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Characterisation of amylase crude extracts of germinated corn (*Kassaï* and *Atp* varieties) and sweet potato flours (*Local* and *1112* varieties).

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Abstract

The high cost and difficulty of access to enzymes of microbial origin push manufacturers in developing countries to seek other sources that are easily accessible and less costly. This led us to characterise the raw extracts of corn malt and sweet potato in order to define the optimal conditions for their applications. To do this, germinated corn and sweet potato flours were used to produce the extracts. Based on these extracts, we carried out a characterisation by determining parameters such as pH and temperature of optimal activity and stability, the effect of ions and EDTA on amylase activity, In vitro digestibility and kinetic parameters. From this analysis, it emerges that the optimum pH of amylase activity of the crude extracts is 6. Amylase crude extracts of germinated corn and sweet potato *1112* variety (yellow) had the same optimal activity temperature (60°C) while the crude extract of sweet potato flour *Local* variety had an optimal activity at 55°C. The optimum stability zone was between 5.5 and 7 and at temperatures between 30 and 60°C for all crude extracts. The activity of the crude extracts increases with Ca^{2+} ions; unlike Cu^{2+} ions which inhibit their activities. The amylase crude extracts have no significant difference ($p>0.05$) in affinity to soluble starch but the hydrolysis rate is more marked with yellow sweet potato flour crude extract (variety *1112*). In view of all these results, we can therefore using the sources of these amylases under these conditions and purifying them.

Keywords: characterisation, amylase, crude extracts, germinated corn, sweet potato flour..

Introduction

Classical β and α-amylases present in higher plant and microorganisms catalyses the hydrolysis of α-1,4-glucosidic linkages of the non- and reducing ends respectively of starch molecules giving maltose, maltotriose, maltodextrine and glucose units (with glucose as major products) (Vihinam and Mantsala, 1989). Generally, grains such as corn are known to contain a varying quantity of amylases (both α- and β -amylases) (Adefila et al., 2012). Unmalted corn has no β-amylase and there is very little when the grain is malted; this affirmation contrast with case of sweet potato (Ipomoea batatas) that is thought to be an important source of β-amylase (Hyung et al., 2003).

These two types of amylase can be developed in corn during malting and employed in enzymatic saccharification of starch in most starch-based industries such as breweries, pharmaceuticals, distilleries, infant food developing industries etc. The major cereals employed in Cameroon industries for malting are maize, sorghum and millet. Malting consists of converting cereal grain to malt under controlled conditions (steeping, germination and kilning) (Tawaba et al., 2013). During this process, the plant hormone, gibberellic acid, is released to activate the aleurone layer and/or the scutellum cells of the grain to produce hydrolytic enzymes (α- and β – amylases in high proportion compared to another enzymes); these activated enzymes break down the complex grain molecules to provide simple molecules for the early development of the plant (Tawaba et al., 2013).

Alpha-amylases are an endoglycosidases (1, 4-α-D-glucan-4-glucanohydrolase; EC 3.2.1.1) produced by plants, animals and microbes, and they play a dominant role in carbohydrate metabolism (Adefila et al., 2012). They catalyse the random hydrolysis of internal α-1,4-D-glycosidic linkages in starch and dextrins, generating smaller dextrins and oligosaccharides with a C_1–OH group in the α-anomeric configuration. Their specificity can vary depending on the source. The α-amylases from fungal and bacterial sources have dominated applications in industrial sectors because they are more stable at high temperature and more active (Pandey et al., 2000).
Beta-amylase (α-1, 4-D-glucan maltohydrolase, EC 3.1.1.2) is an exo-enzyme which works in the non-reducing end of starch molecules. It brock down the amylose and amylopectin for approximately 70% and 55% respectively (Nakamura, 1996). Unlike another enzyme such as α-amylase, α-glucosidase and limit dextrinase which are synthesised de novo during barley germination, β-amylase is synthesized during seed development (Tawaba et al., 2013).

Despite the richness of plants in enzymes and particularly amylases, industrialists in developed countries are more interested in enzymes of microbial origin because of their cost, production time and thermostability. In developing countries like Cameroon, the cost and availability of enzymes of microbial origin force people and industries to turn to locally accessible enzyme sources. These can be obtained as previously mentioned by malting cereals such as corn (Sodipo and Fashakin, 2011) but also unfermented tuber flours such as sweet potato flour particularly rich in amylases (Yadang et al., 2013).

Because of their high availability once extracted and characterized, they can be used in mixture with enzymes of microbial origin in technological processes. This work therefore aims to characterise the crude extracts of amylase corn flour (*Kassaï* and *Atp*) and sweet potato flour (*Local* and *1112*) produced in the locality of Dschang in order to provide optimal conditions for their use in households for the preparation of infant gruels with low consistency and good nutritional value as well as for application on an industrial scale.

Materials and Methods

Plant material: the plant material obtained at IRAD station of Dschang-Cameroon consists of sweet potato tubers (*1112* and *Local* varieties) and corn seeds (*Kassaï* and *Atp* varieties).

Methods

Flour production

The various samples obtained at the Dschang IRAD station were sent to the Biochemistry Laboratory for Medicinal Plants, Food Sciences and Nutrition where they were used to produce the various flours.

Indeed, the sweet potato tubers *1112* and *Local* varieties were transformed into flours according to the method described by Foukam (2016). Indeed, they were washed and peeled then cut into slices (5-7mm thick) with a stainless steel knife. The samples were dried directly in a "venticell" oven set at 50°C. The dried samples were then ground using a “Moulinex” and the flour obtained was sieved (400 µm). The flours were then packed in plastic bags and stored in a desiccator.

Corn seeds were malted using the method described by Traoré et al. (2003). These pre-sorted corn seeds were washed and soaked for 48 hours at 50°C. They were then removed from the water, spread out and mixed with ashes and then for 96 hours, we left the grains mixed with ash in the shade on a cloth that we watered daily until the germination process was well underway and the roots appeared. The corresponding drying was carried out in a "venticell" oven at 50°C for 45 hours. The degerminated seeds were crushed

and the grinding was sieved (Ø=400µm). The germinated flours obtained were then packaged in plastic bags and stored in a desiccator before being used for analysis.

The various flours obtained are used to extract amylases for characterisation.

Germinated corn and sweet potato flours amylase extraction

To 1g of flour was added 4 ml of distilled water. The mixture was homogenized for 1 min using a vortex and centrifuged at 4500 rpm for 30 minutes using a "Heraeus" refrigerated centrifuge. The crude extracts obtained were put in boxes and then stored in a freezer at -10°C for later use (it should be noted that since the extraction buffer was not yet known, the extracts were renewed every 2 days and the enzyme solution diluted every day).

Methods of analysis:

Chemical characterisation of amylase crude extracts

The amylase crude extract were used to determine the pH and minerals content (Ca, Fe, Mg, Na and K) according to the method described by the AOAC (1990). The method described by Fischer and Stein (1961), was used to measure reducing sugars. The titrable acidity was performed according to the method described by AFNOR (1982). The method described by Oshodi et Ekperigin (1989), was used to measure soluble protein in different crude extract. The biuret reactant was used to measure the soluble protein with ovalbumin of white egg as standard.

Study of the amylase activity of flours extracts

The previously obtained crude amylase extracts were diluted to 1/150 and this dilution were used for various tests.

Influence of pH and temperature on the amylase activity of the crude extracts

The activity of amylase extracts was measured in the presence of 1% soluble starch in a buffered medium at different pH ranging from 3.0 to 8.0. The buffer solution used covering this range is sodium citrate (3-5) and sodium phosphate (5-8). The incubation temperature was chosen to be 40°C. The 4x9 bivariate factorial device was used with 4 amylases extracts and 8 pH (3.0; 4.0; 5.0; 5.5; 6.0; 6.5; 7.0 and 8.0).

The influence of temperature was studied by measuring amylase activity over a range of 30 to 90°C. The residual amylase activity was determined using the amyloclastic method of Kéléké et al. (1995), which consisted in pre-incubating 1ml of a 1% (w/v) soluble starch solution with a buffer of 100 mM concentration and pH varying from 3 to 8 with regard to the influence of pH on amylase and preincubate 1 ml of a 1% (w/v) soluble starch solution at temperatures ranging from 30 to 90°C with regard to the influence of temperature on amylase activity in water bath for 20 min. 0.1 ml of diluted enzyme extract was introduced into the tube. The enzymatic reaction was stopped by addition of 1 ml 0,1N hydrochloric acid. 1ml of this reaction medium was collected and added to 1.2 ml 4% lugol (v/v), optical densities were read at 580 nm spectrophotometer. An enzyme unit is defined as the amount of enzyme that converts 1 mg of substrate per minute. This residual or relative activity (RA) is determined according to the formula defined according to the following equation.

RA (%) = Relative activity at given pH or temperature ×100 divided by Maximal or optimal activity

Determination of pH and thermostability temperature

Enzyme extracts (0.1 ml) were preincubated at 40°C with pH buffers ranging from 3-8 for 30 min, after which 1 ml of 1% soluble starch was introduced and the mixture was incubated for 5 min then the amylase activity was determined according to the amyloclastic method of keleke et al. (1995). As for the thermostability temperature, they were heated to temperatures ranging from 30-90°C for 30 and 60 min. after heating, 1 ml of 1% soluble starch was introduced and the mixture incubated for 5 min then the amylase activity was determined according to the amyloclastic method of keleke et al. (1995).

Influence of metal ions and EDTA on amylase activity

The effect of different ions (Ca^{2+}, Na^{+}, Cu^{2+}, Fe^{2+}, Mg^{2+} and K^{+}) at 1 and 5mM concentrations on amylase activity as well as EDTA (chemical agent) at 1 and 40mM concentrations was studied. Indeed, the extracts were pre-incubated in the presence of the various ions and EDTA during 20 min at 40°C. After

this, soluble starch was added and the mixture was pre-incubated for 5 min (time of V_i). The effect of the various ions mentioned above and EDTA on the amylase activity of the various extracts was measured using the amyloclastic method of keleke et al. (1995).

In vitro digestibility of soluble starch by various extracts

In vitro digestibility was done on 1% soluble starch with water as well as different sources of amylases according to the method described by Okou (2005). In vitro digestibility was monitored for 3 hours. 1ml aliquots were collected at regular time intervals (30, 60, 90, 120, 150, 180 minutes) and then amylase activity was determined using the amyloclastic method of keleke et al. (1995) described above.

Determination of kinetic constants

Maximum speed (Vmax) and Constance of Michaëlis (Km)

The kinetic parameters Vmax and Km were determined under the optimal pH and activity temperature conditions of the various amylase extracts. To do this, soluble starch at a concentration ranging from 0-1% was used and the quantity of reducing sugars formed was quantified by Bernfeld's (1955), method. These results allowed us to plot V=f([S]). The amylases obeying a michaelian kinetics, so we proceeded to draw the double inverted Lineweaver-Burk 1/V=f(1/S).

These constants were determined using the regression of the Lineweaver-Burk equation which is a linearisation of the Michaelis-Menten equation

Statistical analysis

The results of the analyses carried out were expressed as averages plus or minus deviations. The means were analyzed by the ANOVA test at the 5% probability threshold and the Duncan test was used to compare the means using SPSS version 20 software. The graphs were drawn using Excel 2013 software.

Results and Discussion

Approximate chemical composition, pH and titrable acidity of the various amylase extracts

Table 1 shows the approximate composition of amylase extracts. It appears that excluding the raw extracts of white sweet potato flour, all the other extracts presented significantly reducing sugar contents ($p>0.05$) similar. This would be due to an increased activity of the amylases of these 3 plants compared to that of the white potato. The soluble protein content is affected only by the nature of the sample. These results can be explained by the extraction method used and the composition of the flours from which the extracts were produced as well as the nature of the proteins present. Ions are factors influencing the activity of enzymes. White sweet potato flour extract has the highest Ca content (81.95 mg/% of DM) compared to germinated yellow corn extract which has the lowest (2.85 mg/% of DM). The Mg content is significantly affected ($p<0.05$) by the nature of the sample and the variety. The highest K content was found in white sweet potato flour extract and the lowest in germinated yellow corn extract. This parameter is affected by the nature of the sample and the variety within the sweet potato samples. This would result from the plant's ability to absorb nutrients during its growth and also from the composition of each plant. Indeed, cereals are rich in phytates which have the ability to chelate ions and thus reduce their bioavailability. Observations similar to those of K are regally observed for Na and Fe ions. Like ions, pH is also a factor influencing amylase activity. It varies from 5.91 to 6.22. It is affected by the nature of the sample, which would be due to the elimination of some of the organic acids formed during soak-fermentation of corn seeds. As for titrable acidity, it is found to be significantly ($p<0.05$) affected by the nature of the sample and the variety within the corn samples. This value in sweet potato samples would be explained by the low synthesis of these compounds during the maturation of these plants on the other hand in corn samples it would be due to the partial elimination during drying of organic acids formed during the process of soak-fermentation of seeds.

Table 1: Approximate chemical composition, pH and titrable acidity of the various amylase extracts

Crude extracts	YSPF	WSPF	GWCF	GYCF
Réducing sugar (% of DM)	12.51±0.29[a]	11.29±0.29[b]	12.92±2,32[a]	12.68±0.46[a]
Soluble protein (mg)	14.91±1.20[a]	16.54±0.16[a]	9.42±0.36[b]	11.20±1.50[b]
Ca (mg/% of DM)	76.65±2.05[b]	81.95±0.92[a]	3.45±0.35[c]	2.85±0.21[c]
Mg (mg/% of DM)	10.88±0.54[b]	7.67±016[c]	10.73±0.22[b]	14.90±0,04[a]
K (mg/% of DM)	174.15±4.74[b]	208.75±2.48[a]	108.40±0.00[c]	108.20±0.28[c]
Na (mg/% of DM)	24.26±0.49[b]	26.70±0.25[a]	0.20±0.00[c]	0.20±0.00[c]
Fe (mg/% of DM)	3.80±0.05[b]	4.64±0.11[a]	0.40±0.04[c]	0.35±0.04[c]
pH	6.11±0.04[b]	5.91±0.00[c]	6.31±0.07[a]	6.22±0.07[a]
Titrable acidity (ml eq of NaOH/% of DM)	3.72±0.16[c]	4.27±0.32[c]	13.20±0.28[a]	7.91±0.16[b]

The values carrying the different letters (a, b, c……) in the same column meaningfully differ ($p<0.05$).
GWCF: Germinated White Corn Flour; YSPF: Yellow Sweet Potato Flour; GYCF: Germinated Yellow Corn Flour; WSPF: White Sweet Potato Flour.

Optimum temperature and pH activity of the various amylase crude extracts

The figures below show the effect of temperature and pH respectively on the amylase activity of plant extracts. With regard to the influence of temperature as represented, we observe that all extracts have an activity greater than 40% between 45-60°C with optimal activity at 60°C for extracts of yellow corn, white corn and yellow sweet potato ; 55°C for extracts of white sweet potato flours. In addition, 2 peaks were observed at 45 and 80°C with corn flours extracts, which would suggest the existence of an enzyme complex in these extracts. We also observe that on both sides of the optimal activity temperatures of the amylase extracts a decline in the activity of the two extracts, which is explained by the loss of native conformation of the enzymes naturally present in the various extracts due to the increase in temperature (Abe et al., 2002). These optimal activity temperatures are similar to those of Klang (2015), which obtained the value of 60°C as the optimal activity temperature of α-amylases extracted from *Burnatia enneandra* and *Abrus precatorius*.

As far as pH is concerned, the four extracts showed maximum activity at pH 6 with a stability zone of between 5.5-6.5. This pH of optimal activity is close to the pH of amylase extracts (6.11, 5.91, 6.31 and 6.22) as shown previously. This range has also been reported by Fahmy et al. (2000), as the pH range of optimal activity of α-amylases extracted and partially purified from *Sakha 69* wheat. These results are also similar to those of lizotte et al (1990), which after investigations obtained an optimal activity of β-amylases extracted from maize between pH 4.5 and 6.2. However, these results are lower than those of Noman et al. (2006) who obtained a slightly alkaline pH (pH 7.3) for *Pachyrus erosus* tuber amylase. These results could be explained by the nature of the samples. In addition, there is a drop in activity below 20% for the four enzymatic extracts, which would mean that at this pH we are witnessing a change in the ionization state of the amino acids involved in catalysis.

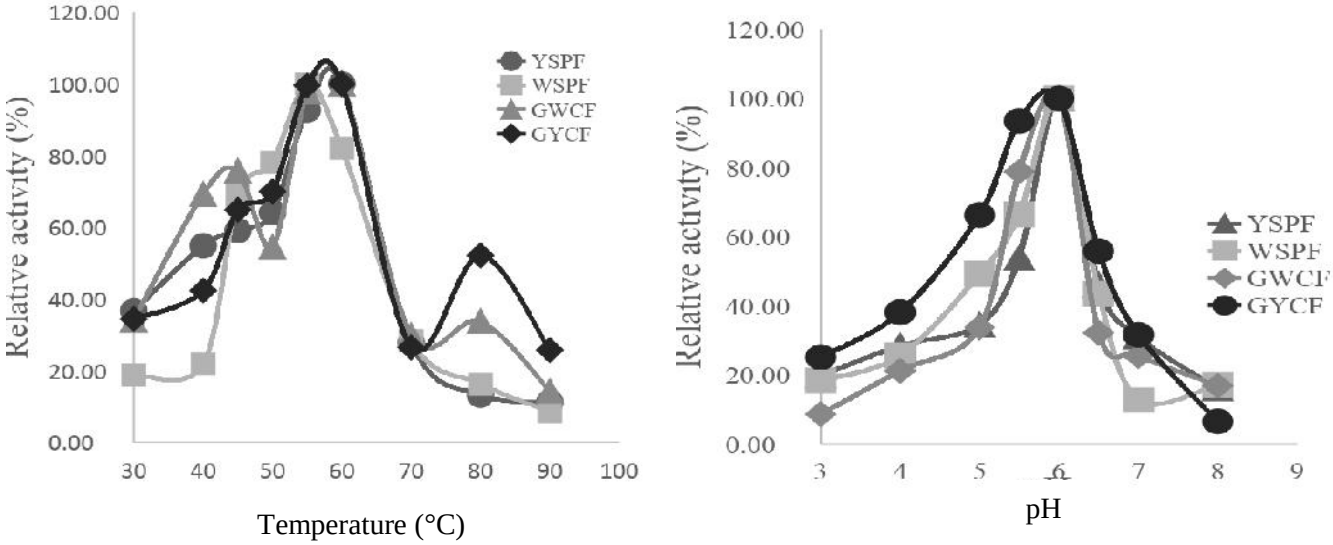

Figure 1: Effect of temperature and pH on the amylase activity of the crude extracts.
GWCF: Germinated White Corn Flour; YSPF: Yellow Sweet Potato Flour; GYCF: Germinated Yellow Corn Flour; WSPF: White Sweet Potato Flour

Influence of temperature and pH on the stability of the various amylase crude extracts

The thermostability and pH stability of the various amylase crude extracts is presented by the curves below (Figure 2). This shows that the sweet potato amylase crude extract maintains a relative activity of 100% up to 55°C and it goes up to 60°C for the other crude extracts. Above these temperatures, there is a drop in germinated corn crude extracts as they retain nearly 40% of their activities after 30 min of heating at 80°C; while sweet potato flour extracts lose up to 70% of their relative activity under the same conditions. There is also an increase in amylase activity of white and yellow germinated corn crude extract at 80°C, which would be due to the enzyme complex with an enzyme active at 80°C but this is very quickly inactivated because at 90°C and after 30 minutes of heating, its activity is less than 20%. The amylases of the crude extract of germinated yellow corn are the most thermostable because they retain more than 50% of activity after 60 min of heating at 80°C; while that of white sweet potato flour are the less thermostable, because they lose 80% of activity under the same conditions. The thermostability temperatures we found are similar to those of Tripathi et al. (2007), who observed that the α-amylase of partially purified extracts of mungo beans was stable (100% RA) up to 60°C (*Vigna radiata*). The stability of the amylase crude extracts was tested after pre-incubation at different pH. Both amylase crude extracts were stable in the pH 5.5-7 range and maintained over 60% relative activity after 20 min incubation at pH 7. However, the activity of amylase crude extracts has radically decreased to less than 40% relative pH 3.0 activity, which demonstrates the labile nature of enzymes at acid pH. Similar results were obtained by Goutam et al. (2010), who demonstrated that purified sweet potato α-amylase had a stability in the pH range of 6 to 8 and lost more than 95% of its relative pH 3 activity.

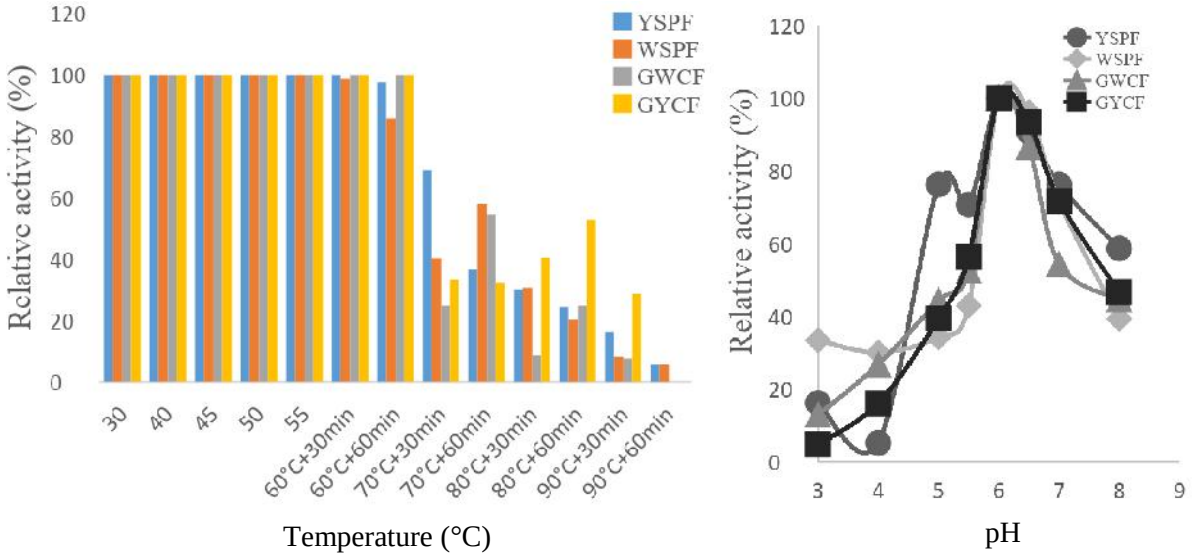

Figure 2: Effect of temperature and pH on stability of amylase crude extracts.
GWCF: Germinated White Corn Flour; YSPF: Yellow Sweet Potato Flour; GYCF: Germinated Yellow Corn Flour; WSPF: White Sweet Potato Flour.

Influence of ions and EDTA on the stability of the various amylase extracts

The effect of metal ions and EDTA on amylase activity is shown in Figure 3. Measurements of the activity of the four amylase crude extracts in the presence of metal ions show inhibition and activation effects depending on the extract used and the type of metal ion. These different ions (Ca^{2+}, Na^{+}, Mg^{2+}, Cu^{2+} and Fe^{2+}) were tested at concentrations of 1 and 5 mM. It shows that Na^{+} at a concentration of 1 mM appears as an activator for amylases present in yellow sweet potato extracts and germinated yellow corn crude extracts while at concentration of 5 mM, it inhibits the activity of amylases present in germinated yellow corn flour extracts but by those present in yellow potato flour extracts. The activity of the other two extracts is inhibited by this ion whatever its concentration. Magnesium (Mg^{2+}) whatever its concentration and iron (Fe^{2+}) has a concentration of 1mM have a positive effect on the amylase activity present in germinated yellow corn flour extracts. These ions, whatever their concentration, appear as common inhibitors of the other 3 amylase crude extracts. It is the same for copper which strongly inhibits the amylase activity of four extracts especially at 5mM because copper sulphate is a heavy metal salt which denatures the protein structures. As for calcium, it appears as an activator of all amylases present in the crude extracts. Generally speaking, it appears that the activity of several amylases is influenced by the presence of metal ions. These ions are cofactors of these enzymes and are therefore called metalloproteins. Generally speaking, it follows that the activity of several amylases is influenced by the presence of metal ions. These ions are cofactors of these enzymes and are therefore called metalloproteins. Amylases and more particularly α-amylases are metalloenzymes whose cofactor is most often calcium. The increase in amylase activity in the presence of Ca^{2+} ions is based on its ability to interact with negatively charged amino acids such as aspartic and glutamic acids; this results in increased stabilisation of the active conformation of the enzyme. In addition to this interaction, calcium is known to play a role in binding the enzyme to the substrate. In our study, Ca^{2+} ions increase the amylase activity of all our extracts, especially when their concentration increases. This led Shipra et al. (2015), claimed that amylase is a calcium-dependent enzyme which hydrolyses complex carbohydrates into glucose and maltose. This ion therefore stabilises the active conformation of the enzyme. Inactivation by metals such as Cu^{2+}, Mg^{2+}, Fe^{2+} and Na^{+} to a lesser extent may be due to their binding to catalytic residues, to a high proportion in the plant or to a replacement of Ca^{2+} which binds the enzyme site (Elarbi et al., 2009). The Role of Ca^{2+} in maintaining the stability and structure of α-amylase is well demonstrated by Parkin (1993). As far as the effect of EDTA is concerned, there is a sharp drop in activity from the first EDTA concentration (1 mM). This can be explained by the fact that EDTA is a chelating agent, i.e. it acts by complexing the metal ions, in particular calcium (co-factors) contained in enzymes, necessary for their activities (Burhan et al., 2003) and this would lead us to believe that the amylases of the four amylase extracts are metalloproteins.

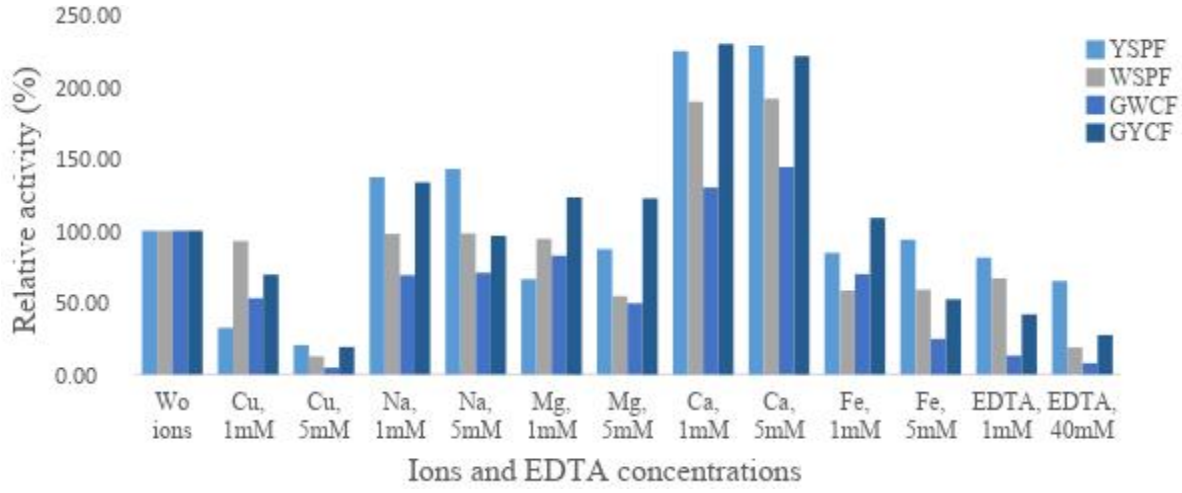

Figure 3: Effect of ions and EDTA on the amylase activity of the crude extracts.
Wo: Without; GWCF: Germinated White Corn Flour; YSPF: Yellow Sweet Potato Flour; GYCF: Germinated Yellow Corn Flour; WSPF: White Sweet Potato Flour.

In vitro digestibility of soluble starch by various extracts

The hydrolysis curves of the soluble starch (10 g/l) by the amylase crude extracts are essentially identical with a drop in the concentration of soluble starch and an increase in the concentration of sugars formed over time. The soluble starch concentration drops rapidly in the first hydrolysis times until it stabilises after 180 minutes. Similarly, an increase in sugar production is noted in the first hydrolysis times with a constant rate observed up to 90 minutes for amylase crude extracts of germinated white corn flour, yellow and white sweet potato and 120 minutes for germinated yellow corn crude extract. This constant speed is called the initial speed and refers to the phase of the reaction where all the molecules of the enzyme are bound to substrate molecules. Under these conditions, the catalytic efficiency of the enzyme is greatest. This speed remains constant as long as the substrate concentration is high and the product concentration low. Then we have a phase in which the speed of the reaction decreases: as the concentration of the product increases, the reverse reaction begins to compete with the one we were measuring (Reaction 1). Finally, in a last phase, the reaction speed becomes zero, the product and substrate concentrations no longer change, we are at equilibrium.

$$E + S \rightleftharpoons ES \rightleftharpoons P + E$$

This kinetics obtained from the determination of the amount of starch remaining in the medium over time gives us an idea of the behaviour over time of the enzymes present in the various amylase flours. The differences observed in the action of crude extracts on soluble starch over time are mainly due to the origin of the starch, the origin of the enzyme and their physico-chemical properties (Nirmala and Muralikrishna, 2003b). This variation in hydrolysis rate was also reported by Klang (2015), who observed that the partially purified α-amylases of *Abrus precatorius* and *Cadaba farinosa* had a constant rate of 10 minutes during hydrolysis of soluble starch and 15 minutes for *Burnatia enneandra* amylase.

As far as the transformation curve of starch over time is concerned, it has the appearance of a Michaelian kinetic. Indeed, we observe that the rate of degradation of starch by enzymes increases up to 90 minutes and stabilises beyond. This stabilization results in the maximum speed of activity being reached and in the quantity of substrate transformed becoming very small in front of the quantity of substrate present.

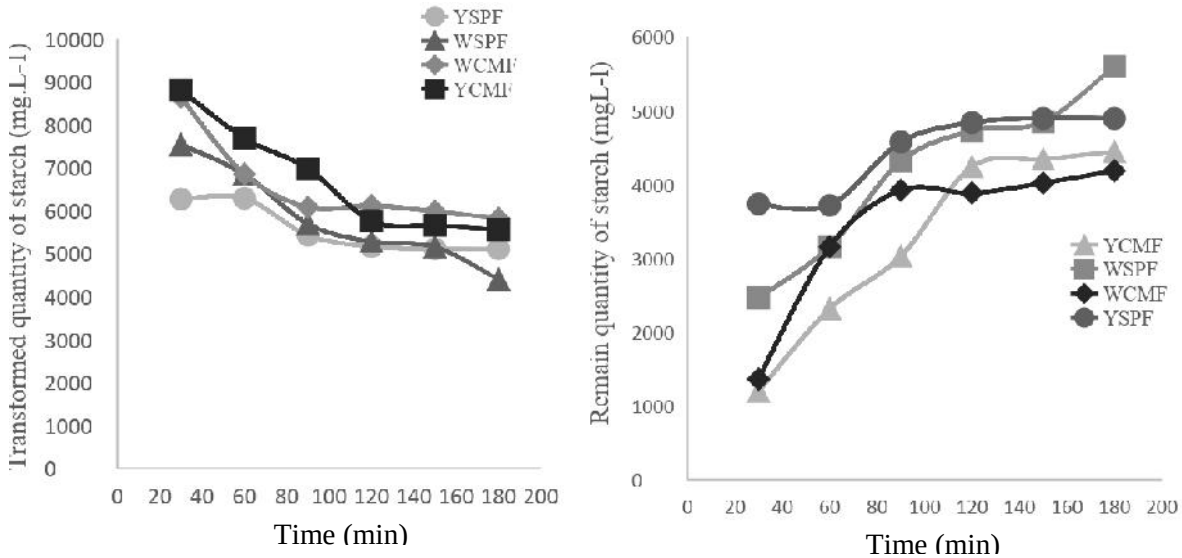

Figure 4: Kinetics of soluble starch hydrolysis and starch transformation with time
GWCF: Germinated White Corn Flour; YSPF: Yellow Sweet Potato Flour; GYCF: Germinated Yellow Corn Flour; WSPF: White Sweet Potato Flour.

Kinetic study

This study consists in determining the kinetic parameters of hydrolysis catalyzed by amylase crude extracts on soluble starch. The hydrolysis reaction is initiated by the addition of amylase extracts (100µl) in the reaction medium (buffer and substrate) and determined by the Bernfeld method (1955). Seven concentrations of soluble starch between 0 mg/ml and 10 mg/ml were used. The results were obtained thanks to the curves of Michaelis-Menten V=f(S) on the one hand and Lineweaver and Burk: 1/V= f(1/S) on the other. From these analyses, the following data emerge. Yellow sweet potato flour extract had the greatest affinity for soluble starch and germinated white corn flour extract had the least affinity. It is annotated that there is no significant difference ($p>0.05$) for this parameter, which means that they statistically have the same affinity for soluble starch. These Km values are higher than those of Klang (2015), which were 3.2; 1.8 and 3.1 respectively for amylases extracted and partially purified from *A. precatorius B. enneandra* and *C. farinosa*. This could be due to the fact that certain factors such as impurities present in our extracts could hinder enzymatic catalysis. The maximum activity rates of the amylase crude extracts show that it takes more time for a molecule present in the amylase extracts of germinated yellow corn to transform a starch molecule into sugars compared to the other three amylases. We note that this parameter is significantly ($p<0.05$) affected by the variety of sweet potato which would be explained by the degree of amylase activity naturally present. It is generally noted that sweet potato crude extracts presented values higher than those of germinated corn, which probably cannot be explained by the fact that the method used (saccharogenic method) for determining these parameters is not advantageous for extracts rich in α-amylases compared to those rich in β-amylases.

Table 2: Kinetic parameters of amylase activity of different extracts

Crude extracts	Vm (mg of maltose/ l of reactionnal mediuml/min)	Km (g/l)
YSPF	3444.44±1503.08^{a}	5.27±2.61^{a}
WSPF	1679.89±727.75^{b}	3.93±2.45^{a}
GWCF	1888.89±192.45^{ab}	7.67±0.58^{a}
GYCF	1191.92±413.65^{b}	4.37±1.70^{a}

The values carrying the different letters (a, b, c……) in the same column meaningfully differ ($p<0.05$).
GWCF: Germinated White Corn Flour; YSPF: Yellow Sweet Potato Flour; GYCF: Germinated Yellow Corn Flour; WSPF: White Sweet Potato Flour.

Conclusion

The characterisation of the amylase crude extracts of the various amylase rich flours was carried out in order to determine the best conditions for combining these flours with gruels in order to reduce the consistency of these. The optimum pH of the four amylase crude extracts is 6 and temperatures 60°C for germinated corn crude extract and sweet potato flour variety *1112* and 55°C for *Local* sweet potato flour variety. Ca^{2+} ions, whatever their concentration in the medium, increase the activity of all the crude extracts. In the presence of soluble starch, the amylase extracts had the same affinity but the hydrolysis rate is higher with sweet potato flour variety *1112*. These different results therefore give us the conditions of use of these crude extracts as well as the flours from which they are derived in the processes where they could be used. In view of all this we can thus envisage a purification of the various extracts in order to propose enzymes directly applicable in industry and thus to compensate for the lack of enzymes of quality.

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Competing interests

Authors have declared that no competing interests exist.

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