Exploitation of Agro-Industrial By-Products as Substrate for Solid-State Fermentation

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Abstract

The agro-food wastes could be appropriate for use as immobilization carrier in the production of different metabolites with added commercial value by solid-state fermentation (SSF).

The objective of this work was the evaluation of the potential of different stone fruit and berry wastes from Romanian food and beverages industry for use as immobilization carrier in SSF. The physical-chemical properties (moisture and water absorption index (WAI)), and the ability of *Rhizopus oligosporus* and *Aspergillus niger* to colonize such solid substrates were used as parameters to select the best by-products that could be used successfully in SSF.

The agro-food wastes used in this study were derived from Transylvanian local regions and from our laboratory, including kernels and pulp/peels of stone-fruits (plum, apricot) and berry press residues (sea buckthorn, *Sambucus nigra* (SN) and *Sambucus ebulus*). The fungal strains were procured from LGC Standards, Germany.

Among the 8 evaluated substrates, only 5 presented higher moisture content than 50%, including apricot peels (AP), residues from plum brandy production (RPBP) (pulp and peel) and berry press residues. The AP and SN berry press residue were the materials with higher WAI values (6.72 and 6.10 (g gel/g dry weight (dw), respectively), whereas the kernels of apricot (2.44 g/g dw) and those from RPBP (2.60 g/g dw) gave the lowest WAI values. The microbiological test showed that the microorganisms (*R. oligosporus* and *A. niger*) had good growth rates when cultivated in berry press residues and stone-fruits pulp/peels.

It could be concluded that the stone-fruit agro-industrial by-products (pulp/peel) and berry press residues have great potential to be successfully used as substrate in SSF.

Keywords: *agro-industrial by-products, Aspergillus niger, solid-state fermentation*

INTRODUCTION

The agricultural and food industries produced annually large amounts of wastes, which can cause serious environmental problems (Rodríguez-Couto, 2008). In recent years have published more and more studies on efficient utilization of agro-industrial by-products for the production of biomolecules (phenolics, lipids etc) and enzymes

using cheap substrates, such as fruit and vegetable processing wastes (apple, grape and tomato pomaces), citrus and pineapple waste and different berries processing wastes (Ajila *et al.*, 2012). Owing to the composition rich in carbohydrates (especially glucose), the agro-industrial residues could be appropriate for use as substrates in solid-state fermentation (SSF) system to produce the industrially relevant compounds.

SSF consists in the growth of microorganisms on solid particles in the absence of free water. The substrate must contain sufficient moisture to allow the microbial growth and metabolism (Thomas *et al.*, 2013). The most important factors that must be taken into account for the development of an efficient SSF process are: the selection of a suitable microorganism and an appropriate solid support, respectively. Microorganisms produce different types of enzymes when grown on different substrates. The most commonly used microorganisms in SSF are the filamentous fungi. The strain of *Aspergillus niger*, is known to produce more than 19 types of enzymes (cellulase, pectinase, protease, etc.) and various added-value bioactive products (citric acid, alcohols, etc.) by SSF of different agro-industrial by-products (Orzúa *et al.*, 2009). *Rhizopus oligosporus* is another important food-grade filamentous fungus, which has been used successfully in SSF. This strain is able to produce high quantities of cellulolytic enzymes when growing on different fruit residues as solid substrates. The cellulolytic enzymes, such as β -glucosidase, are capable of hydrolyzing phenolic glycosides, causing an increase in the phenolic compounds content, which significantly improved the antioxidant potential of the biofermented wastes (Correia *et al.*, 2004; Martins *et al.*, 2011).

Before beginning the SSF process, it is very important to determine some physical-chemical parameters (moisture and water absorption index (WAI)) and microbiological characteristics (microorganism growth rate) of the solid substrates.

This study was carried out to evaluate the potential of different, abundant and cheap, stone fruit and berry wastes from Romanian food and beverages industry for use as immobilization carrier in SSF. The physical-chemical properties (moisture and water absorption index (WAI)), and the ability of *Rhizopus oligosporus* and *Aspergillus niger* to colonize such solid substrates were used as parameters to select the best by-products that could be used successfully in SSF.

MATERIALS AND METHODS

Materials, microorganisms and physico-chemical characterization

The agro-food wastes used in this study were derived from Transylvanian local regions and from our laboratory, including kernels and pulp/peels of stone-fruits (residues from plum (*Prunus*

domestica L.) brandy (RPBP) and juice production (RPJP); apricot (*Prunus armeniaca* L.) peels (AP), and kernels (AK)) and berry press residues (sea buckthorn- *Hippophae rhamnoides* L., ssp. Carpatica (SB); *Sambucus nigra* (SN) and *Sambucus ebulus* (SE)). The fungal strains of *Rhizopus oligosporus* (ATCC-22959) and *Aspergillus niger* (ATCC-6275) were procured from LGC Standards GmbH, Wesel Germany.

Moisture content was determined gravimetrically after drying at 105°C for 12h.

The WAI was determined using the method of Orzua *et al.* (2009). 1.5g of sample was suspended in 15 ml of distilled water in a tared 50 ml centrifuge tube. After stirring for 1 min at room temperature (25°C), has followed the centrifugation at 3000 g for 10 min. The WAI (expressed as g gel/g dry weight) was the weight of gel obtained after removal of the supernatant per unit weight of original dry solids.

Microbiological tests

The assays for determination of the invasion capacity of the microorganisms on the agri-food residues (at three substrate moisture contents: 60%, 70% and 80%) were performed in Petri dishes, based on the protocols described by Robledo *et al.* (2008) and Orzua *et al.* (2009). The spore suspensions of *Rhizopus oligosporus* (ATCC-22959) and *Aspergillus niger* (ATCC-6275) (2×10^7) were inoculated at the center of dishes. Radial growth rate of the fungi was monitored and expressed in mm/h.

Statistical analysis

All treatments were realized by three replications. Dates were expressed as mean $\pm$ S.D and analyzed by ANOVA (Tukey's Multiple Comparison Test; GraphPad Prism Version 4.0, Graph Pad Software Inc., San Diego CA). The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Physico-chemical characterization

According to Martins *et al.* (2011), there are many factors such as particle-size of substrates, supplementation with different nutrients of growth medium, sterilization, moisture content, temperature, pH, agitation and aeration of SSF medium that influence the efficiency of SSF processes. Among these, the moisture content of the solid substrate plays a crucial role in SSF, and has been studied and described by several

authors. Generally, the water content of substrates in SSF may range from 30 to 85%, depending on the agro-food waste (Martins *et al.*, 2011; Thomas *et al.*, 2013).

Figure 1 (A) shows the moisture values obtained in the present work for each solid waste. Among the eight evaluated substrates, only five presented the values of moisture content higher than 50%, including apricot peel (75%), residues from plum brandy production (pulp and peel) (75%) and berry press residues (*Sambucus ebulus*-52%; sea buckthorn- 53% and *Sambucus nigra*- 56%). Lower values of water content may induce the sporulation of the microorganism, while higher levels may reduce the porosity of the system, which can result in oxygen transfer limitation, thereby increasing the risk of bacterial contamination (Pérez-Guerra *et al.*, 2003).

Another parameter that is of interest in SSF is the water absorption index. WAI indicates the solid substrate ability to absorb water, and depends on the availability of hydrophilic functional groups to form bonds with water molecules and on the gel forming ability of macromolecules (Mussatto *et al.*, 2009). As shown in Figure 1 (B), the highest WAI value was found in apricot processing waste (peel) (6.72 g gel/g dm). Statistically higher ($p < 0.05$) values of WAI were also found in SN-BPR (6.10 g gel/g dm), RBPB-pulp and peel (6.08 g gel/g dm), SE-BPR (5.88 g gel/g dm) and SB- BPR (5.73 g gel/g dm) than in kernels of RBPB (2.60 g gel/g dm), RPJP (2.75 g gel/g dm) and AK (2.44 g gel/g dm), respectively (Fig. 1 (B)). The solid substrates with high WAI are preferred for SSF since facilitate the microorganism growth and development (Buenrostro-Figueroa *et al.*, 2013; Orzúa *et al.*, 2009).

Microbiological test

There are several microorganisms, such as fungi, bacteria and yeast which can grow on solid wastes in SSF systems. Generally, fungal and yeast cultures are considered as the most suitable microorganisms for SSF processes, due to of their ability to grow on various solid matrix even in the absence of free water (Orzúa *et al.*, 2009). The choice of the fungus should be linked with the selection of the solid substrate and product (Thomas *et al.*, 2013).

In the present work, eight agro-industrial wastes were submitted to microbiological testes, which consisted in the cultivation of *Rhizopus*

Fig. 1 (A) Moisture content and (B) Water absorption index (WAI) of the agro-industrial wastes: SB-, SN-, and SE-BPR, sea buckthorn-, *Sambucus nigra*- and *Sambucus ebulus*- berry press residues; RBPB, residues from plum brandy production; RPJP, residues from plum juice production; AP, apricot peels; AK, apricot kernel; Results are given as mean $\pm$ SD ($n = 3$); Values with different letters (a-c) are significantly different ($p < 0.05$), using ANOVA "Tukey's Multiple Comparison Test".

oligosporus (ATCC-22959), and *Aspergillus niger* (ATCC-6275) strains, and the evaluation of the fungal growth on the substrates. Table 1 presents the fungal radial growth rate in these eight agro-

Tab. 1. Effect of the moisture content on the microorganism (*Aspergillus niger*-ATCC-6275 and *Rhizopus oligosporus*-ATCC-22959) growth rate by solid-state fermentation in different agro-industrial wastes

Fungal radial growth rate (mm/h)- <i>Aspergillus niger</i> -ATCC-6275								
Moisture content	SB- BPR	RPBP- pulp/ peel	RPBP- kernel	RPJP- kernel	AP	AK	SN-BPR	SE-BPR
60%	0.47±0.02a	0.33±0.02a	is	is	0.26±0.02ab	is	0.44±0.02a	0.33±0.03a
70%	0.46±0.02a	0.35±0.03a	is	is	0.28±0.01a	is	0.42±0.03a	0.32±0.02a
80%	0.38±0.03b	0.36±0.03a	is	is	0.22±0.02b	is	0.38±0.03a	0.31±0.01a
Fungal radial growth rate (mm/h)- <i>Rhizopus oligosporus</i> -ATCC-22959								
60%	0.44±0.01a	0.42±0.03a	is	is	0.38±0.02b	is	0.46±0.03a	0.42±0.02a
70%	0.46±0.02a	0.44±0.02a	is	is	0.46±0.01a	is	0.42±0.02ab	0.40±0.01ab
80%	0.42±0.02a	0.38±0.03a	is	is	0.41±0.02b	is	0.40±0.02b	0.36±0.02b

Note: SB-, SN-, and SE-BPR, sea buckthorn-, *Sambucus nigra*- and *Sambucus ebulus*- berry press residues; RPBP, residues from plum brandy production; RPJP, residues from plum juice production; AP, apricot peels; AK, apricot kernel; Results are given as mean±SD (n = 3); Values with different letters (a-c), in the same column are significantly different (p < 0.05), using ANOVA "Tukey's Multiple Comparison Test". is-insignificant.

industrial by-products, under different initial moisture contents. It can be observed that the initial substrate concentrations significantly (p<0.05) influenced the growth rates of fungi, except in RPBP-pulp/ peel, SN-BPR and SE-BPR for *A. niger*, and SB- BPR and RPBP-pulp/ peel for *R. oligosporus*, respectively (Tab.1), where did not occur significant differences with the variation in the moisture content. Both strains had good growth when cultivated in berry press residues, by-products from plum brandy production (pulp/peel) and in apricot peels, respectively, but their development was very slow, practically insignificant when kernel residues were used as carriers (Tab.1).

Finally, from the eight agro-food wastes evaluated in this work, five of them, namely sea buckthorn-, *Sambucus nigra*- and *Sambucus ebulus*- berry press residues, by-products from plum brandy production (pulp/peel) and apricot peels presented great potential to be used as substrates in SSF. The microbiological test also showed the unsuitability of kernel wastes for use as carrier during SSF process, which could be caused by their low glucose content as carbon source.

CONCLUSION

According to the results obtained from the physico-chemical and microbiological tests it can be concluded that among the 8 agro-food wastes

analyzed, five of them (sea buckthorn-, *Sambucus nigra*- and *Sambucus ebulus*- berry press residues, by-products from plum brandy production (pulp/ peel) and apricot peels) showed great potential to be used as support-substrates in SSF processes. The reuse of such inexpensive agro-industrial residues as immobilization carrier in SSF provides an alternative avenue and value-addition to these under- or non-utilized by-products. The kernels, as by-products of canning ad beverages industry, could be successfully used as substrate in SSF processes after supplementation of fermentation media with different nutrients (especially with sugars as carbon sources).

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