Original Research Article

Functional properties of acetylation-modified starches of three purple maize cultivars from Côte d'Ivoire

ABSTRACT

Acetylation is one of the most used chemical modifications to improve the functional properties of starches. This allows the esterification of native starches by introducing acetyl groups into their structure. The objective of this study was to enhance the value of purple corn from Côte d'Ivoire. Some functional properties and enzymatic digestibility of acetylated and native starches of three cultivars of this maize have been determined. To this end, these maize were harvested in Katiola, a town on the Ivory Coast. The results showed that purple corn starch was modified using 10.2% acetic anhydride. Acetylated starches showed a degree of substitution and an acetyl content between 0.1 and 0.15% and 2.6 to 3.91% respectively. Acetylation increased clarity, swelling and solubility. This increase could contribute to the production of ice cream, frozen fruit, bakery products and sauces. This has also resulted in reductions in demotion trends compared to the corresponding native starches. Concerning the lowest gelling concentration, acetylated starches have recorded low values (1; 2 and 4%) which are very interesting in the food industries for infant foods. For enzymatic digestibility, the starch that has been digested much more quickly in the acetylated form is dark violet (ADP). Overall, these acetylated starches can be recommended in several food industries.

Keywords: Acetylated starch, purple corn, functional properties, enzymatic digestibility, Ivory Coast

1. INTRODUCTION

Starch, synthesized in amyloplasts, form of carbohydrate storage in green plants, is mainly found in seeds, roots, tubers, stems, leaves and fruits [1]. It is composed of two main glucans: amylose and amylopectin. Starch is an important raw material used in food and other industries. It contributes greatly to the textural properties of many foods and has many industrial applications such as thickening and gelling agents [2]. Generally, the performance of starches in food systems was evaluated by their gels textural and physicochemical properties. Thus, gelatinization, pasting, and subsequent gel properties of starch are key functional properties that determine many applications of starch in the food industry [3].

Most often, these native starches are modified according to several processes in order to correct their defects, and there by improve their functional properties (viscosity, appearance and morphology, resistance to enzymes, etc.) for a specific use. Native and modified starches have wide applications [4] as a gelling agent, thickener, emulsifier and encapsulating agent in food products, a sizing agent, a coating agent and an adhesive in the paper industry. Chemical modification such as esterification (insertion of an acetyl group) of native starches not only improves their resistance to retrogradation and syneresis, but also increases freeze-thaw stability and dough clarity [5]. Moreover, it is established that approximately 75% of the global starch industry is made from corn [6]. Maize kernels are basic source of carbohydrates, mainly consisting of starch, making them the most energetic cereals [7, 8, 9].

In Côte d'Ivoire, an important varietal diversity of maize is cultivated by farmers for various reasons. Some of these traditional varieties have socio-cultural significance. This is the case of purple maize cultivated only in Katiola and in the surrounding localities; hence its name "Katiola violet" [10]. Recently studies on the physicochemical and functional properties of the flours and oils of three cultivars of this purple maize were carried out by Akaffou et al. [9]. These corns have been found to be rich and inexpensive sources of starch. However, to date, no study has yet been carried out on the modified starches of these different maize cultivars. Thus, faced with the strong industrial demand for starches and the needs of the Ivorian population. The objective of the present study was to extract starches from the three cultivars of purple corn, subjecting them to a chemical for acetylation, studying some functional properties of these acetylated starches and comparing them to those of native starches from the same sources; analyse the stability of these acetylated starches to enzymatic hydrolysis in order to assess their *in vitro* digestibility with a view to provide relevant information for various uses.

2. MATERIAL AND METHODS

2.1 Raw Materials

Dry kernels of three different varieties of Ivorian purple maize and the ordinary yellow maize (taken as control) were employed as raw material. Ordinary yellow maize kernels were purchased from local market in Abidjan. Purple maize seeds were grown during its appropriate cropping season (raining season from May to July) in 2022 on experimental plots of the National Center of Floristic of the University Felix Houphouet Boigny and Bingerville (5°21′ N and 3°54′ W). After harvesting and drying, 30 kg of each sample were collected and transported to the Biotechnology Laboratory of Felix Houphouët-Boigny University for analysis.

Fig. 1. Purple corn cultivars produced in Ivory Coast. **A**: corn purple red (PR); **B**: corn light purple (LP); C: corn dark purple (DP).

2.2 Starch isolation

Starches were extracted according to Delpeuch et al. **[11]** with some modifications. Corn kernels (1 kg) were washed thoroughly with distilled water and soaked in sodium metabisulfite solution (0.1%) overnight in the refrigerator. The obtained endosperms were mixed, and the paste was mixed with distilled water then dispersed in sodium chloride (4%) solution to separate proteins from starch. the starch obtained in a separating funnel is then dried at 45 ° C for 48 hours in an electric oven (VENTICELL, Fisher Bioblock Scientific, Allemagne). The dried product was crushed and sieved with a 250 µm mesh sieve.

2.2 Acetylation

The method of Ganiyat et al. [12] was used. 100 g of native purple corn starch were dispersed in 500 mL of distilled water and homogenized using a magnetic stirrer for 20 min. The pH of the mixture was adjusted to 8.0 by using a sodium hydroxide solution (1 M). Acetic anhydride (10.2 g) was added slowly (with stirring) over 1 h while maintaining the alkaline pH between 8 - 8.5. This alkaline starch suspension was left to incubate on the bench at room temperature (28 °C) for 5 min. Then, the pH of the starch suspension was brought to 4.5 with hydrochloric acid (1 M). This acid solution was filtered through Whatman number 42 paper and washed four times with distilled water. The pellet obtained was dried in a ventilated oven at 45 °C for 48 h. The dry sample was ground in a porcelain mortar, then sieved with a 100 µm mesh sieve to obtain a fine powder which was packaged in a previously dried and hermetically sealed glass jar. The latter was stored in a desiccator at room temperature (28 ± 2 °C) until use.

2.3 Acetylation percentage (Acetyl %) and substitution degree (SD)

The determination of the degree of substitution of acetylated starches was carried out according to the method of Smith [13]. A homogeneous ethanolic starch suspension (native and acetylated) of 6.6% (w/v) contained in Erlenmeyer flask, was heated with stirring in a water bath (Fisher scientific) at 50 °C for 30 min. The suspension was allowed to cool to room temperature (28 ± 2 °C) for 10 min, then diluted in 25 mL of sodium hydroxide solution (1 M) with discontinuous stirring. After this dilution, the starch suspension was left at room temperature (28 ± 2 °C) for 72 h with constant stirring. The excess sodium hydroxide was then titrated with hydrochloric acid solution (1 M) in the presence of phenolphthalein, a coloured indicator. The blank (native starch) and the sample (acetylated starch) were titrated at the same time.

2.3.1 Acetyl percentage

$$\boxed{\mathbf{Acetyl}\ (\%) = \frac{\left(\mathbf{V_{blank}} - \mathbf{V_{sample}}\right) \times \mathbf{Molarity\ HCL} \times \mathbf{M\ acetyl\ X\ 10^{-3}}}{\mathbf{Weight\ of\ the\ sample}} \times \mathbf{100}}$$

2.3.2 Substitution Degree (SD)

$$\boxed{\mathbf{SD}\ (\%) = \frac{\mathbf{M\ glucose} \times \%\ \mathbf{acetyl}}{\mathbf{M\ acetyl\ X\ 100} - ((\mathbf{M\ acetyl} - \mathbf{1})\ \mathbf{X}\ \%\ \mathbf{Acetyl})}}$$

V_{blank}: Volume of blank; V_{sample}: Volume of sample; M_{acetyl}: Molar mass of acetyl; $M_{glucose}$: Molar mass of glucose.

2.4 Functional parameters of starches

2.4.1 Paste clarity

The method described by **Zheng and Sosulki [14](1998)** was used for the determination of the paste clarity. Aqueous dispersions (1%) of starch were boiled at 100°C and constantly shaken for 30 min. The paste was cooled at ambient temperature and stored at 4°C for 4 weeks. The transmittance was measured at 650 nm every week by using a spectrophometer (Jenway 7315 spectrophotometer, Angleterre).

2.4.2 Smallest gelling concentration

the slightly modified method of Coffman and Garcia [15] was used for the determination of the smallest gelling concentration. Aqueous starch suspensions (1, 2, 4, 6, 8 and 10%; w / v) contained in test tubes were homogenized by manual shaking for 2 min at room temperature (28 ± 2 ° C), then heated in a boiling water bath for 1 hour. Then the tubes were quickly cooled to 4 ° C in a refrigerator for 30 min. The minimum gel concentration was defined as that of the suspension whose gel could not slip or fall when the tube was inverted.

2.4.3 Syneresis

Stability of starches samples to freezing was determined according to Singhal et Kulkarni [16]. Ten (10) grams of gel sample were conditioned in a plastic tube at 4°C for 4 weeks. Freezing stabilities were performed every week by measuring the percentage of water expelled after centrifugation at 5000 g for 30 min.

2.4.4 Solubility and swelling power

Swelling power and solubility of the starch were determined according to the method described by Leach et al. [17].

2.4.5 Dispersibility

The dispersibility (D) of the starches was determined according to the method described by Mora-Escobedo et al. [18]. Aqueous starch suspension (5% W/V) contained in a graduated cylinder is stirred carefully by hand for 2 min (avoid losing part of the solution). The dispersibility of starch is defined as the difference between the total volume of particles just after manual stirring and the volume of deposited particles recorded at time t (min).

2.4.6 Water absorption, oil absorption

The method described by Sosulki [19] was used for the measurement of oil absorption. A 10% dry starch (w / v) dispersion is prepared with distilled water or oil in centrifuge tubes. The mixtures thus formed are stirred with a vortex for 2 min and centrifuged at 4000 rpm for 20 min. The supernatants were removed, and the weight of hydrated starches is determined.

2.5 Enzymatic digestibility

An aliquot (100 μL) of starch suspension (2%; w / v) was mixed with 80 μL of sodium acetate buffer (100 mM pH 5.0) and 20 μL enzymatic extract diluted to 1 / 400. Several tubes were prepared and incubated at 37 ° C in a water bath at different times (0,5, 1, 2, 4, 8, 20 and 24 h). Reducing sugars obtained by enzymatic hydrolysis was determined according to Bernfeld's method [20].

2.6 Statistical analysis

All the experiences were performed in triplicate and the data were analyzed using EXCELL and XLSTAT 2018. Differences between means were evaluated by Duncan's test. The significance level was set at p value < 0.05.

3. RESULTS AND DISCUSSION

3.1 Acetylation percentage (Acetyl %) and substitution degree (SD)

The results of the acetyl percentages and the degree of substitution are shown in **table 1**. Analysis of the samples revealed that the percent acetylation of the three varieties studied evolves in line with the degree of substitution. Note that the red violet corn starch has the best acetylation rate of 3.91% compared to about 2.6% for the other two starches. As for the degree of substitution of the different starches, the values obtained (0.1 and 0.15) are statistically identical for the three types of starches.

Acetyl percentage and substitution degree are important parameters of acetylation-modified starch. The substitution degree rates recorded for the studied starches (0.10 to 0.15%) comply with the standard which requires a substitution degree less than 0.2 for starches intended for food uses [21]. As a result, these acetylated starches would be raw materials of choice for food industries such as bakeries and pastry shops. The acetyl contents (2.6 to 3.91%) with the acetic anhydride addition level of 10.2% are higher than those (between 1.01 and 2.80%) obtained by Vasanthan et al. [22] for potato, waxy corn, wheat, pea and lentil starches. They are like those reported by Sodhi and Singh [23] after use of 6% acetic anhydride. In fact, these authors obtained acetylation values sited between 2.26 and 3.68% for rice starches. These differences in acetyl content may be due to the structure of the starch granules.

Table 1. Acetylation percentage (% AC) and substitution degree (SD) of starches from the three purple corn cultivars treated with 10.2% acetic anhydride

STARCHES	%AC	DS
DP	2.6	0.1
PR	3.91	0.15
LP	2.6	0.1

DP: Dark Purple; PR: Purple Red; LP: Light Purple.

3.2 Dispersibility and smallest gelling concentration of starches

The results of the dispersibility are given by **Table 2**. The proportions oscillated between 58.82 and 64.71% with a relatively high dispersibility degree for the native starches (64.71%). In contrast, acetylated starches exhibited slightly lower dispersibility rates, ranging from 58.82 for the starch from the light purple maize cultivar (ALP) to 62.75% for that from the red purple cultivar (APR). The high dispersibility of native starches and the purple red cultivar acetylated starch (APR) is a property of great interest in the food industries. Indeed, it participates in the reconstitution of a mixture in water. The dispersibility of weaning food ingredients ranged from 63 to 79 [24].

The native and acetylated starches of the light purple and dark purple cultivars have the lowest concentrations, which are 1% and 2% respectively. As for the starch of the purple red cultivar, it showed concentrations of 2 and 4%, respectively for the native and acetylated samples (Table 2). These results of the smallest gelling concentration obtained (1%, 2% and 4%) for native and acetylated starches are lower than those for bean starches which are between 6 and 10% [25]. These purple corn starches have a good gel profile. The ability of starches to gel readily when heated is a desirable quality in the food industries for infant foods.

Table 2. dispersibility and smallest gelling concentration of native and acetylated starches from three cultivars of purple corn produced in Ivory Coast

Starches	Dispersibility (%)	Smallest gelling concentration (%)
NDP	64.71	2
NPR	64.71	2
NLP	64.71	1
ADP	60.78	2
APR	62.75	4
ALP	58.82	1

NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

3.3 Paste clarity

The clarity of the starch solutions is shown in **Fig. 2**. Overall, all native and acetylated starches exhibited opaque gels however acetylated starches exhibited higher clarities (1.1 to 1.5%) than native starches (0.7 to 0.9%). During storage in the refrigerator for native starches, there is a decrease in clarity during the first three weeks. Beyond that, this clarity becomes almost stable until the end of the fourth week. In contrast, for acetylated starches, there was no change in clarity during the 1st week of storage. The decrease in clarity is mainly observed during the second week. This decrease in clarity is most pronounced (approximately 25% decrease) in acetylated starch from the light purple corn cultivar (**Fig. 2**). Beyond week 2, clarity stabilizes until the end of week 4 of storage for acetylated starches.

The transmittance (%) after starch acetylation treatment of the three purple corn cultivars was higher compared to native starches. This effect of acetylation on the clarity of starch gels is identical to that reported by Lawal [26] and Singh et al. [27] for various types of starches. This could be explained by a greater swelling of the acetylated starch granules which allow more light to pass through the granules which are reflected [28]. This phenomenon could also be explained by the dissociation of starch granules and their ability to reflect light [27]. The introduction of the acetyl group in the native starch granules would cause repulsion between the starch molecules and the inter-chain association which is apparently reduced [29, 30]. This resulted in higher transmission than native starches. This improvement in starch clarity is necessary for the manufacture of ice creams, baked goods and sauces which exhibit better texture [31].

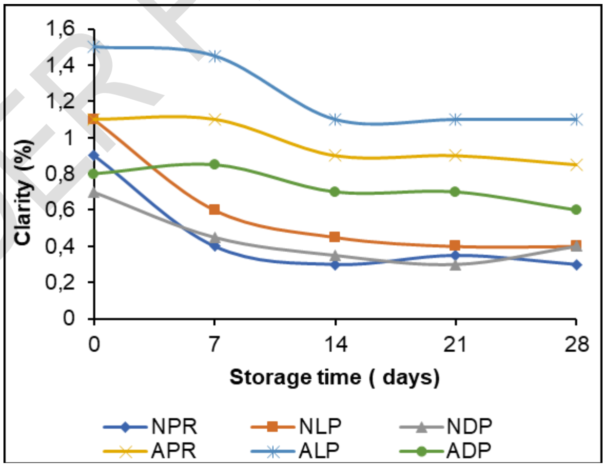

Fig. 2. Evolution of native and acetylated starch pastes clarity 4 °C

NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

3.4 Syneresis

The results of syneresis are illustrated in **Fig. 3**. The syneresis of purple corn starch gels revealed that the water rejection occurs from the 1st day and increases steadily until the 28th day (4th week) during storage in the refrigerator as well for the starches native (27.58 to 52.84%) and acetylated (17.08 to

35.43%). Overall, the synereses of native starches (NPR, NDP, NLP) are respectively higher than those of acetylated starches (APR, ADP, ALP). The highest syneresis is observed in the native starch of dark purple corn (52.84%). The syneresis of acetylated starch gels from purple corn is lower compared to that of native starches. This acetylation effect is identical to that obtained by Fei et al. [28] on normal corn starch and by Sodhi and Singh [23] for starches of several rice cultivars. This improvement in syneresis after acetylation could be attributed to the presence of acetyl groups which increases the water retention capacity of starch gels during refrigeration [32].

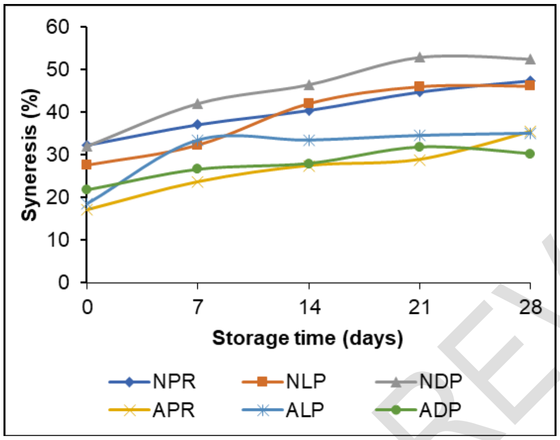

Fig. 3. Evolution of native and acetylated starches syneresis at 4 °C

NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

3.5 Swelling power and Solubility

The results of the swelling and the solubility of the starch granules are shown in **Fig. 4**. The swelling power of the native and acetylated starch granules of the different purple maize cultivars is strongly noticeable between 50 and 70 °C. The extreme values recorded in this temperature interval vary between 2 and 11 g/g. Above 70 °C, the swelling capacity is less accentuated. This increase in the swelling power of granules with the increase in temperature reaches a maximum of 10.9 to 12.55 g/g for native starches and from 12.59 to 13.24 g/g for acetylated starches at 90 °C. Acetylated starches have higher swelling power and higher solubility than native starches. The shapes of the starch solubility curves are comparable to those of swelling, namely an increase in the solubility index with temperature (50 to 90 °C) to reach maxima of 13.46 to 19.35% for native starches and from 19 to 26% for acetylated starches.

Acetylation treatment increased the swelling and solubility of purple corn starch granules. This phenomenon has already been observed and reported by Aning [33] and Singh et al. [27] on the starches of different corn varieties. These increases in solubility and swelling power can be explained by the introduction of hydrophilic acetyl groups allowing the water molecules retention due to their ability to form hydrogen bonds [31]. The retention of the water which penetrates the granules, thus increases the swelling and promotes gelatinization. When the swelling increases, the clarity of the gels is favoured, thus corroborating the improvement in transmittance observed at the level of acetylated starches [34, 35]. It is a sought-after property in the manufacture of certain confectionery products, in pastry and bakery.

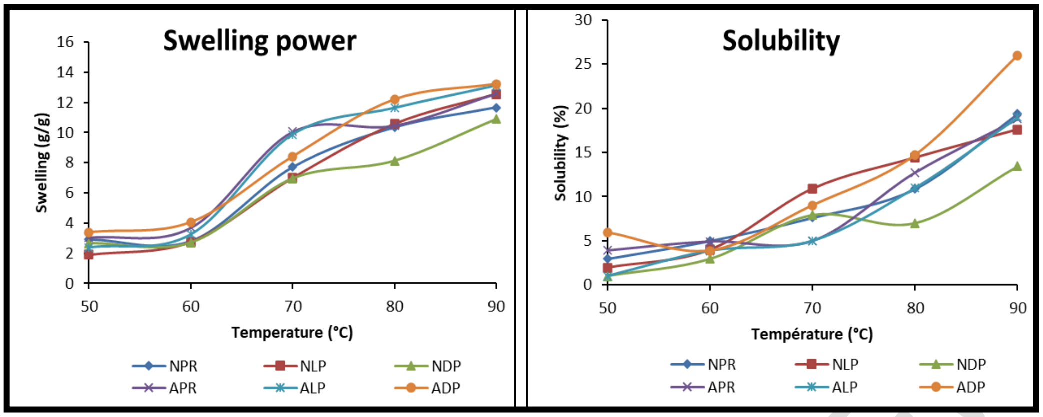

Fig. 4. Swelling power and solubility of starches from the three purple corn cultivars at 4 °C

NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

3.6 Hydration capacity and oil absorption

Tables 3 and **4** respectively show the hydration capacity and the oil absorption capacity as a function of the variation in the starch concentration. The results showed that the binding capacities are significantly different. Overall, they decrease with increasing starch concentration. The hydration capacity is higher at each concentration for acetylated starches than for native starches. The values decrease from 315.785 ± 7.08% to 218.505 ± 1.66% for acetylated starches and from 249.305 ± 3.09% to 204.35 ± 0.30% for native starches, respectively for starch concentrations varying from 2 to 10% (w / v). The oil absorbency values of the six starch samples ranged from 65.59 ± 1.24 to 139.655 ± 7.08%.

The increase in hydration capacity after acetylation treatment is identical to that obtained on cocoyam starch (*Xanthosoma sagittifolium*) by Lawal [26]. This increase could be due to an easy increase in water percolation in some areas starch due to intra-granular structural disorganization caused by steric effects and the breakdown of hydrogen bonds [36].

Table 3. Hydration capacity of native and acetylated starches from the three purple corn cultivars as a function of starch concentration

Starch Concentration	***Hydration capacity* (%)**				
	2%	4%	6%	8%	10%
NLP	249.305±3,09^{b}	226.37±9,15^{c}	212.53±7,01^{d}	208.605±1,94^{b}	204.35±0,30^{e}
NDP	255.99±11,84^{b}	231.715±13,50^{c}	223.35±0,45^{c}	223.415±6,03^{a}	212.045±2,61^{d}
NPR	253.59±9,89^{b}	229.34±4,72^{c}	225.045±1,82^{c}	203.705±2,43^{b}	205.57±1,13^{e}
ALP	306.735±2,60^{a}	272.775±11,90^{a}	240.77±8,81^{b}	233.58±1,98^{a}	228.6±1,64^{b}
ADP	293.895±15,56^{a}	273.99±3,72^{a}	251.325±2,81^{a}	238.21±7,48^{a}	233.165±0,23^{a}
APR	315.785±7,08^{a}	251.625±1,94^{b}	251.495±6,58^{a}	226.35±3,14^{a}	218.505±1,66^{c}

Values given are the averages of at least three experiments ±SE. Values followed by different superscript on the same column are significantly different (P=0.05). NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

Table 4. Oil absorption capacity of native and acetylated starches from the three purple corn cultivars as a function of starch concentration

Starch Concentration	**Oil absorption capacity (%)**				
	2%	4%	6%	8%	10%
NLP	90.145±6,87^{d}	113.34±4 ,24^{c}	78.2±3,58^{d}	66.5±0^{c}	78.19±0,71^{c}
NDP	191.755±4,66^{a}	122.635±3,87^{c}	97.695±3,26^{b}	90.27±1,80^{a}	85.385±0,70^{b}
NPR	139.655±7,08^{b}	117.175±2,58^{c}	86.59±0,11^{c}	85.415±1,18^{a}	74.05±1,98^{d}
ALP	136.135±2,10^{b}	125.375±0,70^{c}	86.855±1,21^{c}	82.96±2,98^{a}	72.665±1,83^{d}
ADP	127.815±1,27^{c}	131.86±5,15^{b}	106.475±0,23^{a}	89.885±5,14^{a}	93.33±0,14^{a}
APR	136.815±0,26^{b}	140.9±1,56^{a}	89.425±0,45^{c}	74.69±2,29^{b}	65.59±1,24^{e}

Values given are the averages of at least three experiments ±SE. Values followed by different superscript on the same column are significantly different (P=0.05). NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

3.7 Digestibility of native and acetylated starches

The enzymatic hydrolysis results are shown in **Fig. 5**. The glucose concentration gradually increases between 0 h and 4 h. It remains almost constant from 4 to 24 h. During the first four hours of hydrolysis, the reducing sugar quantities released are greater in the acetylated starches. Between 4 and 24h, this trend is not always observed. The concentration of glucose obtained with native light purple starch (NLP) is higher compared to that obtained from its acetylated starch (ALP). The enzymatic hydrolysis of purple corn starches is identical to that obtained by Huijun et al. [37] on normal and waxy maize which showed little change due to acetylation. However, the starch which was digested much faster in the acetylated form is that of dark purple (ADP). This type of starch also showed better solubility compared to the other starches tested.

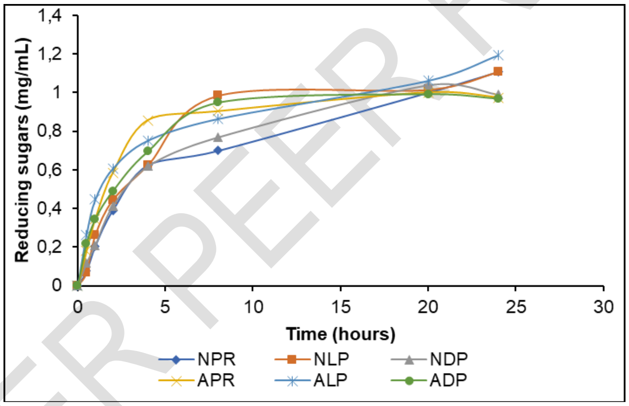

Fig. 5. *In vitro* enzymatic hydrolysis of native and acetylated starches from the three purple corn cultivars

NDP: Native Dark Purple; NPR: Native Purple Red; NLP: Native Light Purple; ADP: Acetylated Dark Purple; APR: Acetylated Purple Red; ALP: Acetylated Light Purple.

4. CONCLUSION

Acetylation of the starches from the three purple corn cultivars resulted in a good acetylation percentage and substitution degree. The results obtained revealed that acetylated starches generally exhibit better properties than natives. The esterification therefore led to a significant improvement in the techno-functional properties of the native starch of these corn (less retrogradation, reduced gelatinization time, less syneresis, swelling and high solubility) making the gel more stable. Among the three acetylated starches, that of the light purple corn gives the best swelling power, solubility, clarity, oil absorption and hydration capacity. Moreover, it has great digestibility. The studied starches could therefore be used in the food industry for infant foods, the manufacture of ice cream, frozen fruits, bakery products and sauces. In the non-food industry, they could valuably be recommended for the coating of tablets and the manufacturing of biodegradable films.

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