where it was minimized the mean squared error between the experimental and the calculated data for the time evolution of RS. After obtaining this set of parameters (Table 2), it was possible to draw the solid lines in Fig. 2.

The goodness of the curve fitting was tested by correlating the RS calculated with the kinetic model against the experimental one. As regard to the coefficient of determination (Table 2), a value of R^2 greater than 0.9 was obtained in all cases but for red kidney bean ($R^2 = 0.742$).

The kinetic constant k references the rapidity of the RS production process, red kidney beans being the fastest (2.49 h^{-1}) and kidney beans the slowest (0.084 h^{-1}). Furthermore, considering the asymptotic behavior of the curves, a new parameter, $t_{90\%}$, can be defined so that it represents, in a better understanding mode, the rapidity of the reaction. The value of $t_{90\%}$ indicates the time of hydrolysis necessary in order to achieve the 90% of the difference between RS_∞ and RS_0. In order to calculate $t_{90\%}$, from Eq. (2) it can be obtained:

$$\ln\left(\frac{RS_\infty - RS}{RS_\infty - RS_0}\right) = -k \cdot t \quad (4)$$

If $t = t_{90\%}$ then $(RS_\infty - RS)/(RS_\infty - RS_0) = 0.1$, thus:

$$t_{90\%} = \frac{2.303}{k} \quad (5)$$

For this parameter (Table 2), red kidney beans achieved 90% of the total production within 0.92 h, followed by lentils, which take 1.87 h. Finally, the degree of slowness which is involved to the kidney beans was the highest with a $t_{90\%}$ of 27.49 h.

3.3. Influence of the amylose content

It has been previously reported that the greater the amylose percentage in starch, the greater the RS production (Berry, 1986; Sievert & Pomeranz, 1989). In this paper, a linear correlation ($R^2 = 0.905$) between the amylose content of raw legume flours and the relative increment of RS during hydrolysis, $(RS_\infty - RS_0)/RS_0$, was obtained (Fig. 3.)

The highest relative increment (101%) corresponded to lentil, for which the amylose content was also the highest (27.9 ± 1.1%). On the other hand, since red kidney bean flours possessed the lowest percentage of amylose (21 ± 0.6%) they achieved the lowest increase (22%). Concerning about kidney beans (amylose content: 26.1 ± 0.7%), the relative increment of RS was 81%. This value was followed by faba bean with a 75% of increment (amylose content: 23.73 ± 0.4%). Finally, regarding chickpea flour, with an amylose content of 23.0 ± 1.0%, it suffered an increase of 55%.

The fact that the amylose content is solely correlated with the yield and not with the RS of raw legumes can be explained by the existence of RS2. During the thermal treatment the amount of RS2, which is related to the existence of granules of starch, is reduced because those granules swelled and got broken. Hence, the resistant starch of raw flours is not only affected by the amount of amylose but also by the starch structure.

However, in the case of RS3 the amount of amylose is linearly correlated with the relative increase of RS; fact which confirms the strong relationship between amylose percentage and RS3 production.

4. Conclusions

Although the result of thermal treatment varied according to the species (due to the structure of the starch), in the case of hydrolysis all the legumes had the same behavior: linear and constant trends at short and long reaction times, respectively. Hence, a kinetic model composed of three parameters: k, RS_0 and RS_∞, was developed. The kinetic constant k, which refers to the velocity of the RS production,

ranged within 0.08 h^{-1} for kidney bean and 2.49 h^{-1} for red kidney bean. Additionally, RS_0, which represents the initial content of RS in the legumes flours varied from 4.67%, in the case of chickpea flours, to 2.86% for lentils. The other constant, RS_∞, is related to the RS value at long times of hydrolysis. Its value is ranged between 7.26% for chickpeas and 4.70% for red kidney beans. Moreover, the coefficient of determination R^2 calculated was higher than 0.9 for all the legumes tested but for red kidney bean and the error was less than 10% in all cases.

Finally, the relative increase of RS, $((RS_\infty - RS_0)/RS_0)$, was successfully correlated to the amount of amylose in raw legumes ($R^2 = 0.905$).

Acknowledgements

This research was funded by the projects CTQ2008-02978 and CTQ2011-23009 from the Spanish Ministry of Science and Innovation.

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