Revue Africaine d'Environnement et d'Agriculture
2018; 1(1), 19-24
http://www.rafea-congo.com
Dépôt légal: JL 3.01807-57259

Modeling effects of African Yam Bean (*Stenostylis stenocarpa* (Hochst. ex A. Rich.) Harms) extract on the digestibility of African Yam Bean flour by an extracted barley malt α-amylase

Arthur Amisi Kapepa*, Willy Kasembe, Patrick Baguma Kalimba, Jean-Claude Bwanganga Tawaba

University of Kinshasa. Agricultural Faculty. P.O. Box. 14071. Kinshasa 1 (DRC). E-mail: arthuramisi@hotmail.fr; arthur.amisi-kapepa.1@ulaval.ca

Reçu le 12 mai 2018, **accepté** le 22 août 2018

ABSTARCT

In this study, the African Yam Bean flour inhibitors of α-amylase were extracted using 5 solvents, namely: acetone / water mixture (70/30; V/V), acetone / water mixture (50/50; V/V), distilled water, water / acetic acid mixture (90/10; V/V), distilled water/acetic acid mixture (80/20; V/V). The digestibility of resulted flours was shown to be significantly affected by the extraction procedure. The acetone/water mixture (70/30; V/V) extraction presented the highest starch digestibility by barley malt α-amylase. The acetone/water mixture (70/30; V/V) extract was also responsible of reducing the hydrolysis rate of corn starch. The kinetic parameters of inhibition were determined by studying the effect of increasing corn starch concentration (2, 3 and 4 g/l) used as a substrate on the initial velocity of the reducing sugars release (V_0) in the presence of the various concentrations of acetone / water mixture (70/30; V/V) extract. The results obtained show that the inhibition of α-amylase by the African Yam Bean acetone/water mixture (70/30; V/V) extract on the digestibility of Corn starch (66 % amylose) is of competitive type.

Key-words: African Yam Bean, modeling, α-amylase, inhibition, digestibility.

RESUME

Modélisation des effets d'extrait de haricot igname (*Stenostylis stenocarpa*) sur la digestibilité de la farine de haricot igname par l'α-amylase du malt d'orge. Dans cette étude, les inhibiteurs de l'α-amylase de la farine de haricot igname ont été extraits à l'aide de 5 solvants, à savoir: le mélange acétone/eau (70/30; V/V), le mélange acétone/eau (50/50; V/V), l'eau distillée, le mélange eau/acide acétique (90/10; V/V), le mélange eau distillée/acide acétique (80/20; V/V). La procédure d'extraction a montré que la digestibilité des farines obtenues était significativement affectée. L'extrait obtenu après extraction à l'aide du mélange acétone/eau (70/30; V/V) présentait la digestibilité la plus élevée de l'amidon par l' α-amylase de malt d'orge. L'extrait de mélange acétone/eau (70/30; V/V) était également responsable de la réduction du taux d'hydrolyse de l'amidon de maïs. Les paramètres cinétiques de l'inhibition ont été déterminés en étudiant l'effet de l'augmentation de la concentration en amidon de maïs (2, 3 et 4 g/l) utilisé comme substrat sur la vitesse initiale de libération des sucres réducteurs (V_0) en présence des différentes concentrations de mélange acétone/eau (70/30; V/V). Les résultats obtenus montrent que l'inhibition de l' α-amylase par le mélange acétone/eau (70/30; V/V) du haricot igname sur la digestibilité de l'amidon de maïs (66 % amylose) est de type compétitif.

Mots clés : Haricot igname, modélisation, α-amylase, inhibition, digestibilité.

1. INTRODUCTION

Starch plays an important role in both animal and human nutrition. It is known that the functionality of a starch type and its quality can be determined by the physicochemical properties that include water Binding capacity, gelatinization temperature, swelling power and solubility, freeze-thaw stability, paste clarity, paste viscosity, retrogradation and gel strength (Adebowale and Lawal, 2002). Previous

studies of these properties have been conducted for the starch of two local cultivars of African Yam Bean from the Democratic Republic of Congo by Malumba *et al.* (2016).

The digestibility of starch is also an important food property. The lack of starch digestibility that can be measured as the in vivo ingested portion (Clark *et al.*, 1984) or as a rate of in vitro released simple sugars after digestion by amylase extract (Colonna and Champ, 1990) and determines its quality in a food system (the of rate of the splitting off of simple glucose units). For food purposes, rapidly digestible starch (RDS), slowly digestible starches (SDS) and resistant starches (RS) are distinguished, as a function of the rate and of the extent of starch digestion (Englyst *et al.*, 1992) SDS and RS are considered to be slower release of glucose in the intestinal tract resulting in low glycemic. It is known that continuous intake of high glycemic index (GI) foods can lead to obesity, diabetes and cardiovascular diseases. Thus, low GI foods have been suggested to be beneficial in preventing diseases associated with various metabolic disorders. Ideally, the starch of a food should have a better digestive utilization rate, but the release of the simple sugars should be slow so that it is considered a true appetite suppressant and does not induce high insulin secretions.

African Yam Bean starch is often considered to have low digestibility and is known to affect the digestibility of other foods consumed at the same time. This problem of digestibility may depend on its structure (resistant starch) or the presence of inhibitors of hydrolytic enzymes.

Indeed, if this digestibility is low following only the presence of the inhibitors, their extraction will improve and the latter will exert the same action on another type of starch (e.g.corn starch). The aim of this work is to study the effect of inhibitor extracts of African Yam Bean on the in vitro digestibility of African Yam Bean flour and corn starch.

Thus, we determined the influence of extraction by solvents with different polarity (acetone /water mixture (70/30; V/V), acetone / water mixture (50/50; V/V), distilled water, water / acetic acid mixture (90/10; V/V), water / acetic acid mixture (80/20; V/V)) on the digestibility of African Yam Bean flour by a barley malt α-amylase extract. The in vitro digestibility of the flours obtained after extraction by different solvents was compared. The effect of increasing extract concentration on the digestibility of corn starch (66 % amylose) was also evaluated.

2. MATERIALS AND METHODS

A local cultivar of African Yam Bean was obtained in the western of the D.R. of Congo. African Yam Bean flour was obtained by grinding and sieving (mesh size: 0.5 mm). The standard barley malt flour used for the extraction of α-amylase (Megazyme CERALPHA Control Malt Flour 335 U/g) and corn starch (66 % amylose) were from Magazyme International Ireland.

2.1. Extraction of inhibitor compounds

Five solvents were used to extract the inhibitor compounds of the African Yam Bean flour, namely: acetone / water mixture (70/30; V/V), acetone / water mixture (50/50; V/V), distilled water, water / acetic acid mixture (90/10; V/V), distilled water / acetic acid mixture (80/20; V/V). Extraction (three times) was carried out in the following manner: 5 g of African Yam Bean flour was placed in a tube containing 20 ml of solvent. The tube was regularly stirred and the mixture was allowed to extract for about 30 minutes and centrifuged at 1600 rpm for 5 minutes.

2.2. Extraction of α-amylase

The α-amylase was extracted from the standard barley malt (335 U of α-amylase per gram of flour) using a malate buffer (1 M sodium malate, 1 M Sodium chloride, 40 mM calcium chloride and 0.1 % sodium azide diluted 5/100, pH adjusted to 5.4). So, 0.5 g of malt flour was placed in 10 ml of malate buffer for 20 minutes followed by centrifugation at 1600 rpm for 5 minutes.

2.3. Measurement of hydrolysis rate

Two grams of African Yam Bean flour were dissolved in one liter of water and the solution was used as a substrate, the corn starch solution (66 % amylose) 2 % in distilled water was used as a control. Two hundred microliters (200 µl) of substrate solution were mixed with 200 µl of enzyme extract and allowed to react for exactly 5 minutes at 40 °C. The reaction was stopped by adding 3 ml of phosphate buffer (Trisodium phosphate 20 % w/v, pH 7). The released sugars were assayed using the dinitrosalicylic acid (DNS) procedure (Bernfeld, 1955) with minor modifications. Indeed, to 1 ml of each sample was added 1 ml of DNS solution (solution containing 1 g of 3,5-dinitrosalicylic acid, 30 g of sodium and potassium tartrate and 1.6 g of sodium hydroxide dissolved in this order in 100 ml of distilled water and stored in the dark at 4 °C for a maximum of 15 days). The mixture was stirred before being incubated for 5 minutes at 100 °C. The tubes were then cooled in ice-water and 10 ml of distilled water were added before reading the absorbance at 540 nm using a spectrophotometer. The rate of

hydrolysis was expressed in mmol of glucose equivalents released per minute.

The calibration line was plotted as follows: to 1000 µl of solution containing 0, 0.5, 1, 1.5, 2 and 2.5 g glucose per liter were added 1000 µl of DNS solution. The solutions were stirred before being incubated at 100°C for 5 minutes and then cooled in ice-water. Ten milliliters (10 ml) of distilled water were then added to each solution and the absorbance was measured at 540 nm using a spectrophotometer (UV-VIS Heidolph UNIMAX 2010).

2.4. Inhibitory effect of acetone / water mixture (70/30; V/V) extract

The residual acetone of acetone / water mixture (70/30 V/V) extract was evaporated in a Rotavapor centrifuge evaporator to eliminate the acetone fraction. The extract was then re-dissolved in 10 ml of distilled water. The starch solutions (2 grams per liter) containing respectively 0-100-300-300-400-500 and 600 µl of extract were prepared and to 200 µl of each solution were added 200 µl of the enzyme extract. The mixture was allowed to react at 40°C for exactly 5 minutes. The released reducing sugars were assayed by the DNS method as described above and the rate of hydrolysis was determined and expressed in mmol glucose equivalents released per minute.

2.5. Inhibition kinetics

The kinetic parameters of inhibition were determined by studying the effect of increasing corn starch concentration (2, 3 and 4 g/l) used as a substrate on the initial velocity of the reducing sugars release (V_0) in the presence of the various concentrations of acetone / water mixture (70/30; V/V) extract (100, 200, 300 and 400 µl). K_m, $K_{m\ app}$ and V_{max} were extrapolated using the Lineweaver-Burk's Double Reciprocal plot which were calculated from the experimental results according to Michaelis-Menten kinetics.

2.6. Data analysis

Analysis of the data (ANOVA, Tukey's Honestly Significant Difference test, linear modeling) was carried out using the Minitab 16 software.

3. RESULTS AND DISCUSSION

Extracts were obtained from the African Yam Bean flour usng five solvents as described in the section Materials and methods (section 1). The digestibility of extracted African Yam Bean flours by barley malt α-amylase extract (Megazyme CERALPHA Control Malt Flour 335 U/g) expressed as Released Glucose Equivalent (µmol/min) is presented in Table 1.

Table 1. Digestibility of extracted African Yam Bean flours by barley malt α-amylase extract (Megazyme CERALPHA Control Malt Flour 335 U/g)

Extraction solution	Released Glucose Equivalent (µmol/min)
Acetone/water 70/30	320.0±9,0[a]
Acetone/water 50/50	217.3±7,0[b]
Distilled water	200.7±9,0[c]
Distilled water/acetic acid 80/20	66.7±5,0[d]
Distilled water/acetic acid 90/10	50.0±7,0[e]
S = 5 R-sq = 99.84 % R-sq (adj)= 99.77 %	

These results show that the solvent nature significantly affects the digestibility of the African Yam Bean flours, extraction with the acetone/water mixture (70/30; V/V) giving the highest flour digestibility. These results show that there are possibly inhibitors in African Yam Bean which significantly affect flour digestibility by α-amylase. The effect of the acetone / water mixture (70/30; V/V) extract on the digestibility of a corn starch containing 66 % amylose is presented in Fig. 1.

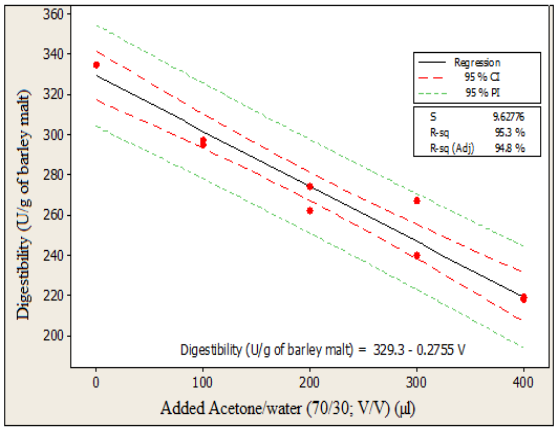

Figure 1. Effect of African Yam Bean acetone / water (70/30) extract on the hydrolysis rate of corn starch. v = ml of added acetone/water (70/30:v/v) extract.

The results in Fig. 1 show that the higher the concentration of acetone/water mixture (70/30; V/V) extract, the lower the digestibility of the corn starch. The loss of digestibility of the corn starch reaches up to 41.3 % after addition of 600 µl of acetone/water mixture (70/30; V/V) extract per milliliter of corn starch solution. The loss of digestibility obtained in this study was lower than those obtained by Chethan *et al*. (2008) when using purified phenolic compounds (gallic acid, protocatechuic acid, p-Hydroxybenzoic acid, p-Coumaric acid, vanillic acid, syringic acid, Ferullic acid, trans-cinnamic acid and quercetin).

It has been shown by Lemlioglu-Austin *et al.* (2012) that the extracts obtained with acetone/water mixture (70/30; V/V) (assumed to be the total phenolic content) from sorghum sounds decreased the hydrolysis of sorghum starch.

The regression analysis of $1/V_0$ as a function of 1/ [S] is presented in Tables 2A, 2B, 2C, 2D and 2E for the different acetone / water mixture (70/30; V/V) extract concentrations and the regression lines are presented in Fig. 2.

Table 2A. Analysis of the regression of 1 / V_0 as a function of 1 / [S] without addition of acetone / water (70/30) extract

Predictor		Coeff	CoefErT	T	P
Constant		0.00204892	0.00008684	23.60	0.000
1/[S]		0.00191080	0.0002311	8.27	0.001
S = 0.0000588316	R-sq = 94.5 %	R-sq (adj) = 93.1 %			
ANOVA					
Source	**DF**	**SC**	**MS**	**F**	**P**
Regression	1	2.36655E-07	2.36655E-07	68.37	0.001
Residual Error	4	1.38446E-08	3.46116E-09		
Total	5	2.50499E-07			

The regression equation is:
$1/V_0 = 0.00205 + 0.00191\ 1/[S]$ (Equation 1)

Table 2B. Analysis of the regression of 1 / V_0 as a function of 1 / [S] after addition of 100 ml of acetone / water (70/30) extract

Predictor		Coeff	CoefErT	T	P
Constant		0.00200629	0.00004674	42.93	0.000
1/[S]		0.0021526	0.0001244	17.31	0.000
S = 0.0000316653	R-sq = 98.7 %	R-sq (adj) = 98.4 %			
ANOVA					
Source	**DL**	**SC**	**CM**	**F**	**P**
Regression	1	3.00323E-07	3.00323E-07	299.52	0.000
Residual Error	4	4.01077E-09	1.00269E-09		
Total	5	3.04334E-07			

The regression equation is:
$1/V_0 = 0.00201 + 0.00215\ 1/[S]$ (Equation 2)

Table 2C. Analysis of the regression of 1 / V_0 as a function of 1 / [S] after addition of 200 ml of acetone / water (70/30) extract

Predictor		Coeff	CoefErT	T	P
Constant		0.00202117	0.00003722	54.30	0.000
1/[S]		0.00240404	0.00009906	24.27	0.000
S = 0.0000252188	R-sq = 99.3 %	R-sq (adj) = 99.2 %			
ANOVA					
Source	**DF**	**SC**	**MS**	**F**	**P**
Regression	1	3.74593E-07	3.74593E-07	588.99	0.000
Residual error	4	2.54395E-09	6.35987E-10		
Total	5	3.77137E-07			

The regression equation is:
$1/V_0 = 0.00202 + 0.00240\ 1/[S]$ (Equation 3)

Table 2D. Analysis of the regression of 1 / V_0 as a function of 1 / [S] after addition of 300 ml of acetone / water (70/30) extract

Predictor		Coeff	CoefErT	T	P
Constant		0.00202075	0.00004860	41.58	0.000
1/[S]		0.0026568	0.0001293	20.54	0.000
S = 0.0000329250	R-sq = 99.1 %	R-sq (adj)= 98.8 %			
ANOVA					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	4.57506E-07	4.57506E-07	422.03	0.000
Residual error	4	4.33622E-09	1.08405E-09		
Total	5	4.61842E-07			

The regression equation is:
$1/V_0 = 0.00202 + 0.00266\ 1/[S]$ (Equation 4)

Table 2E. Analysis of the regression of 1 / V_0 as a function of 1 / [S] after addition of 400 ml of acetone / water (70/30) extract

Predictor		Coeff	CoefErT	T	P
Constant		0.00200063	0.00006411	31.21	0.000
1/[S]		0.0029921	0.0001706	17.54	0.000
S = 0.0000434354	R-sq = 98.7 %	R-sq (adj) = 98.4 %			
ANOVA					
Source	**DF**	**SC**	**MS**	**F**	**P**
Regression	1	5.80266E-07	5.80266E-07	307.57	0.000
Residual error	4	7.54654E-09	1.88663E-09		
Total	5	5.87812E-07			

The regression equation is:
$1/V_0 = 0.00200 + 0.00299\ 1/[S]$ (Equation 5)

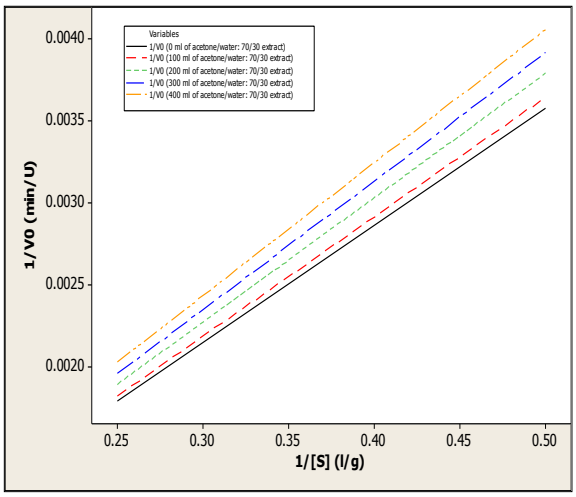

Figure 2. Regression lines of $1/V_0$ as a function of 1/[S] (Lineweaver-Burk Double Reciprocal plot).

An increase in concentration of acetone/water mixture (70/30; V/V) extract changes both the x-intercept (K_m) and the slope, but doesn't change the y-intercept ($1/V_{max}$)). V_0 and [S] represent respectively the initial velocity and the starch concentration.

The results in Fig. 2 and Tables 2 and 3 suggest the competitive nature of inhibitors whose effect can be overcome by increasing the concentration of the substrate suggesting that inhibitors in acetone/water mixture (70/30; V/V) are possibly substrate analogs. Indeed, it is known that a number of compounds can bind to proteins non-covalently and these interactions are responsible for competitive inhibition of α-amylase (Rawel *et al.*, 2005; Li *et al.*, 2009). The increase in substrate concentration has not greatly altered the maximum velocity (y-intercept of the Lineweaver-Burk Double Reciprocal plot) whose values have an average of 495.1 mmol of Glucose Equivalent/min and a standard deviation of 4.6. When 1/[S] approaches 0, V_0 is independent of the presence of inhibitors whereas there is an apparent increase of the Michaelis-Menten constant K_m.

It is known that the extent to which [S] must be increased to completely overcome the inhibition depends upon the concentration of inhibitor present [I], its affinity for the enzyme K_i, and the K_m of the enzyme for its substrate. For this type of inhibition, the expression of the initial velocity is given by the Eq. 6:

$$V_0 = V_{max}\frac{[S]}{K_m(1+\frac{[I]}{K_i})+[S]} \quad \text{(Equation 6)}$$

The affinity of the inhibitors for the enzyme (K_i) can then be calculated according to Eq. 7.

$$K_i = \frac{K_m[I]}{K_{m\ app}-K_m} \quad \text{(Equation 7)}$$

Indeed, during a competitive inhibition, the total enzyme concentration is the sum of those in the free enzyme and those in the enzyme involved in the enzyme-substrate and enzyme-inhibitor complexes.

It has been shown that the presence of huge amount of phenolic compounds extracted by an acetone/water (70/30, V/V) mixture affects sorghum starch digestibility (Chethan *et al.*, 2008). It also has been shown that two abortive complexes could be produced: the amylase–phenolic complex (EI) resulting from binding of the phenolic compound to the active site and the amylase–starch–phenolic complex (ESI) in which phenolic compound is bound at a secondary binding site other than the active centre, which is accessible only after starch binding as occurred at the active centre suggesting an uncompetitive type of inhibition for individual phenolic compounds (Chethan *et al.*, 2008).

The mixed competitive inhibition obtained in this study is a global response of the huge amount of the extracted components that can be strongly influenced by a small category of inhibitors. The compounds responsible for this inhibition must be individually tested after HPLC purification as in Chethan *et al*. (2008). Also, since the African Yam Bean starch is often used for human consumption in DR Congo, the heat treatments can be planned to improve the digestibility and other functional properties of the starch considerably.

4. CONCLUSION

This study revealed that the digestibility of African Yam Bean flour is lower than the one obtained after inhibitors extraction. The acetone / water mixture (70/30; V/V) extraction leads to flour with the highest digestibility. They also show that the digestibility of corn starch (66 % amylose) decreases when the concentration of added African Yam Bean acetone / water mixture (70/30; V/V) extract increases. The Lineweaver-Burk's Double Reciprocal plot allowed the determination of the kinetic parameters V_{max}, K_m and $K_{m\ app}$ while revealing that the inhibition of α-amylase by the African Yam Bean acetone / water mixture (70/30; V/V) extract on the digestibility of Corn starch (66 % amylose) is of competitive type. This study can be completed by the chemical structures study of amylase inhibitors and the pH and thermal stability of Enzyme-Inhibitor complexes.

Acknowledgments

Authors thank the "NGO Feed My People" for financial support.

References

Adebowale K. O. & Lawal O. S., 2002. Effect of annealing and heat-maoisture conditioning on the physicochemical characteristics of bambara groundnut (*Voandzeia subterranean*) starch. *Nahrung/Food*, 46, 311-316.

Bernfeld P., 1955. Amylases α and β. *Method Enzymology*, 1, 149-158.

Chethan S., Sreerama Y.N. & Malleshi N.G., 2008. Mode of inhibition of finger millet malt amylases by the millet phenolics. *Food Chemistry*, 111, 187-191.

Clark J., McNaughton J.E. & Stark J.R., 1984. Metabolism in marine flatfish. I. Carbohydrate digestion in Dover sole (*Solea solea L.*). *Comp. Biochem. Physiol*., 77B, 821-827.

Colonna P. & Champ M., 1990. Significance of starch damage in feeds. *Sci. Aliments*, 10, 877-897.

Englyst H.N., Kingman S.M. & Cummings J.H.,1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46(2), 33-50.

Lemlioglu-Austin D., Turner N. D., McDonough C. M. & Rooney L. W., 2012. Effects of Sorghum [*Sorghum bicolor* (L.) Moench] Crude Extracts on Starch Digestibility, Estimated Glycemic Index (EGI), and Resistant Starch (RS) Contents of Porridges. *Molecules*, 17, 11124-11138.

Li Y., Gao F., Shan F., Bian J. & Zhao C., 2009. Study on the interaction between flavonoid compounds and α-amylase by fluorescence spectroscopy and enzymatic kinetics. *Journal of Food Chemistry*, 10, 1750-3841.

Malumba P., Bungu M.D., Katanga K.J., Doran L., Danthine S. & Béra F., 2016. Structural and physicochemical characterization of *Sphenostylis stenocarpa* (Hochst. ex A. Rich) Harmes tuber starch. *Food Chemistry*, 212, 305-312.

Rawel H.M., Meidtner K. & Kroll J., 2005. Binding of selected phenolic compound to proteins. *Journal of Agriculture and Food Chemistry*, 53, 4228-4235.