Enzymatically hydrolysed, acetylated and dually modified corn starch: physico-chemical, rheological and nutritional properties and effects on cake quality

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Abstract Corn starch was treated by enzymatic hydrolysis with *Aspergillus oryzae* S2 α -amylase, acetylation with vinyl acetate, and dual modification. The dual modified starch displayed a higher substitution degree than the acetylated starch and lower reducing sugar content than the hydrolysed starch. The results revealed that the cooling viscosity and amylose content of those products decrease ($P < 0.05$). An increase in moisture, water, and oil absorption capacity was observed for the acetylated starch and, which was less pronounced for the enzymatically hydrolysed starch but more pronounced for the enzymatically hydrolysed acetylated product. The latter product underwent an increase in resistant starch content, which is induced by a rise in hydrolysis time to attain about 67 % after 1 h of reaction. The modified starch samples were added to cake formulations at 5 and 10 % concentrations on a wheat flour basis and compared to native starch. The results revealed that when applied at 5 % concentrations, the modified starches reduced the hardness, cohesion, adhesion and chewiness of baked cakes and enhanced their elasticity, volume, height, crust color, and appearance as compared to native starch. These effects were more pronounced for the cake incorporating the dually modified starch.

Keywords Corn starch, α -amylase · *Aspergillus oryzae* S2 · Acetylated · Dual modification · Cake properties

Introduction

Starch is an agricultural raw material and essential dietary carbohydrate for human nutrition (Maarel and Leemhuis 2013). Three categories of digestible starches are distinguished by the rate at which glucose is formed and absorbed in the blood (Zhang and Hamaker 2009). Rapidly digestible starches (RDS) are hydrolysed in the small intestine within the first 20 min of digestion, whereas slowly digestible starches (SDS) acquire more time to degrade. Resistant starch (RS) generally escapes digestion in the small intestine and passes through the large intestine as dietary fiber for fermentation by bacteria, where it helps to maintain colon health and protect against disease (Miao et al. 2014; Jiang et al. 2014). RDS can induce a rapid increase in blood glucose and insulin levels, which can lead to various health complications, such as obesity, diabetes and cardiovascular diseases. The literature broadly classifies RS into three different types, namely RS1, RS2, and RS3 (Hoover and Zhou 2003). RS1 resistant starch is physically inaccessible to digestive enzymes due to attachment in structures such as intact cells in legumes. RS2 consists of native crystalline regions of starch granules and retrograded amylopectin. RS3 has been reported to consist predominantly of retrograded amylose. An additional type of RS, type RS 4, has been identified as resulting from chemical modifications (Fuentes-Zaragoza et al. 2011).

Native starches have been used in various food and non-food industries for decades. They have often been reported to present a number of undesired properties, including is insolubility in cold water, crumbling after heating, and loss of viscosity (Sandhu et al. 2015). Moreover, the cooling of a heated

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native starch suspension often leads to the formation of a disordered, firm and opaque gel due to hydrogen bonding between parallel oriented amylose and/or long side chains of amylopectin and syneresis. The latter process, termed as retrogradation, is irreversible and causes the loss of gel structure (Luchese et al. 2015).

Several methods have been proposed to solve the inconveniences caused by the inherent limitations of native starch, particularly through the modification of its chemical and/or physical profile to maximize its positive attributes and minimize its negative properties. Acetylation is one of the most common methods used for starch modification; it involves the substitution of some hydroxyl groups by acetates groups (Xie et al. 2005). Corn acetate starch, which contains 0.5 to 2.5 % acetyl content, has a lower gelatinization temperature and a higher peak viscosity than unmodified starch (Raina et al. 2006; Wickramasinghe et al. 2009; Colussi et al. 2014). Acetylation also improves the stability and clarity of native starches by increasing the degree of swelling and dispersion of starch granule in the cooling cycle, and also reduces retrogradation (Han et al. 2013; Luchese et al. 2015). Because of their desirable properties, acetate starches have been used as binders in soups, snacks, canned, and refrigerated foods. However, acetate starches generally lack resistance to high temperature, acidity, and shear stress, which is undesirable for several food applications (Xie et al. 2005).

Various enzymes can be used in starch hydrolysis to alter starch structure and achieve desired functionality (Maarel and Leemhuis 2013; Caliari et al. 2014). Alpha-amylases (EC 3.2.1.1), key additives for dough and bakery products, are endo-acting amylases that randomly hydrolyse α -(1–4) glycosidic bonds of starch polymers, resulting in oligosaccharides with varying lengths and different α -limit dextrins (Sahnoun et al. 2015). They increase the level of fermentable sugars in the dough, thus promoting the fermentation of yeast and the formation of Maillard reaction products, which, in turn, intensify the flavour and crust colour of bakery products (Goesaert et al. 2005; Sahnoun et al. 2013). Alpha-amylases also increase dough resistance, elasticity, and softness (Patel et al. 2012) and decrease dough crumb firmness and hardness (Kim et al. 2006; Hemalatha et al. 2010; Raghu and Bhattacharya 2010; Gomes-Ruffi et al. 2012). They have, however, been reported to reduce dough stability and extensibility, which may be attributed to the presence of high amounts of amylase-reducing polysaccharides. The latter break gluten disulphide bridges and, hence, contribute to the modification of wheat protein. The addition of oxidizing agents would, therefore, be necessary (Kim et al. 2006; Sahnoun et al. 2013). Nevertheless, and to the authors' knowledge, no studies have so far investigated the optimization of the improver mixture of both oxidizing and reducing agents. Acetyl groups incorporated in starch could act as electron donors (oxidant) and, hence, improve dough stability and

extensibility attribute to the modification of gluten wheat protein.

Considering the promising opportunities that starch modification might offer for the production of starch with tailored properties, this study was undertaken to investigate corn starch modification by acetylation with vinyl acetate, enzymatic hydrolysis with *Aspergillus oryzae* S2 α -amylase, and dual modification. It also aimed to evaluate the effect of those starch modifications on the physico-chemical properties and rheological attributes of the final starch end-products. The modified corn starches were assayed in cake preparations, and the changes they brought in terms of the texture and properties of the baked cakes were investigated.

Materials and methods

Materials

The experimental assays of the present study were performed using Amyloglucosidase (3300 U/ml) (Sigma Chemical, St. Louis, USA) and 181,481.48 U/g of *Aspergillus oryzae* S2 concentrated crude extract that was free from aflatoxins (Sahnoun et al. 2011) and formulated with dried starch as previously described elsewhere (Sahnoun et al. 2013). Commercial corn (*Zea mays*) starch, purchased from the Egyptian starch and glucose company, Cairo, was used as native starch. It contained 0.76 % protein ($N \times 5.70$), 0.71 % ash, 0.58 % lipid, and 91 % carbohydrates. Salt (sodium chloride), sugar milk, shortening egg, baking powder and wheat flour, containing 10.47 % protein ($N \times 5.70$), 0.61 % ash, 14 % moisture, and 24 % moist gluten, were purchased from the local market of Sfax, Tunisia, and used for cake preparation.

Methods

Starch characterization

Total carbohydrates were determined after total acid hydrolysis (Israilides et al. 1978). Protein content was evaluated by the Kjeldahl method (Pearson 1970). Lipid content was determined gravimetrically after Soxhlet extraction using hexane solvent (Riedel-De Haën, Germany) (AOAC 1984). Dry matter was determined by oven drying at 105 °C to constant weight, and ash content was estimated by the combustion in a muffle furnace at 550 °C for 12 h. All contents were expressed as matter weight/weight. Tests were performed in triplicates. Moisture content of natives and modified starches was evaluated as weight loss after drying at 105 °C until constant weight, and pH was measured by a pH-meter (Eutech Cyberscan 1000, Singapore). Amylose content was

measured by the colorimetric method of iodine binding capacity as described by Dura et al. (2014).

Acetylation with vinyl acetate

Vinyl acetate was used in accordance with the specifications issued by the Food and Drug Administration (FDA) for the preparation of organic and inorganic monoesters of starch intended for use in food (Xie et al. 2005). Corn starch acetylation (esterification) was performed according to a slightly modified version of the method described by Saartrat et al. (2005). In brief, native corn starch (100 g, dry basis) was placed in a 500 ml beaker, and 225 ml of distilled water were added at 25 °C. The mixture was stirred for 1 h until a homogeneous slurry was formed. The pH was adjusted to 8.0 by the drop-wise addition of a 3 % aqueous sodium hydroxide solution. After that, 6 g of vinyl acetate were added drop-wise, with the concurrent addition of 3 % sodium hydroxide under continuous stirring to keep the pH at 8.0–8.4. When the addition of the vinyl acetate was complete, the pH was adjusted to 4.5 with 0.4 N HCl to stop the reaction. The slurry was filtered under vacuum, washed with twice volumes of distilled water, and dried at 45 °C for about 24 h.

Preparation of enzymatically hydrolysed of native and acetylated starch

The slurry was preheated at 10 % (w/w) of (native or acetylated starch maize) under stirring condition until reaching the optimal active temperature (50 °C) of *Aspergillus oryzae* S2 alpha amylase, which was added at 5800 U/g dry weight of starch. The pH of the mixture was fixed at optimal pH activity (pH 5.6). After a 10-min hydrolysis phase, the reaction was stopped by heating at 70 °C for 10 min, and the solution was then cooled to room temperature. The starch was collected by centrifugation at $5000 \times g$ for 10 min, and dried at 45 °C for 24 h.

The reducing sugars (ReS) of the product (1 %) were determined as glucose equivalents by the DNS method (Miller 1959) using a calibration curve of glucose solutions with different concentrations.

Rheological properties analysis

The starch suspension (6 %, v/v) was solubilised in a boiling water bath for 30 min. Viscosity (Pa.s) was determined with a Stress Tech Rheologica Rheometer (reologica instruments AB, Lund, Sweden) using a steel cone plate (C40/4) under a constant shear rate of 100 s^{-1} after cooling to 20 °C.

In vitro digestion

The digestibility of the starch was analyzed according to a slightly modified version of the procedure described by Jiang et al. (2014). In brief, Amyloglucosidase (solution I) (2.16 ml) was diluted to 6.0 ml. Alpha amylase (solution II) was prepared by suspending *Aspergillus oryzae* S2 alpha amylase (6.6 g) in water (80.0 ml) under magnetic stirring for 10 min and then centrifuging the mixture for 10 min at $1500 \times g$. A fraction (54.0 ml) of the supernatant was transferred into a beaker. Solution III was prepared by mixing solution I (6.0 ml), solution II (54.0 ml) and water (4.0 ml). A starch sample (200 mg) was dispersed into 15 ml of phosphate buffer (0.2 M, pH 5.2) by vortexing, and the solution was equilibrated at 37 °C for 5 min. Enzyme solution III (5.0 ml) was then added, and the whole mixture was shaken at (37 °C, 150 rpm). Hydrolysed solution (0.5 ml) was taken at different time intervals and mixed with 4 ml of absolute ethanol to denature the enzymes. The glucose content of the hydrolysate was determined using the DNS method. Hydrolysed starch (%) was calculated by multiplying the glucose content by a factor of 0.9. Each sample was analyzed in triplicate. The values of different carbohydrate nutritional fractions (RDS, SDS and RS) were calculated by combining the values of G_{20} (glucose liberated after 20 min), G_{120} (glucose liberated after 120 min), FG (free glucose), and TG (total glucose) using the following formulas:

$$\begin{aligned}\%RDS &= (G_{20} - FG) \times 0.9 \times 100 \\ \%SDS &= (G_{120} - G_{20}) \times 0.9 \times 100 \\ \%RS &= (TG - FG) \times 0.9 \times 100 - \%RDS - \%SDS\end{aligned}$$

Acetyl content and the degree of substitution (DS)

The acetyl content (expressed as % on dry basis) and degree of substitution (DS) of the corn acetylated starch were determined as described by Saartrat et al. (2005). An amount of 5 g of starch sample was transferred to a 250 ml beaker and dispersed in 50 ml distilled water. A few drops of phenolphthalein indicator were added and titrated with sodium hydroxide 0.1 N till the appearance of pink color. An amount of 25 ml of 0.45 N NaOH was then added, and the whole mixture was shaken for 30 min. The excess alkali was titrated with 0.2 N HCl till the disappearance of the pink color. The 25 ml of 0.45 N NaOH was titrated as blank. The acetyl content (%) and degree of substitution (DS) were calculated using the following equations:

$$\% \text{ Acetyl content} = \frac{(b-s) \times N \times 0.043 \times 100}{W \text{ (dry basis)}}$$

Where b refers to the volume of 0.2 N HCl utilized to titrate blank (ml), s to the volume of 0.2 N HCl utilized to titrate

sample (ml), N to the normality of 0.2 N HCl, and W to the mass of the sample (g).

$$DS = \frac{(162 * \%AC)}{[4300 - (42 * \%AC)]}$$

Water holding capacity (WHC)

An amount of 1 g of starch was vigorously mixed with 30 ml of distilled water in a pre-weighed 50 ml polypropylene centrifuge tube for 1 min. The tube was placed at 30 °C for 1 h, followed by cooling in ice bath, and then centrifuged for 15 min at 10,000×g. The supernatant was removed, and the tube with the precipitate was weighed (Mi et al. 2014). The WHC was calculated as g of water bound per g of sample on a dry basis.

Oil absorption capacity (OAC)

Oil absorption capacity (OAC) was determined according to a slightly modified version of the De la Hera et al. (2013) method. In brief, 0.5 g of starch sample was mixed with 6 ml of corn oil in pre-weighed centrifuge tubes. The contents were stirred for 1 min to diffuse the sample in the oil. After a holding phase of 30 min, the tubes were centrifuged for 25 min at 4000×g. The separated oil was removed with a pipette, and the tubes were inverted for 25 min to drain the oil before reweighing. Fat absorption capacity was calculated as g of oil bound per g of sample on a dry basis.

Preparation of cake

Cake samples were prepared using a slightly modified version of the procedure described by Karaoglu et al. (2001). The cake mix and preparation method are listed in Table 1, and Fig. 1,

Ingredients	(%)
Flour *	29.42
Sugar	26.48
Milk	17.65
Semisolid margarine	11.77
Egg white	11.77
Egg yolk	2.35
dry baker's yeast	0.50
Salt	0.05
Starch**	
*% Flour levels decreased when amount of starch was added	
**Amount of starch was determined as stated in trial plan

Fig. 1 flowchart for cake process conditions

respectively. The final mix (60 g) was placed in greased pans and baked in an oven for 28 min at 180 °C.

Evaluation of cake texture

Cake texture was evaluated by the texture profile analysis method using a texture analyzer (Lloyd Instruments, Fareham, UK) set at a 0.05 (N) detection range and equipped with an aluminium 19-mm diameter cylindrical probe and 1000 (N) load cell. Sample slices of 2 cm thickness were compressed to 50 % of their initial height at a 10 mm/s speed in a 'texture profile analysis' (TPA) double compression test with a 30 s delay between the 1st and 2nd compression. The samples were cut by an electric knife to ensure uniform shapes. Primary attributes [hardness, cohesion, and springiness] and secondary mechanical properties [adhesion and chewiness] were determined from the TPA curves in accordance with well established procedures (Nishinari et al. 2013). Hardness refers to the peak force of the first compression cycle; cohesiveness corresponds to the ratio of the positive areas of the second cycle to the area of the first cycle; adhesion designates the work necessary to pull the compressing plunger away from the sample. The

height during the time that elapses between the end of the 1st compression cycle and the start of the 2nd compression cycle is defined as springiness. Chewiness is the product of hardness X cohesiveness X springiness. Assays were performed after cooling for 2 h at room temperature. The TPA values expressed the averages of three different determinations.

Loaf specific volume

The specific volume of cake was determined by rapeseed displacement as previously described elsewhere (Sahnoun et al. 2013). Specific volume corresponded to the quotient of bread volume (cm³)/bread weight (g). Improvement of bread loaf specific volume was calculated as follows:

$$\text{Improvement} = [1 - (\text{specific volume of bread}) / (\text{specific volume of control sample})] \times 100$$

All determinations were performed in triplicates, and results were expressed as mean values.

Statistical analysis

For the statistical analysis, we applied the statistical package SPSS program (version 17). To ensure the equality of the variances, a Levene test was accomplished. The normal distribution was then checked by the P-Plot function. A one-way ANOVA test was performed to analyze differences between the means. The data were then submitted to a Duncan's LSD test to evaluate the critical differences between the groups. Significance was set at ($P < 0.05$) in all cases.

Result and discussion

Physicochemical properties of modified starch

The results revealed that the degrees of substitution (DS) and acetyl content (% AC) displayed for the dual modified starch was higher than the acetylated one (Table 2). This increase in (% AC) and (DS) recorded for the hydrolysed acetylated starch could be attributed to the increase of the free hydroxyl groups likely to be involved in acetylation by the preliminary alpha-amylase hydrolysis. Furthermore, the results presented in Table 2 revealed that the hydrolysed acetylated starch

underwent a decrease in terms of reducing sugars content compared to the hydrolysed starch. This decrease could be ascribed to the acetylation effect of starch free hydroxyl functions responsible to the reducing power.

The moisture contents of the acetylated and dual modified starches increased significantly ($p < 0.05$) compared to native starch (Table 2). This increase could be related to the percentage of acetylation and presumably attributed to the introduction of hydrophilic groups into the native starch. This result is in agreement with previous reports on rice (Raina et al. 2006) and new cocoyam (Olayide 2004) starches. The increase recorded for the moisture of starch samples treated with *Aspergillus oryzae* S2 alpha amylase could be attributed to the release of hydrolysis products with different degrees of polymerization, freer OH groups, and higher water absorption capacity than native starch. The dual modified starch exhibited a more pronounced moisture content increase, which could be ascribed to the hydrophilic synergistic power given by each modification step.

A significant ($p < 0.05$) decrease in amylose content was observed for the starch treated by enzymatic hydrolysis (Table 2). This decrease was a normal consequence of α -amylase hydrolysis of α -(1, 4) linkages inside the starch amylose and amylopectin polymers (Sahnoun et al. 2011). With 6 % vinyl acetate, acetylation was noted to induce a marked decrease in amylose content (Table 2). This decrease was presumably due to the disruption of intermolecular starch granules and helical structure of amylose. Similar results were previously reported

Table 2 Physicochemical characteristics of native, enzymatically hydrolysed, acetylated, and enzymatically hydrolysed and acetylated corn starch. Data expressed as means $\pm$ SD. $n = 3$. Means followed by the same letter within column are non-significantly different ($P < 0.05$)

Starch sample	Viscosity (Pa·s)	Moisture (%)	Amylose (g/100 g DM)	ReS (g/L)*10 ⁻²	Acetyl content (%)	RS (%)	DS*10 ⁻²
Native	1 $\pm$ 0.01 ^a	13 $\pm$ 0.13 ^a	26 $\pm$ 0.33 ^c	0.21 $\pm$ 0.01 ^a	0 $\pm$ 0 ^a	1 $\pm$ 0.25 ^a	0 $\pm$ 0 ^a
Enzymatically hydrolysed	0.70 $\pm$ 0.04 ^c	15.30 $\pm$ 0.14 ^b	20.30 $\pm$ 0.24 ^a	23.10 $\pm$ 0.20 ^c	0 $\pm$ 0 ^a	1.20 $\pm$ 0.21 ^a	0 $\pm$ 0 ^a
Acetylated	0.30 $\pm$ 0.02 ^b	17.50 $\pm$ 0.12 ^c	21 $\pm$ 0.26 ^b	0.18 $\pm$ 0.01 ^a	1.83 $\pm$ 0.01 ^b	12 $\pm$ 0.34 ^b	7.00 $\pm$ 0.10 ^b
Enzymatically hydrolysed and acetylated	0.12 $\pm$ 0.02 ^d	19.20 $\pm$ 0.13 ^d	20 $\pm$ 0.37 ^a	12.60 $\pm$ 0.30 ^b	2.14 $\pm$ 0.01 ^c	25 $\pm$ 0.28 ^c	8.20 $\pm$ 0.10 ^c

Superscripts letters were defined by Duncan's multiple range test

DM: dry matter, ReS: reducing sugars content, RS: resistant starch content, DS: degree of substitution

Fig. 2 water holding capacity of native, enzymatically hydrolysed, acetylated, and enzymatically hydrolysed acetylated corn starch at 60, 70, 80 and 90 °C. Values with the same letter are non-significantly different ($P < 0.05$)

(Xie et al. 2005; Wickramasinghe et al. 2009) for acetyl concentration of 5 %. A controversial effect was, however, described in the work of Singh et al. (2004) which involved acetyl concentrations of less than 4 %. In fact, the substitution starts in the more amorphous regions and proceeds to the more crystalline regions of the granule (Chen et al. 2005; Gray and BeMiller 2004; Huang et al. 2007). The decrease in the amylose content for the case of the hydrolysed acetylated starch was the result of a synergistic effect of the two modifications.

The results recorded for the water holding capacity (WHC) of the native and modified starches heated at a temperature range of 60 to 90 °C are shown in Fig. 2. The findings revealed that the WHC values of the native and modified corn starches increased with the increase of temperature. In fact, at high temperatures, the hydrogen bridges stabilizing the semi-crystalline structure of starches are broken and replaced by water molecules (Tester and Karkalas 1996). The hydrolysed acetylated starch displayed the highest WHC value. This could presumably be attributed to the modification effect. Certainly, acetylation induced repulsion between the starch molecules by steric effects, thus facilitating the weakening of starch granules (Raina et al. 2006). Furthermore, the bulky acetyl groups could have prevented the inter-chain association and improved the access of water to the amorphous area, thus enhancing the leaching of amylose and the swelling and hydration of starch

(Wickramasinghe et al. 2009; Mi et al. 2014). The increase observed for the WHC values of the enzymatically hydrolysed starch could be ascribed to the generation of large amounts of polysaccharides-reducing products having a better solubilisation and water absorption than the native starch (Dura et al. 2014). The strong WHC recorded for the hydrolysed acetylated starch at 90 °C was a novel attractive feature that supported its promising candidacy for potential application in various food industries, including the confectionary industry.

The oil absorption capacity of the hydrolysed acetylated corn starch underwent a 2.3 fold improvement compared to that of native starch (Fig. 3). This provided further support for its promising candidacy for application as an alternative substitute to emulsifiers in the food industry. The improvement observed in oil absorption could be due to the modification of the structural arrangement of starches following the fixing of acetyl groups and hydrolysis of the native starch, respectively. In this context Konowal et al. (2012) showed that the enzymatically hydrolysed derivatives of acetylated potato starch induced a reduction in the surface/interfacial tension of both the air/water and toluene/water interfaces.

The results also revealed that the modified starches underwent a decrease in their final viscosity after cooling, which was more pronounced for the enzymatically hydrolysed acetylated starch derivatives, followed by the acetylated and

Fig. 3 Oil absorption capacity of native, enzymatically hydrolysed, acetylated, and enzymatically hydrolysed acetylated corn starch. Values with the same letter are non-significantly different ($P < 0.05$)

Fig. 4 Kinetics of resistant starch formation as a function of the duration of the preliminary enzymatically hydrolysis step of the dual modified corn starch

then the hydrolysed starches. The decrease in the viscosity of corn acetate starch after cooling could presumably be attributed to starch depolymerisation due to acetate mutual repulsion among polymer chains and decrease in their to water absorption ability. Various studies have previously reported on a similar decrease in the viscosity of acetylated starch as compared to native starch (Colussi et al. 2014; El Halal et al. 2015). The reduction observed for the viscosity of enzymatically hydrolysed starch after cooling could be ascribed to enzyme digestibility. In fact, the viscosity of starch after cooling was inversely related to the digestibility of enzymatically hydrolysed starches (Miao et al. 2014).

The resistant starch content of the acetylated starch was noted to increase by up to 12 %, reaching the desired range for starch use on an industrial scale (Than et al. 2007). The combination between enzymatic hydrolysis and acetylation showed an increase in resistance to digestibility, reaching 67 % of resistant starch content after 1 h of preliminary hydrolysis phase (Fig. 4). Accordingly, a tailored starchy food

containing the desired amount of RS could be produced by controlling the reaction time of the *Aspergillus oryzae* S2 α -amylase hydrolysis. The increase observed for the resistance to digestibility of the dual modified starch could be attributed to the abundance of the bulky acetyl group attached to the C2 position in the glucose unit of starch. This particular attachment was enhanced by the preliminary enzymatic hydrolysis time, which reflected the exposure of starch C2-glucose free site. In fact, this typical substitution would sterically hinder the correct positioning of the dual modified starch into the active site of α -amylase during its digestion treatment. It would also hamper the proton donation from the catalytic glutamic acid residue belonging to the amylase cleavage site into the glycosidic bond oxygen during hydrolysis. Furthermore, the powerful electron-withdrawing of the carbonyl oxygen of the acetyl group would result in the creation of a positive charge on C2. Thus, on the glucose unit, there would be two electron deficient poles (positively charged C1 and C2) toward the nucleophilic carboxylate anion. Consequently, the α -retaining double dislocation hydrolysis mechanism that starts from the proton donation by glutamic acid to the glycosidic oxygen and from the carboxylate anion nucleophilic attack of aspartate on C1 (Coutinho and Henrissat 1999) would take place more slowly.

Application and effect of modified starch on cake properties

Given their attractive properties and attributed, the modified starches presented in this work were assayed in the cake making process at two concentrations, namely 5 %, and 10 %. The effects on the cake samples were compared to those of native starch using the protocol described in the Materials and

Table 3 Effect of modified addition on textural properties, physical characteristic of cake prepared with native, enzymatically hydrolysed, acetylated and enzymatically hydrolysed and acetylated corn starch at

Starch sample	Hardness (N)	Cohesiveness	Elasticity (mm)	Adhesion (N)	Chewiness (Nmm)	Firmness (N/mm)	Volume (cm ³)	Height (cm)
5 %								
Native	4.04 ± 0.05 ^d	0.32 ± 0.02 ^c	9.28 ± 0.08 ^a	2.08 ± 0.08 ^a	18.68 ± 0.09 ^d	0.87 ± 0.02 ^d	560 ± 2 ^a	4.3 ± 0.1 ^a
Enzymatically hydrolysed	2.58 ± 0.08 ^b	0.26 ± 0.02 ^b	10.58 ± 0.05 ^b	1.37 ± 0.04 ^b	13.29 ± 0.05 ^c	0.68 ± 0.03 ^c	592 ± 3 ^b	5.8 ± 0.1 ^b
Acetylated	3.23 ± 0.01 ^c	0.25 ± 0.01 ^b	10.88 ± 0.07 ^c	1.27 ± 0.05 ^b	11.98 ± 0.07 ^b	0.62 ± 0.01 ^b	598 ± 4 ^c	6.4 ± 0.2 ^c
Enzymatically hydrolysed and acetylated	2.01 ± 0.07 ^a	0.20 ± 0.01 ^a	11.64 ± 0.05 ^d	0.67 ± 0.09 ^c	10.34 ± 0.07 ^a	0.57 ± 0.01 ^a	626 ± 2 ^d	6.9 ± 0.2 ^d
10 %								
Native	7.63 ± 0.04 ^d	0.41 ± 0.02 ^d	8.09 ± 0.06 ^a	2.43 ± 0.04 ^d	24.53 ± 0.04 ^d	1.17 ± 0.04 ^c	530 ± 1 ^a	3.7 ± 0.3 ^a
Enzymatically hydrolysed	4.83 ± 0.03 ^c	0.36 ± 0.01 ^c	10.09 ± 0.05 ^c	1.80 ± 0.03 ^c	16.42 ± 0.09 ^c	1.01 ± 0.01 ^b	571 ± 1 ^b	5.1 ± 0.1 ^b
Acetylated	4.34 ± 0.03 ^b	0.32 ± 0.02 ^b	9.89 ± 0.03 ^b	1.66 ± 0.03 ^b	15.55 ± 0.04 ^b	0.96 ± 0.02 ^b	577 ± 2 ^c	5.8 ± 0.1 ^c
Enzymatically hydrolysed and acetylated	4.23 ± 0.08 ^a	0.29 ± 0.01 ^a	10.22 ± 0.07 ^d	1.53 ± 0.05 ^a	14.31 ± 0.03 ^a	0.86 ± 0.03 ^a	581 ± 1 ^d	5.6 ± 0.1 ^c

Superscripts letters were defined by Duncan's multiple range test

Fig. 5 Cake prepared with **a** native, **b** enzymatically hydrolysed **c**, acetylated, and **d** enzymatically hydrolysed acetylated corn starches at 5 % and 10 % concentrations

Methods section. The effects of the different starch compositions on the textural parameters of wheat flour cake are presented in Table 3. The results revealed that the application of the modified starches at a concentration of 5 % induced a decrease in the hardness, chewiness, cohesion, and adhesion of the baked cakes (Table 3). This decrease was more pronounced for the cake incorporating hydrolysed acetylated, acetylated, and hydrolysed starches, respectively.

The decrease observed in the hardness of the modified starches is consistent with the results previously reported by Khalil et al. (2001) who found a significant and negative correlation between the moisture and water retention power of starch and the hardness of the cooked product. The decrease in cohesion is generally related to the loss of intramolecular interaction ingredients. The reduction recorded for the cohesion of the cake baked with hydrolysed starch was expected since

amylase breaks down starch into lower molecular weight dextrans and polysaccharides with lower cohesive forces than amylose and amylopectin constituent chains (Grabowski et al. 2006). The decrease in the cohesion of the cake samples cooked with acetylated starch was presumably due to the substituent effect which reduced the interaction between starch molecules and, hence, induced swelling power (Wickramasinghe et al. 2009).

The results also revealed a decrease in the adhesion of the cake samples containing hydrolysed starch. This decrease could be ascribed to a reduction in the polysaccharide external interaction chains due to high water-holding and moisture. In fact, water interferes in the formation of gluten-starch complexes of wheat flour, which are responsible for maintaining crumb adhesion and hardening. This may happen in analogy to emulsifiers that fix to protein with their

Fig. 6 Aspects of crumb prepared with native (**a**), enzymatically hydrolysed (**b**), acetylated (**c**), and enzymatically hydrolysed acetylated (**d**) corn starches at a concentration of 5 %

polar end, pointing their hydrophobic end to the exterior, and thus avoiding interaction with the polar starch polymer (Popper et al. 2007). The cake samples cooked with acetylated starch also showed a decrease in adhesion of, which is consistent with the results previously reported by Raina et al. (2006).

Further results indicated that the elasticity of the cake samples cooked with the modified starches underwent an improvement. This effect was more pronounced for the hydrolysed acetylated, followed by the acetylated and hydrolysed starches, respectively. The improvement recorded for the elasticity of the cakes baked with hydrolysed starch could be attributed to the amylase hydrolysis of starch network during swelling, leading to reduced dough strength and induced compressive stress (Gambaro et al. 2006). The increase in dough and crumb elasticity caused by α -amylase treatment was previously reported for several other bakery products (Kim et al. 2006; Hemalatha et al. 2010; Raghu and Bhattacharya 2010; Patel et al. 2012; Gomes-Ruffi et al. 2012). We can therefore note that the textural limitations of acetate starches can be overcome by enzymatic hydrolysis.

When used at a concentration of 5 %, the hydrolysed acetylated starch derivative yielded into better results in terms of crust color and cake appearance than the acetylated starch and the hydrolysed one (Fig. 5). This optimum of cake appearance could be the result of the synergistic improver effect of the enzymatically hydrolysed and acetylated starch products.

The functionality of the enzymatic hydrolysed starch products during baking could be related to the decrease of dough viscosity during gelatinization, thereby increasing dough volume in the oven. This could, however, reduce dough stability and extensibility, which could be attributed to the break down of gluten disulphide bridges as a result of the presence of high amounts of amylase-reducing polysaccharides. In conjunction with the improver effect of the hydrolysis, the ameliorative action of acetylation could be attributed to the aggregative properties generated by gluten proteins due to the oxidation of free thiol groups. This result proved the optimum appearance of cake samples having a number of cells in the crumb and showing favorable expansion of carbon dioxide during cooking obtained with the dually modified starch at a concentration of 5 % (Fig. 6). The better results were also recorded in terms of cake volume and height with the dually modified starch. The application of the modified starches at a concentration of 10 % brought no positive effects on the set of cake properties under investigation (Table 3).

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References

- AOAC 1984 Official Methods of Analysis. 14th edn, Association of Official Analytical Chemists, Arlington, VA, 1141
- Caliari M, Júnior MSS, Beleia ADP (2014) Infant dairy-cereal mixture for the preparation of a gluten free cream using enzymatically modified rice flour. *LWT Food Sci Technol* 59:1033–1040
- Chen Z, Huang J, Suurs P, Schols HA, Voragen AGJ (2005) Granule size affects the acetyl substitution on amylopectin populations in potato and sweet potato starches. *Carbohydr Polym* 62:333–337
- Colussi R, Pinto VZ, Halal SLM, Vanier NL, Villanova FA, Silva RM, Zavareze ER, Dias ARG (2014) Structural, morphological, and physicochemical properties of acetylated high-, medium-, and low-amylose rice starches. *Carbohydr Polym* 103:405–413
- Coutinho PM, Henrissat B (1999) Carbohydrate-active enzymes: an integrated database approach. Recent advances in Carbohydrate Bioengineering, Gilbert HJ, Davies G, Henrissat B, Svensson B (eds), The Royal Society of Chemistry, Cambridge, 3–12.
- De la Hera E, Gomez M, Rosell CM (2013) Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties. *Carbohydr Polym* 98:421–427
- Dura A, Błaszczak W, Rosell CM (2014) Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydr Polym* 101:837–845
- El Halal SLM, Colussi R, Pinto VZ, Bartz J, Radunz M, Carreño NLV, Dias ARG, Zavareze EDR (2015) Structure, morphology and functionality of acetylated and oxidized barley starches. *Food Chem* 168: 247–256
- Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, Sayas E, Navarro C, Fernández López J, Pérez-Alvarez JA (2011) Resistant starch as prebiotic: a review. *Starch/Stärke* 63(7):406–415
- Gambaro A, Gimenez A, Ares G, Gilardi V (2006) Influence of enzymes on the texture of brown pan bread. *J Texture Stud* 37:300–314
- Goesaert H, Brijs K, Veraverbeke WS, Courtin CM, Gebruers K, Delcour JA (2005) Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends Food Sci Tech* 16:12–30
- Gomes-Ruffi CR, da Cunha RH, Almeida EL, Chang YK, Steel CJ (2012) Effect of the emulsifier sodium stearoyl lactylate and of the enzyme maltogenic amylase on the quality of pan bread during storage. *LWT Food Sci Technol* 49:96–10
- Grabowski JA, Truong VD, Daubert CR (2006) Spray-drying of amylase hydrolysed sweetpotato puree and physicochemical properties of powder. *J Food Sci* 71(5):E209–E217
- Gray JA, BeMiller JN (2004) Development and utilization of reflectance confocal laser scanning microscopy to locate reaction sites in modified starch granules. *Cereal Chem* 81(2):278–286
- Han F, Gao C, Liu M, Huang F, Zhang B (2013) Synthesis optimization and characterization of acetylated corn starch with the high degree of substitution. *Int J Biol Macromol* 59:372–376
- Hemalatha MS, Prasada Rao UJS, Leelavathi K, Salimath PV (2010) Influence of amylases and xylanase on chemical, sensory, amylograph properties and microstructure of chapatti. *LWT Food Sci Technol* 43(9):1394–1402
- Hoover R, Zhou Y (2003) In vitro and in vivo hydrolysis of legume starches by α -amylase and resistant starch formation in legumes—a review. *Carbohydr Polym* 54:401–417
- Huang J, Schols HA, Jin Z, Sulmann E, Voragen AGJ (2007) Pasting properties and (chemical) fine structure of acetylated yellow pea starch is affected by acetylation reagent type and granule size. *Carbohydr Polym* 68:397–406
- Israilides CJ, Grant GA, Han YW (1978) Sugar level, fermentability, and acceptability of straw treated with different acid. *Appl Environ Microb* 36:43–46

- Jiang H, Miao M, Ye F, Jiang B, Zhang T (2014) Enzymatic modification of corn starch with 4- α -glucanotransferase results in increasing slow digestible and resistant starch. *Int J Biol Macromol* 65:208–214
- Karaoglu MM, Kotancilar HG, Çelik I (2001) Effects of utilization of modified starches on the cake quality. *Starch/Stärke* 53:162–169
- Khalil HPSA, Ismail H, Rozman HD, Ahmad MN (2001) The effect of acetylation on interfacial shear strength between plant fibres and various matrices. *Eur Polym J* 37:1037–1045
- Kim JH, Maeda T, Morita N (2006) Effect of fungal α -amylase on the dough properties and bread quality of wheat flour substituted with polished flours. *Food Res Int* 39:117–126
- Konował E, Lewandowicz GY, Thanh-Blicharz JL, Prochaska K (2012) Physicochemical characterisation of enzymatically hydrolysed derivatives of acetylated starch. *Carbohydr Polym* 87:1333–1341
- Luchese CL, Frick JM, Patzer VL, Spada JC, Tessaro IC (2015) Synthesis and characterization of biofilms using native and modified pinhao ~ starch. *Food Hydrocoll* 45:203–210
- Maarel MJECV, Leemhuis H (2013) Starch modification with microbial alpha-glucanotransferase enzymes. *Carbohydr Polym* 93:116–121
- Mi J, Liang Y, Lu Y, Tan C, Cui B (2014) Influence of acetylated potato starch on the properties of dumpling wrapper. *Ind Crop Prod* 56:113–117
- Miao M, Xiong S, Jiang B, Jiang H, Cui SW, Zhang T (2014) Improved the slow digestion property of maize starch using partially β -amylolysis. *Food Chem* 152:128–132
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428
- Nishinari K, Kohyama K, Kumagai H, Funami T, Bourne MC (2013) Parameters of texture profile analysis. *Food Sci Technol Res* 19(3): 519–521
- Olayide S, Lawal 2004. Composition, physicochemical properties and retrogradation characteristics of native, oxidized, acetylated and acid-thinned new cocoyam (*xanthosoma sagittifolium*) starch. *Food Chem* 87: 205–218.
- Patel MJ, Ng JHY, Hawkins WE, Pitts KF, Chakrabarti-Bell S (2012) Effects of fungal α -amylase on chemically leavened wheat flour doughs. *J Cereal Sci* 56:644–651
- Pearson D (1970) The chemical analysis of foods, seventh edn. Churchill Livingstone, Edinburgh, pp. 6–25
- Popper L, Gmbh M, Co Kg 2007. "Future of flour" the comprehensive manual of practical information on cereals, flour and flour improvement. Hard back, english, 480 pages, published by verlag agrimedia.
- Raghu KS, Bhattacharya S (2010) Finger millet dough treated with α -amylase: rheological, physicochemical and sensory properties. *Food Res Int* 43:2147–2154
- Raina CS, Singh S, Bawa AS, Saxena DC (2006) Some characteristics of acetylated, cross-linked and dual modified Indian rice starches. *Eur Food Res Technol* 223:561–570
- Saartrat S, Puttanlek C, Rungsardthong V, Uttapap D (2005) Paste and gel properties of low-substituted acetylated canna starches. *Carbohydr Polym* 61:211–221
- Sahnoun M, Bejar S, Sayari A, Triki MA, Kriaa M, Kammoun R (2011) Production purification and characterization of two α -amylase isoforms from a newly isolated *Aspergillus oryzae* strain S2. *Process Biochem* 47(1):18–25
- Sahnoun M, Naili B, Elgharbi F, Kammoun R, Gabsi K, Bejar S (2013) Effect of *Aspergillus oryzae* CBS 819.72 α -amylase on rheological dough properties and bread quality. *Biologia* 68(5):808–815
- Sahnoun M, Kriaa M, Elgharbi F, Ayadi D-Z, Bejar S, Kammoun R (2015) *Aspergillus oryzae* S2 alpha-amylase production under solid state fermentation: optimization of culture conditions. *Int J Biol Macromol* 75:73–78
- Sandhu KS, Sharma L, Kaur M (2015) Effect of granule size on physicochemical, morphological, thermal and pasting properties of native and 2-octenyl-1-ylsuccinylated potato starch prepared by dry heating under different pH conditions. *LWT Food Sci Technol* 61:224–230
- Singh J, Kaur L, Singh N (2004) Effect of acetylation on some properties of corn and potato starches. *Starch* 56:586–601
- Tester RF, Karkalas J (1996) Swelling and gelatinization of oat starches. *Cereal Chem* 73(2):271–277
- Than JL, Blaszcak W, Lewandowicz G (2007) Digestibility vs structure of food grade modified starches. *Food Sci and Technol* 10:1–3
- Wickramasinghe HAM, Yamamoto K, Yamauchi H, Noda T (2009) Effect of low level of starch acetylation on physicochemical properties of potato starch. *Food Sci Biotechnol* 18:118–123
- Xie SX, Liu Q, Cui SW (2005) Starch modification and applications. Copyright by Taylor & Francis Group, LLC
- Zhang G, Hamaker BR (2009) Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. *CRC Critical Reviews in Food Sci Nutr* 49:852–867