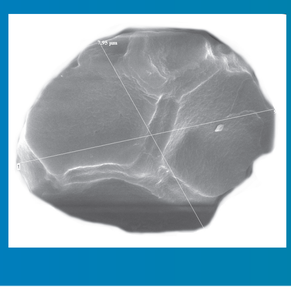
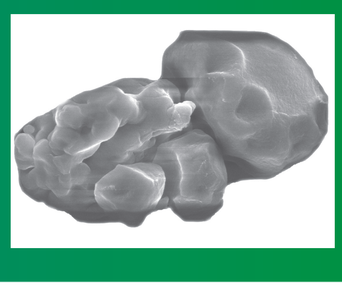


Potential of the small-granule cassava starch mutation for the bioethanol industry



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Introduction

Cassava is a poor-man crop. It is, however a very important source of food energy (starch) in tropical and subtropical regions of the world. It is the most productive starch-producing crop in tropical regions (productivity per area). The deep macrostructure of the small-granule starch mutation identified in 2006[1] was reported in 2012[2]. This mutation resulted in a deeply modified branching pattern of amylopectin as well as other starch characteristics and properties. These modifications include changes in starch granule ultrastructure (e.g. decreased starch crystallinity), a weakly organized structure, and increased susceptibility to mild acid raw starch hydrolysis (fastest and most efficient hydrolysis of all studied native starches).

Cassava is, along with sugarcane, the most promising feedstock for bio-ethanol production in tropical and subtropical regions of the world. The advantage of cassava is that fermentation may be conducted with higher dry matter contents to produce higher concentration of ethanol which, in turn, reduces the costs of distillation and ethanol production. The aim of this study was to compare the rate of hydrolysis of this raw new starch and other starchy sources and to optimize the hydrolysis and fermentation conditions for production of ethanol from very high-gravity (VHG) cassava mash by *Saccharomyces cerevisiae* during simultaneous saccharification and fermentation (SSF) processing.

Materials and Methods

The methodology described by Holm *et al.* (1985)[3], was used to evaluate de hydrolysis pattern of different starches using porcine pancreatic enzyme (SIGMA) and commercial Stargen2 (Genecor). Very high gravity (VHG) and simultaneous saccharification and fermentation (SSF) were implemented in the laboratory as reported by Zhao *et al.* (2009)[4].

Results and Conclussions

The small size of the granule, its rough surface and the easier acidic hydrolysis allowed us to suggest that this mutation could offer interesting advantages for the production of ethanol. Work on the enzymatic hydrolysis clearly show that both with the pancreatic enzyme **(Figure 1A)** or industrial enzyme StarGen II **(Figure 1B)** the new small-granule starch (5G160-13) hydrolyses much faster. The difference in rate of hydrolysis cannot be attributed solely to the particle size because taro starch (average granule size 4 µm) has a slower hydrolysis compared with the small-granule cassava starch (average granule size 6.5 µm).

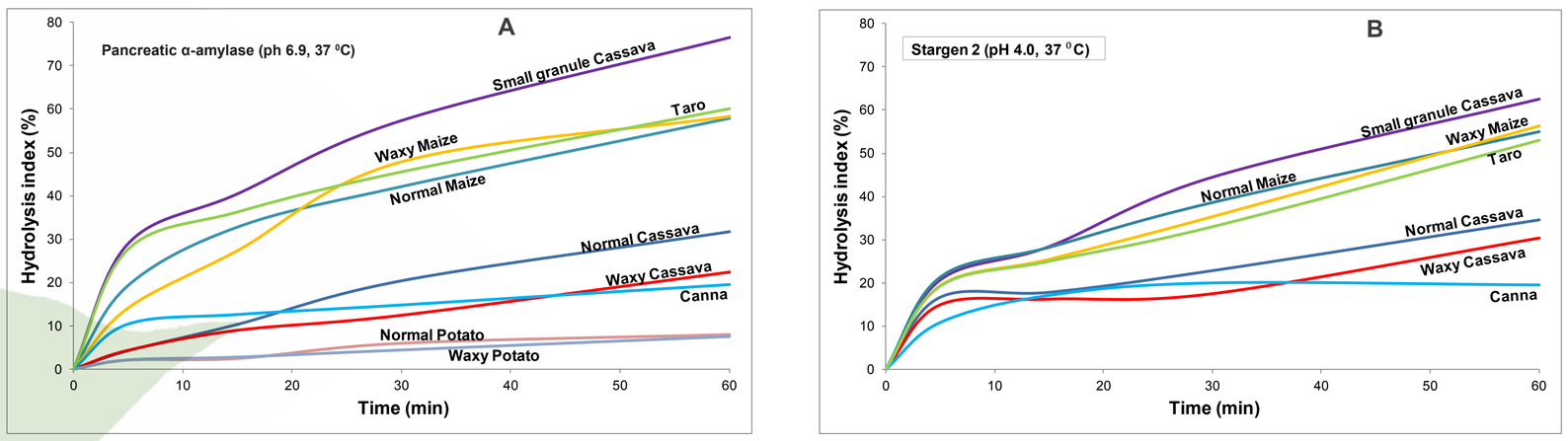

Figure 1 A, B. Hydrolysis based on different enzymatic alternatives of the most relevant starch sources for the production of bio-ethanol. (Average 5 repetitions)

Recent cassava crosses produced segregating progenies whose starch had the small-granule characteristics, but the amylopectin content ranged from 19 to 42%.
Despite the high levels of amylose measured (19 to 42.4%), some clones have even higher rates of hydrolysis (AM 1995-14) compared with the parental clone (5G160-13), **Figure 2.**

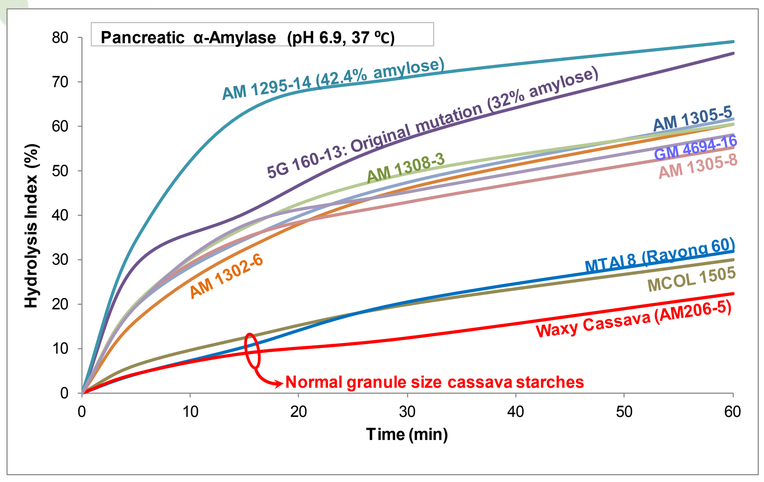

Figure 2. Hydrolysis patterns of different cassava clones

The fermentation of native starch showed that the rate of glucose production by SSF is faster for the original small-granule mutation (5G-160-13) than for normal and waxy starches. Therefore, a reduction of the concentration of enzyme to ferment the starch can be envisioned, thus reducing ethanol production costs **(Figure 3)**.

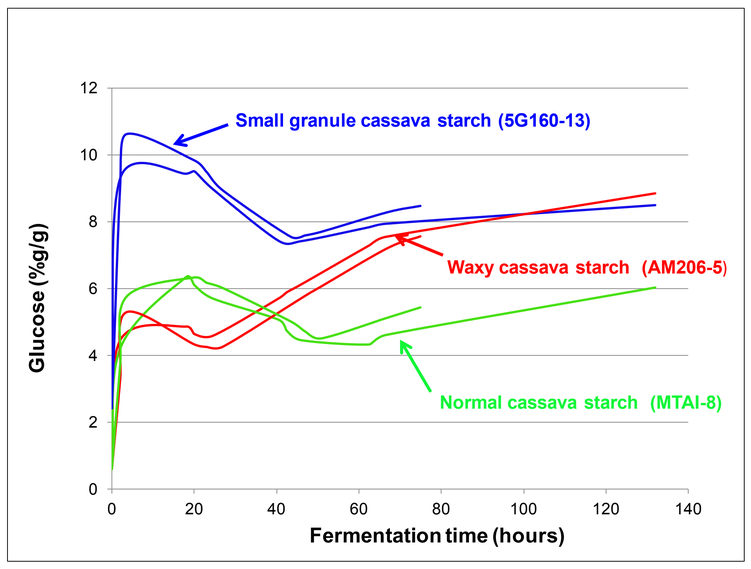

Figure 3: Glucose production of during SSF of VHG cassava starch for ethanol production

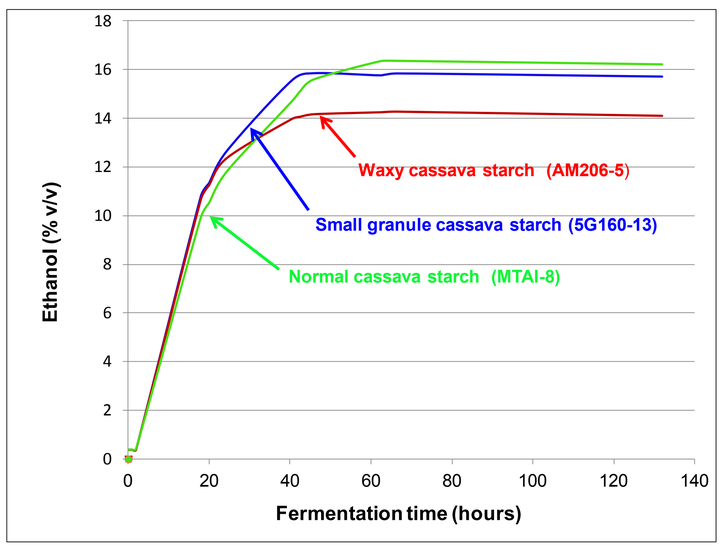

Figure 4: Ethanol production during SSF of VHG cassava starch for ethanol production

Very high gravity fermentation allows reaching 16% of ethanol in the fermentation slurry **(Figure 4)**. This ethanol concentration becomes toxic to yeast and optimization of fermentation should take this factor into account. There is no significant difference in ethanol yield between the various starches tested; the big difference was the ease of hydrolysis of the small-granule starch 5G160-13.

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