International Journal of Biotechnology and Food Science
Vol. 3(4), pp. 49-56, July 2015
ISSN: 2384-7344
Research Paper

# HPAEC-PAD profiles of maltooligosaccharide produced by hydrolysis of sorghum starches using amylases from various sources

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Accepted 10th May 2015

***Abstract.*** Starches isolated from white and pigmented sorghum (Sorghum *bicolor* (L.) Moench) were used to determine enzymatic activity of starch hydrolysis by fungal (*Aspergillus Oryzae*) and bacterial (*Bacillus Subtilis*) α-amylase and oligosaccharides profiles of hydrolysate was determinate in same conditions by High Performance Exchange Anion Chromatography with Pulsed Amperometric Detection (HPEAC-PAD). Pure starches from potato, amylose and amylopectin were used for comparison. Oligosaccharide compositions ranging from glucose (DP1) to maloheptaose (DP7) as well as the significantly effect of α-amylase source and starch structure were determined.

**Keywords:** Sorghum starches, α-amylases, maltooligosaccharides, HPAEC-PAD.

**ABBREVIATIONS: FAO,** Food and Agriculture Organization of the United Nations; **E1,** fungal α-amylase from *Aspergillus oryzae*; **E2,** bacterial α-amylase from *Bacillus subtilis*; **MOS,** maltooligosaccharide; **ST2,** potato starch; **ST4,** amylase; **ST5,** amylopectin; **WSI,** starch of white sorghum from In saleh; **PSI,** starch of pigmented sorghum from In saleh; **PSA,** starch of pigmented sorghum from America.

## INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), as other cereals, should be an optimal crop for food and beverages also for industrial application. Several previous reviews done by McDonough et al. (2000), Rooney and Waniska (2000) and Taylor et al. (2006) reported its utilization for bioindustrial products such as ethanol, starch, and plastics.

Grains of sorghum are rich in starch. According to Food and Agriculture Organization of the United Nations FAO (1995), the content is ranging between 55.6 and 75.2%. Many sorghum landraces were cultivated in hyper arid regions of Algeria (Gast and Adrian, 1965) and still under cultivation. Starch contents in kernels were about 65 to 67%. In white sorghum kernel, it was slightly higher than pigmented one and exhibited interesting rheological and thermal properties because of their genotypic and environmental effect (Boudries et al., 2009; Boudries et al., 2014). Starch digestion is also affected by these factors. Many authors as (Adejumo et al., 2013) reported that susceptibility and mode of amylase action depend on the starch and enzyme system. The production technology of starch hydrolysates is influenced by the shape and size of starch grain as well as the quantity of amylose to amylopectin, the content of fat, proteins and nonstarch polysaccharides.

Amylases are the most important industrial enzyme

used in bread making, brewing (malting) and maltose syrups. The α-Amylase (1,4-D-glucan-glucanohydrolase; EC 3.2.1.1) randomly hydrolyzes the α-1,4 glucosidic linkages in polysaccharides, resulting in short chain dextrins, maltose and eventually glucose.

As endo-enzymes, α-amylases contain fast liquefaction and weak saccharification abilities. Due to the random cutting of starch glucosidic α-1,4-linkages of the amylose molecule, unbranched dextrins of medium chain length (oligosaccharides) are formed and the thin fluid solution obtained upon α-amylase-catalyzed starch hydrolysis is iodine negative (Bruchmann and Fauveau, 2010).

It is expected that linear amylose will be degraded to maltose and maltotriose, if amylose contains odd number of glucosyl units. The outer linear branches of amylopectin are degraded by β-amylase to maltose. The enzyme action is arrested as it reaches the branching point yielding limit dextrins (Tharanathan, 2002).

Hydrolysis of Starch by α-amylases produces a mixture of branched α-dextrins, short linear oligosaccharides and glucose (Guzmán-Maldonado and Paredes-López, 1995; Krzyżaniak et al., 2003). The products of hydrolysis with the same dextrose equivalent can significantly differ in their carbohydrate composition (Krzyżaniak et al., 2003; Griffin and Brooks, 1989) and the chemical and physical properties of maltodextrins aqueous solutions depend on their oligosaccharide profile. Viscosity, crystallinity and sweetness as relation between polymerization degree and functional properties could be cited.

Amylases with ability to synthesize maltooligosaccharides, MOS, from starch have been reported from *Bacillus stearothermophilus* US100, *Bacillus subtilis*, *Brachybacterium* sp. strain LB25 and *Bacillus acidicola*. Only few strains viz. *B. subtilis* and *Bacillus* sp. GM8901 produce maltotriose and maltotetraose (Kumar and Khare, 2012).

MOS has been used in number of food industries as low sweetener, anti-hygroscopic agent, tunicating agent and humectants. Particularly, maltoheptaose is highly demanded as high value-added material in the medical field (Il-Shik, 1997). Maltotriose and maltotetraose producing amylases are highly desirable for application in bread making and other food industries (Kumar and Khare, 2012). Some syrup of starch hydrolysed MOS has been commercialized as well as pure separated MOS.

The influence of enzyme on starch interested many authors as Takasaki (1985), Sarikaya et al., (2000) and Słomińska et al. (2003) had cited some of their contributions. Many studies were interested in enzymatic hydrolysis of potato, cassava, corn and rice but no significant studies treated sorghum and pearl millet starches digestion, so this article focused on hydrolysis of starch isolated from sorghum cultivars by fungal and bacterial α-amylases and determined the maltooligosaccharide profile in initial conditions.

The analyzing of products of enzymatic hydrolysis allows obtaining information about the structure and composition of starch molecules as well as about the mechanism of alpha-amylase action.

## MATERIALS AND METHODS

### Starch isolation and purification

The kernels of three landraces of sorghum with varying degrees of pigmentation were used in this study. White WSI and pigmented (red) sorghum PSI cultivated in a hyper arid region of In Saleh (Algeria) and the third landrace pigmented (brown) sorghum PSA imported from USA and marketed in Ghardaïa (Algeria).

Starch was isolated from sorghum cultivars by alkali extraction of protein as proposed by Beta et al. (2000), Beta and Corke (2001) and Pérez Sira and Amaiz (2004), and described in detail by Boudries et al. (2009).

Three pure starches from potato (Merck A1252), ST2, amylose from maize (sigma S-4180), ST4 and amylopectin from maize (sigma S-6976), ST5 were used for comparison.

### Starch gelatinization

$1 \pm 10^{-4}$ g of starch were dispersed in 100ml (1% w/v) of Milli-Q water and gelatinized by boiling for 20 min at slow stirring.

### Enzyme preparations

Starch hydrolysis was carried out with fungal α-amylase from *Aspergillus Oryzae* (Fluka, 10065, 26U/mg), E1, and bacterial α-mylase from *Bacillus subtilis* (Fluka, 10070, 55 U/mg), E2, preparations. The concentrations of α-amylase used were: 10 and 14 U respectively for E1 and E2. The concentrations were obtained to give kinetic model of Mickaelis-Menten. 1 U corresponds to the amount of enzyme which liberates 1 µmol maltose per minute at pH 6.0 and 25°C (starch acc. to Zulkowsky, Fluka No. 85642, as substrate).

### Enzymatic hydrolysis

Enzymatic hydrolysis reactions were conducted according Sigma method (1997), using 1 ml of gelatinized starch (1% db) and 1 ml of enzyme solution in 25 ml closed Pyrex bioreactors at 20°C, pH = 6.9 (20 mM sodium phosphate Buffer with 6.7 mM sodium chloride). The hydrolysis was stopped by boiling for 5 min.

### HPAEC-PAD analysis of maltooligosaccharides

MOS composition was determined by HPAE-PAD model ICS-300 Dionex chromatograph equipped with an CarboPac® PA1100 column (4 × 250 mm), a pre-column CarboPac® PA1100 (5 × 50 mm) and pulsed amperometric detection (Dionex Corp., USA).

The samples were respectively eluted with Milli-Q water

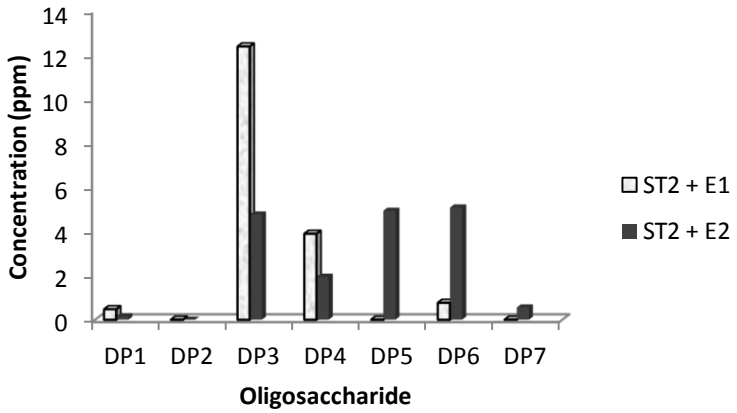


**Figure 1.** MOS profile obtained from potato starch, ST2, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase, E1, and bacterial (*Bacillus subtilis*) α-amylase, E2.

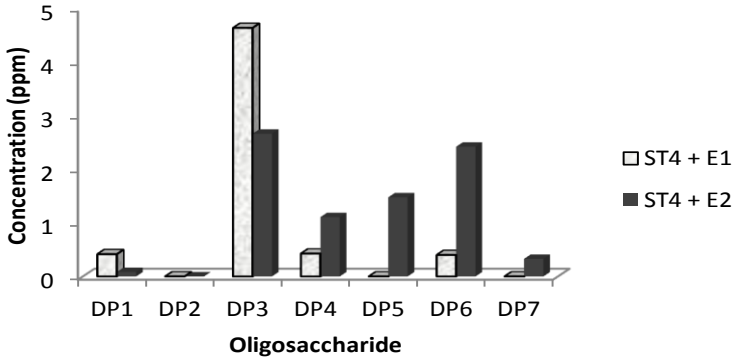


**Figure 2.** MOS profile obtained from amylose, ST4, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase, E1, and bacterial (*Bacillus Subtilis*) α-amylase, E2.

(dilute factor 1/100), filtered through a 0.45 µm nylon filter, transferred into glass vials and loaded onto a column at a volume of 25 µl.

For the MOS analysis, the samples were eluted with a mixture of eluent A (NaOH 100 mM) and eluent B (NaOH 100 mM/NaAc 600 mM) at a flow rate of 1 ml/min at 35°C. Their identification was done on the basis of retention time ($t_r$) and the concentrations were determined by using the measurement of area of peaks and computer integration HPCnem.

D-(+) glucose, DP1, maltotriose, DP3, Maltotetraose, DP4, maltopentaose, DP5, Maltohexaose, DP6, and Maltoheptaose, DP7, from SUPELCO, Bellefonte PA, USA and maltose, DP2, from sigma (M8378) were used as standards with concentrations of 2, 5, 10, 20 and 50 ppm.

## RESULTS AND DISCUSSION

The results of enzymatic reactions and HPAE-PAD of products were exploited to show the effect of source of α-amylase and that of botanical origin of starch. Overall, the obtained profiles of MOS presented some disparity as observed by other authors (Sarikaya et al., 2000).

### Effect of source of α-amylase

The histograms giving the composition of individual oligosaccharide (DP1-DP7) obtained after enzymatic hydrolysis of starch from potato ST2, amylopectin ST4, amylose ST5, local white sorghum, WSI, local pigmented sorghum, PSI, and imported pigmented sorghum, PSA, by fungal E1 and bacterial E2 α-amylase were illustrated respectively at Figures 1 to 6.

The composition of the different hydrolysates showed that of the two amylases were susceptible to degrade starches onto OMS however their profiles in quantity and quality were significantly different. The composition of individual MOS was similar for almost amylolytic systems starch-amylase. Oligosaccharides as DP3, DP4, DP5,

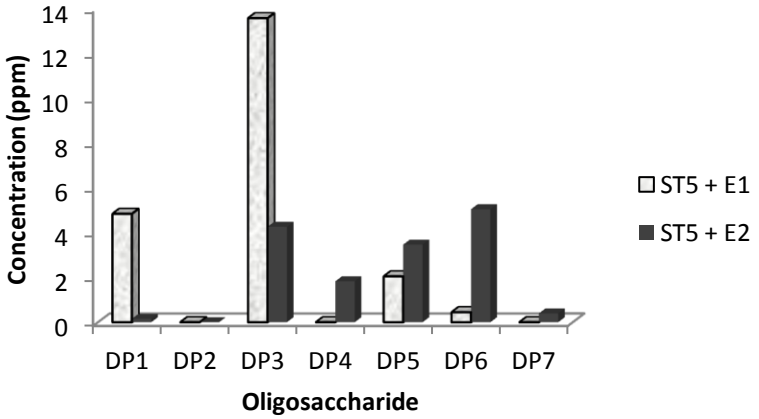


**Figure 3.** MOS profile obtained from amylopectin, ST5, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase, E1, and bacterial (*Bacillus subtilis*) α-amylase, E2.

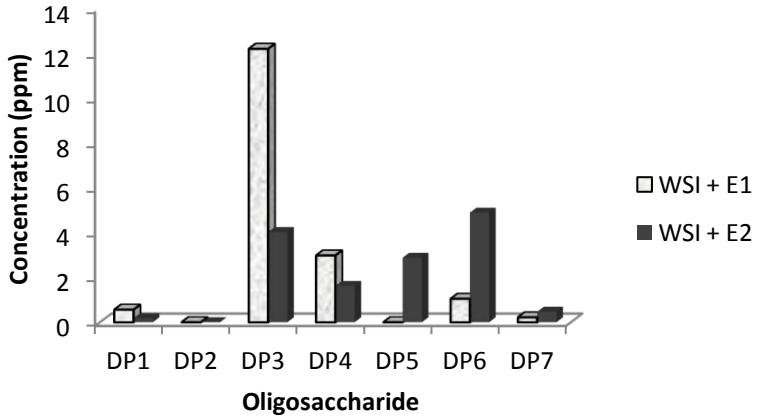


**Figure 4.** MOS profile obtained from local white sorghum starch, WSI, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase, E1, and bacterial (*Bacillus subtilis*) α-amylase, E2.

DP6, were produced with significant amount unlike DP1, DP2 and DP7. However the sugar profiles showed significant differences in concentration according to type of amylase. Overall, maltotriose was present in the mixtures of fungal amylase hydrolysate with the highest concentration representing about 70% of the mixture. Using bacterial amylase, it represented about 30%.

Amylose hydrolysis using individually fungal and bacterial α-amylase gave the lowest concentration because of its linear structure of α1,4-linked glucose which has only one non-reducing end unlike amylopectin which has one reducing end and many non-reducing ends knowing that alpha amylase catalyses the hydrolysis of starch into non-reducing ends. Amylose is more resistant to digestion than other starch molecules and is therefore an important form of resistant starch because of its tightly packed structure.

The hydrolysis of starch isolated from white and pigmented sorghum gave similar MOS profiles in composition but significant differences in concentrations as noticed for potato starch, amylose and amylopectine. α-amylase from *Bacillus subtilis* was less efficient than from *Aspergillus oryzae* on sorghum starches in DP1, DP3 and DP4 maltooligosaccharides production. Comparatively to the pure commercial starches, sorghum starches hydrolysis produced maltoheptaose in addition to other sugars.

## Effect of kind of starch

The effect of the structure of starch on MOS profiles of α-amylase hydrolysates can clearly be shown at Figures 7 to 10. It appeared that fungal α-amylases produced hydrolysates with the same composition in individual MOS for all kind of starches tested. It seems that genotype of sorghum (white and pigmented) and grown environment (Algeria and USA) did not affect the

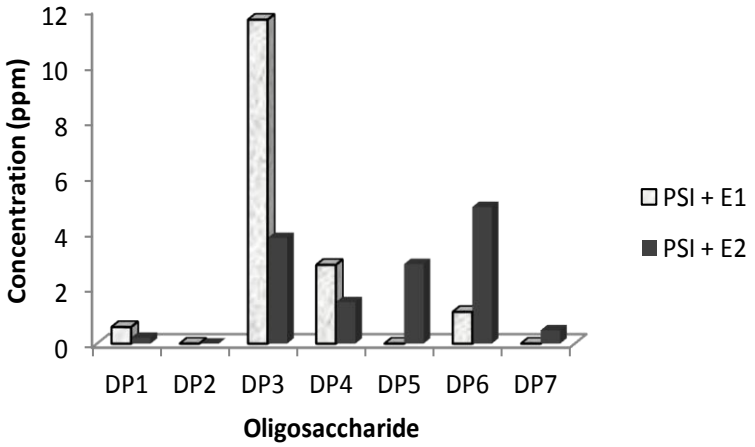


**Figure 5.** MOS profile obtained from local pigmented sorghum starch, PSI, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase, E1, and bacterial (*Bacillus subtilis*) α-amylase, E2.

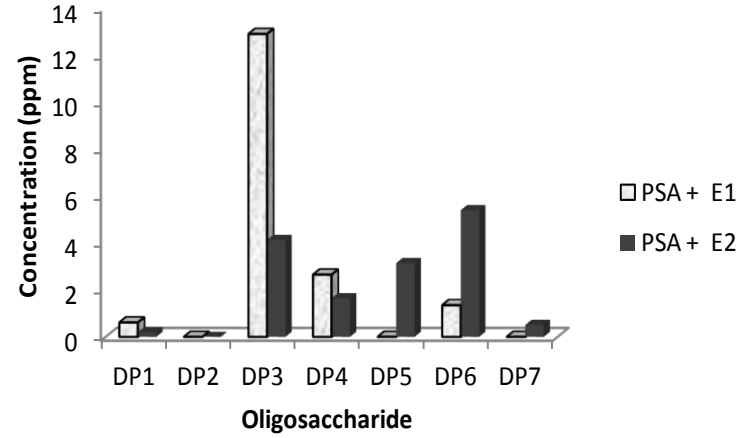


**Figure 6.** MOS profile obtained from American pigmented starch, ST2, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase, E1, and bacterial (*Bacillus subtilis*) α-amylase, E2.

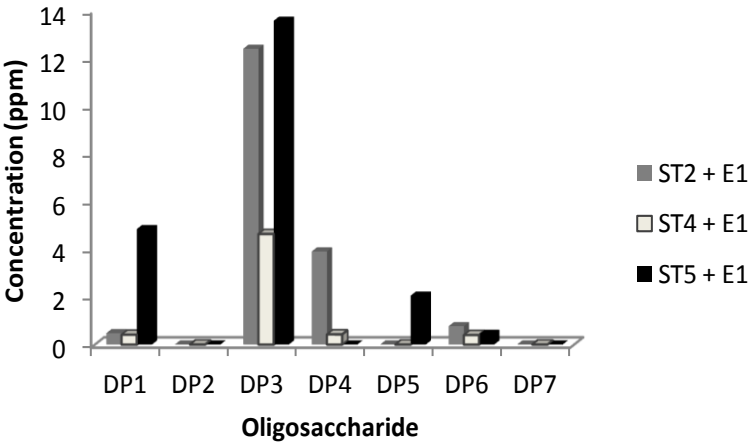


**Figure 7.** MOS profile obtained from potato starch, ST2, amylose, ST4, and amylopectin, ST5, hydrolysis by fungal (*Aspergillus oryzae*) α-amylase.

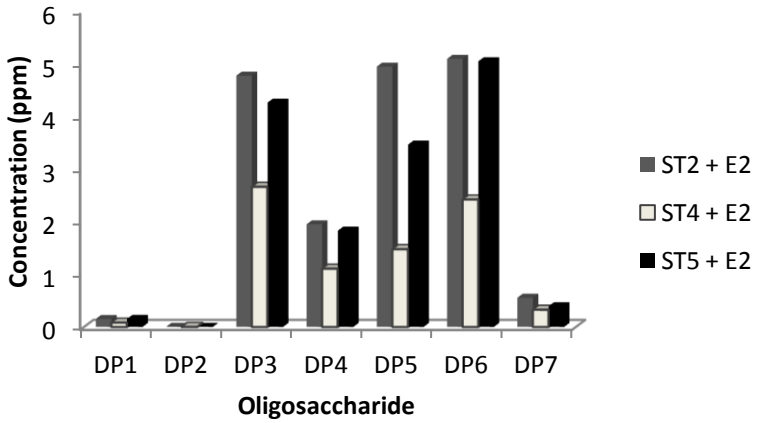


**Figure 8.** MOS profile obtained from potato starch, ST2, amylose, ST4 and amylopectin, ST5, hydrolysis by bacterial (*Bacillus subtilis*) α-amylase.

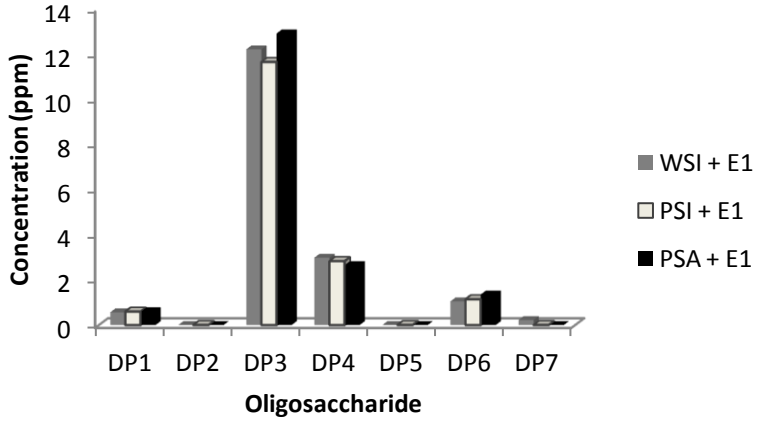


**Figure 9.** MOS profile obtained from local white sorghum, WSI, local pigmented sorghum starch, SPI, and American pigmented sorghum hydrolysis by fungal (*Aspergillus oryzae*) α-amylase.

α-amylase hydrolysis products and so type of α-amylase attack, but their concentrations were significantly different so the efficiency of attack appeared to be affected by the structure of starch. It was the same finding when α-amylase from *B. subtilis* was used.

The amount of the different MOS liberated from amylose was relatively the lowest. Bacterial α-amylase hydrolyzed potato starch and amylopectin in the same way. Many researchers reported differences of susceptibility between cereal and potato starches and confirm that cereal starch granules are susceptible whereas potato starch are resistant to hydrolysis (Słomińska et al., 2003).

There was no significant difference in MOS for white and pigmented sorghum starch in quality and quantity using the same amylase but comparatively, α-amylase of *B. subtilis* was less efficient on sorghum starch than that of *Aspergillus orizae* about 2.3 times. Maltotriose was the main MOS-forming amylase present in each system α-amylase-starch hydrolysate. Takasaki (1985) did the same observation using α-amylase from *B. subtilis.* Duedahl-Olesen et al. (2000) reported two other enzymes from *Bacillus* sp. MG-4 and *Streptomyces griseus.* The conformation of MOS liberated, concluded that the enzyme from *B. subtilis* is a kind of α-amylase exhibiting the endomechanism (Takasaki, 1985; Duedahl-Olesen et al., 2000).

Generally, maltose and maltotriose are the major end products of starch degradation (Sarikaya et al., 2000). Many studies revealed the presence of maltose in the starch hydrolysis medium for many starch from different botanical origin and amylase from different microorganism (Kouame et al., 2004; Cotta, 1992).

Maltotriose and maltotetraose were liberated from all starches at significant amount except amylopectin. It would be deduced that hydrolysates of sorghum and pearl millet starches can provide an application in bread making industry. Maltose was not liberated in almost

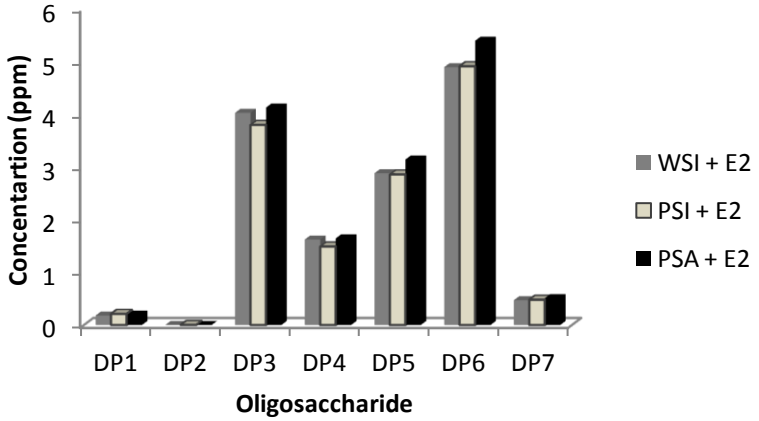


**Figure 10.** MOS profile obtained from local white sorghum, WSI, local pigmented sorghum starch, SPI, and American pigmented sorghum hydrolysis by bacterial (*Bacillus subtilis*) α-amylase

hydrolysates and glucose was in weak amounts. This may suggests that there was no inhibitory effect because of these saccharides. Inhibition of glucose and maltose against the α-1,4 hydrolysis activity of barley α-amylase and *Pyrococcus* sp. ST04 maltose-forming α-amylase was reported respectively by Lim et al. (2003) and Jung et al. (2014). For having specific composition in glucose and maltose, the combination of α-amylase and other amylases as glucoamylase and β-amylase is recommended as tested by Kouame et al. (2004).

## CONCLUSION

The results could bring out the relationship between the type of amylase used for starch hydrolysis, structure of starch molecule and chemical composition of MOS obtained:

i) Individual MOS composition was similar for almost enzymatic systems starch-α-amylase, but their concentrations were significantly different.
ii) Amylose hydrolysis using individually fungal and bacterial amylase gave the lowest concentration because of its linear structure of α1,4-linked glucose.
iii) Maltotriose was the main MOS present in hydrolysate of each system amylase-starch suggesting that amylase has endoaction.
iv) α-amylase from *Bacillus subtilis* was less efficient than that of from *Aspergillus orizae* on starches.
v) No significant differences in MOS profiles for white and pigmented sorghum starches for individual amylase.

The chemical composition of maltodextrins influences the properties as viscosity and sweetness of their aqueous solutions. Indeed, the polymerization degree of oligosaccharide molecules affects sweetness of their solutions. Below DP 7 the sweetness is perceptible. So starches isolated from white and pigmented sorghum had abilities to give sweetener solutions even at initial conditions.

Further studies are necessary to know completely the mechanism of enzyme attack during hydrolysis reactions to control the composition and properties of starch hydrolysates and so improve end-products for food (digestion) as well as non-food industrial applications of these cereal starches.

## ACKNOWLEDGEMENTS

The authors wish to thank Virginie Byttebier and Michel Paquot from Department of Chemistry and Bio-industries, Gembloux Agro.Bio.Tech of Liege University, Belgium for their assistance and will also like to pay tribute to late Lynda Kheloufi (phd student) who contributed to this work.

The Belgium technical cooperation is gratefully acknowledged for its financial support.

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