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SHORT COMMUNICATION

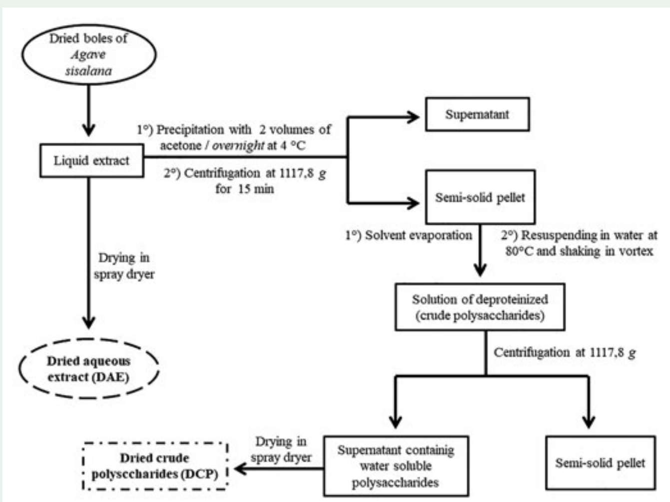
Aqueous extracts of *Agave sisalana* boles have prebiotic potential

Alexsandra Conceição Apolinário[a,b], Antonio Diogo Silva Vieira[a], Susana Marta Isay Saad[a], Attilio Converti[c], Adalberto Pessoa Jr[a] and José Alexsandro da Silva[d]

[a]Department of Biochemical and Pharmaceutical Technology, University of São Paulo, São Paulo, 05508-000, Brazil; [b]Graduate Program of Pharmaceutical Sciences, State University of Paraíba, Campina Grande, Paraíba, Brazil; [c]Department of Civil, Chemical and Environmental Engineering, Chemical Engineering Pole, Genoa University, Genoa, Italy; [d]Department of Agrarian and Exact Sciences, Campus IV – UEPB, Post-Graduate Program in Agroindustry Systems, Federal University of Campina Grande, Pombal, Paraíba, Brazil

ABSTRACT

This work aimed at evaluating the prebiotic potential of the aqueous extract and crude polysaccharides from *Agave sisalana* boles by an *in vitro* screening. Crude polysaccharides were obtained from the aqueous bole extract by precipitation with acetone and resuspension in water. The liquid extract and the polysaccharide solution were then spray dried and submitted to thermal analysis and quantification of metabolites. Prebiotic activity was checked on probiotic strains belonging to the *Lactobacillus* genus using inulin, fructo-oligosaccharides, fructose and glucose as positive controls. The powder of *A. sisalana* bole extract, which has recently been identified as a rich source of inulin, exhibited higher potential of fermentation compared with crude polysaccharides.



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CONTACT A. Converti converti@unige.it Department of Civil, Chemical and Environmental Engineering, University of Genoa, Pole of Chemical Engineering, Via Opera Pia 15, I-16145, Genoa, Italy

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1. Introduction

A prebiotic is defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Roberfroid 2007). Non-digestible carbohydrates are considered prebiotics when they respect the following criteria: a) resistance to gastric acidity and mammalian enzymes; b) vulnerability to fermentation by gut bacteria and c) capacity to enhance viability and/or action of beneficial microorganisms (Al-Sheraji et al. 2013). Many new functional foods have been manufactured with health promotion purposes by combining traditional foods with herbal extracts in the form of dietary supplements or dairy foods.

It has been demonstrated that the aqueous extract of *Agave sisalana* boles has high contents of sugars (Apolinário et al. 2014) and inulin (Apolinário et al. 2017), which suggests a prebiotic application of this species that has features typical of plants adapted to dry environments. This work aimed at evaluating, by an *in vitro* screening, the prebiotic potential of dried aqueous extract and crude polysaccharides obtained by spray drying *A. sisalana* boles.

2. Results and discussion

Previous studies (Apolinário et al. 2014, 2017) demonstrated that the aqueous extract of *A. sisalana* boles is rich in sugars and contains inulin with mean degree of polymerization of 10, which is similar to those extracted from other commercial sources such as *Agave tequilana* or *Agave atrovirens*.

About the important issue of the influence of plant age on fructan composition, it is widely accepted that younger plants have increased free sugars content. Thus, with enough free sugars and inulin available in young plants, they could be used effortlessly for enzymatic breakdown and fermentation. Selective harvesting would allow the plant to continue growing for future use depending on its ripening degree. This is the case, for instance, of *A. tequilana* used to produce beverages, whose plants are harvested when they are 8–12 years old (Arrizon et al. 2010; Close et al., 2017). In this sense, *A. sisalana* is an economically advantageous option, being possible sugar extraction from relatively young (6-years old) plants.

According to the thermograms illustrated in Figure S1 (Supplementary Material), decomposition of the dry powder of aqueous extract (DAE) of *A. sisalana* boles occurred in three steps, at 27.1–117.4 °C, 123.9–243.9 °C and 642.4 °C, while that of dry crude polysaccharides (DCP) isolated from this extract in four steps, at 27.2–143.6 °C, 251.7–421.1 °C, 600.5–693.0 °C and 875.0 °C.

The first event, responsible for a small initial mass loss in both powders, can be ascribed to water loss. The higher loss observed for DCP compared with DAE can be explained by moisture desorption from the saccharide structure (Bothara and Singh 2012). The second mass loss event pointed out relevant differences between the powders, in that DAE exhibited smaller stability than DCP, likely due to decomposition of additional thermosensitive hydrosoluble metabolites (proteins, pigments, phenolics). This decomposition occurred in DCP in two steps: the former, showing the typical behavior of polysaccharides, can be ascribed to thermal decomposition and

Table 1. Contents (mg/g) of different classes of metabolites contained in dry powders of aqueous extract (DAE) and crude polysaccharides (DCP) obtained by spray drying boles of *A. sisalana*.

Sample	Total phenols	Flavonoids	Total sugars	Free sugars
DAE	$5.67^{a} \pm 0.09$	$0.60^{b} \pm 0.02$	$859.3^{b} \pm 129.9$	$48.0^{b} \pm 1.1$
DCP	$16.32^{b} \pm 0.09$	$10.94^{a} \pm 0.20$	$531.1^{a} \pm 35.5$	$99.0^{a} \pm 3.0$

$p < 0.05$ for all values. Means in the same column followed by different letters were statistically different.

depolymerization of fructan branched chains, while the latter to final pyrolytic decomposition of unpurified polysaccharides still containing contaminants (Espinosa-Andrews and Urias-Silvas 2012; Xie et al. 2013). The mineral residue resulting from DAE carbonization (last event) was more abundant than that of DCP likely because crude polysaccharides were obtained by precipitating only water-soluble substances. Temperature ranges of decomposition events suggest good thermostability of both materials, even though polysaccharides were more stable.

Table 1 lists the contents of different metabolites detected either in DAE or DCP. Even though total and free sugars were majority in both powders, it was possible to quantify total phenols and flavonoids.

The lower content of total sugars in DCP compared with DAE was likely the result of significant mass losses due to multistep precipitation with acetone to recover crude polysaccharides, while the higher contents of other metabolites in DCP may be ascribed to the fact that all soluble compounds present in the starting liquid extract were subject to precipitation, hence concentrating in this powder.

Figure S2 (Supplementary Material) illustrates the results of growth of different *Lactobacillus* species and strains on the carbon sources contained either in DAE or DCP, using different carbohydrates as positive controls and a modified MRS medium lacking in carbohydrates as the negative one. Glucose allowed for the highest growth of lactobacilli, being the preferred carbon source for most lactic acid bacteria. These results agree with those of Srinivas et al. (1990) who found maximum viable counts of *Lactobacillus acidophilus* strains in glucose. Tsujikawa et al. (2013) observed that *Lactobacillus paracasei* grew well in glucose, fructose, sucrose and inulin-type fructans, which would explain the comparable growth of our strain in glucose and inulin. Watson et al. (2013) reported better growth of *Lactobacillus rhamnosus* in glucose than in fructo-oligosaccharides or inulin and outstanding growth of two strains of *Lactobacillus casei* in glucose, but, depending on the strain, low or no growth in fructo-oligosaccharides and inulin.

As expected, crude polysaccharides behaved as a worse carbon source than positive controls, whereas DAE exhibited an intermediate performance. Additional metabolites other than polysaccharides and monosaccharides may have concentrated during DCP preparation affecting the fermentation. On the other hand, DAE showed higher total sugar content than DCP, thus improving the fermentation.

Crude polysaccharides could indeed be purified but these additional steps would imply significant yield reduction. The widespread use of extracts, juices or other mixtures with prebiotic properties suggests that expensive and time-consuming steps to isolate and purify carbohydrates are not necessary, being the use of materials rich in fructans or other polysaccharides satisfactory. In this study DAE from *A. sisalana* exhibited potentially prebiotic properties; however, a deep optimization study along with additional tests are required to ascertain safety and efficiency.

Studies on other species resulted in good perspectives, e.g. *Agave tequilana* stems used to recover agave insoluble dietary fiber showed promising effects as functional ingredient (Sáyago-Ayerdi et al. 2014). Pascoal et al. (2013), investigating the prebiotic properties of lyophilized onion-containing diets by *in vitro* and *in vivo* fermentations, observed increases in the production of short-chain fatty-acids and molar proportions of propionate and butyrate as well as simultaneous decreases in the molar proportion of acetate and pH.

3. Conclusion

This study suggests that the extract of boles from *A. sisalana* exerts a prebiotic effect linked to its significant sugar content; therefore, it could be an economically-viable material for both the pharmaceutical and food industries. No isolation and purification of crude polysaccharides were necessary. Anyhow, complete fermentation tests, *in vivo* assays and studies of optimization of the involved technology should be carried out to obtain a final standardized product.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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