Cassava bagasse fermented by Rhizopus spp. for potential use as animal feed

Article *in* New Biotechnology · September 2009

DOI: 10.1016/j.nbt.2009.06.654

also for the target for biotechnological manipulation of product accumulation in *A. chrysochlorus*.

doi:10.1016/j.nbt.2009.06.653

3.2.21

Cassava bagasse fermented by *Rhizopus* spp. for potential use as animal feed

C. Sriherwanto[1,2,*], **C. Koob**[1], **B. Bisping**[1]

[1] University of Hamburg, Department of Chemistry, Institute of Food Chemistry, Division of Food Microbiology and Biotechnology, Biocenter Klein Flottbek, Ohnhorststrasse 18, 22609 Hamburg, Germany

[2] Agency for the Assessment and Application of Technology (BPPT), Biotech Center, Building 630, Puspiptek Serpong, Tangerang 15314 Indonesia

Rhizopus spp. are known as fungi used in the production of tempe, a traditional Indonesian foodstuff produced by solid state fermentation of hulled soybeans. Studies on tempe have shown that fungal growth has positive influences on the content of free amino acids, fatty acids, oligosaccharides, water-soluble and fat-soluble vitamins in fermented soybeans. This has generated interest in producing highly nutritious feedstuff for animals using the similar fungal fermentation on poorly nutritious agroindustrial wastes such as cassava bagasse, which is naturally rich in starch and fibre. Previous studies have shown that optimized fermentation conditions led to good growth of Rhizopus spp. on cassava bagasse as substrate and the resulting fermented product contained up to 10% protein. Its influence on the vitamin content has not been reported yet. In comparison to unfermented raw substrate, the fermented cassava bagasse was found to contain increased concentrations of riboflavin, thiamine, biotin, nicotinic acid, vitamin B_6 and folic acid. The content of these water-soluble vitamins are comparable to some major feedstuffs of plant origin normally used as components of feedstuffs in animal husbandry.

doi:10.1016/j.nbt.2009.06.654

3.2.22

Antigens production in plants as edible vaccines: a great promise

M. Cámara[*], **E. Cebadera**

University Complutense Madrid, Madrid, Spain

Vaccines have been one of the most far-reaching and important public health initiatives of the 20th century. Advancing technology, such as oral DNA vaccines, intranasal delivery and edible plant-derived vaccines, may lead to a future of safer and more effective immunization. Edible vaccines, in particular, might overcome some of the difficulties of production, distribution and delivery associated with traditional vaccines.

Significant challenges are still to be overcome before vaccine crops can become a reality. However, while access to essential healthcare remains limited in much of the world and the scientific community is struggling with complex diseases such as HIV and malaria, plant-derived vaccines represent a new idea that appears to hold great promise. Current research is focused at mixing viral or bacterial DNA in a formula, which is then inserted into the edible plant. When a plant takes on the bacteria, therapeutic DNA becomes stitched into the plant's genetic makeup and as the plant grows, its cells start to produce whatever proteins the new genes are designed to make. When the plant or fruit is eaten, immunization starts, prompting the body to produce the appropriate antibodies.

The success of immunization strategies depends principally on reducing the susceptible proportion of the population to levels below which disease can remain endemic. Despite advances in medical science, the goal of herd immunity remains logistically, if not economically, unattainable for most of the world's population, largely because of constraints on vaccine production, distribution and delivery.

Various technical obstacles, regulatory and non-scientific challenges, though all seem surmountable, need to be overcome. Therefore, there are many questions which need to be answered before developing a plant-based vaccine. At the present, at least three successful human clinical trials have shown that adequate doses of antigen can be achieved with plant-based vaccines. To determine the right dosage, one needs to consider the person's weight, age; fruit and plant's size, ripeness and protein content. The amount to be eaten is critical, too low a dose would fail to induce antibodies and too high a dose would, instead, cause tolerance. Regulatory concerns would include consistency, uniformity of dosage and purity.

Edible plant-derived vaccine may lead to a future of safer and more effective immunization. They would overcome some of the difficulties associated with traditional vaccines, like production, distribution and delivery. However, edible vaccines hold great potential, especially in Third World countries where transportation costs; poor refrigeration and needle use complicate vaccine administration.

doi:10.1016/j.nbt.2009.06.655

3.2.23

Plant polyphenols modified chitosan to inhibit human myeloperoxidase chlorinating activity

M. Díaz González[*], **G. Rocasalbas**, **A. Francesko**, **T. Tzanov**

Universitat Politècnica de Catalunya, Colom 1, Terrassa, Spain

Chronic wound fluids are characterised by elevated level of proteolytic enzymes, especially of neutrophil origin. Myeloperoxidase (MPO) is the main neutrophil enzyme. This enzyme catalyses the oxidation of chloride ions by hydrogen peroxide resulting in hypochloric acid (HOCl) production. The cytotoxicity of this reaction allows the killing of bacteria in the first line of defense. However, the HOCl generated also reacts with most biological molecules, including protease inhibitors. The inactivation of protease inhibitors has a direct effect in the protease—antiprotease balance, promoting the proteolytic damage of healthy tissues. One strategy for chronic wound healing and prevention is the development of bio-polymer coatings incorporating myeloperoxidase

Cassava bagasse fermented by *Rhizopus* spp. for potential use as animal feed

Universität Hamburg

Catur Sriherwanto[1,2], Cornelia Koob[1] and Bernward Bisping[1]

1). University of Hamburg, Dept. of Chemistry, Institute of Food Chemistry, Division of Food Microbiology and Biotechnology, Biocenter Klein Flottbek, Ohnhorstrasse 18, 22069 Hamburg, Germany. Email: bisping@uni-hamburg.de
2). Agency for the Assessment and Application of Technology (BPPT), Biotech Center, Building 630, Puspiptek Serpong, Tangerang – 15314, Indonesia. Email: c.sriherwanto@yahoo.de

ABSTRACT:

***Rhizopus* spp. are known as fungi used in the production of tempe, a traditional Indonesian foodstuff produced by solid state fermentation of hulled soybeans. Studies on tempe have shown that fungal growth has positive influences on the content of free amino acids, fatty acids, oligosaccharides, water soluble and fat soluble vitamins in fermented soybeans. This has generated interest in producing highly nutritious feedstuff for animals using the similar fungal fermentation on poorly nutritious agroindustrial wastes such as cassava bagasse, which is naturally rich in starch and fibre. Previous studies have shown that optimized fermentation conditions led to good growth of *Rhizopus* spp. on cassava bagasse as substrate and the resulting fermented product contained up to 10% protein. Its influence on the vitamin content has not been reported yet. In comparison to unfermented raw substrate, the fermented cassava bagasse was found to contain increased concentrations of riboflavin, thiamine, biotin, nicotinic acid, vitamin B_6 and folic acid. The contents of these water soluble vitamins are comparable to some major feedstuffs of plant origin normally used as components of feedstuffs in animal husbandry.**

MATERIALS & METHODS:

The fermentation substrate, cassava bagasse, is a starch rich lignocellulosic, solid residue from cassava starch (tapioca) extraction process (Figure 1). It contains 9.9% moisture, 77.2% starch, 9.3% fibre, 0.2% crude lipid, 1.3% crude protein and 1.1% ash. The substrate was pregelatinized by mixing 200 g cassava bagasse with 300 ml distilled water, autoclaving at 121°C for 15 minutes. After drying overnight at 60-70°C, the dried flakes were ground mechanically to pass through 1 mm sieve.

The pregelatinized substrate was moisturized up to 68% with a solution containing 0.084% KH_2PO_4, 1.7% $(NH_4)_2SO_4$ and 1.6% urea. Thirty one grams of moisturized substrate was inoculated with 1 ml 10^5 spores/ml of 5 strains of *Rhizopus* spp. previously selected for its ability to grow well on cassava bagasse as carbon source (Figure 2). These strains were *Rhizopus oryzae* EN (EN), *R. oryzae* Fi (Fi), *R. oryzae* Mala (Mala), *Rhizopus oligosporus* Tebo (Tebo) and *R. oryzae* ZB (ZB). The fermentation was carried out at 30°C for 120 hours.

Analyses of the fermented samples were done to determine the concentrations of true protein, residual carbohydrate as well as water soluble vitamins, including thiamine, riboflavin, niacin, vitamin B_6, biotin and folic acid.

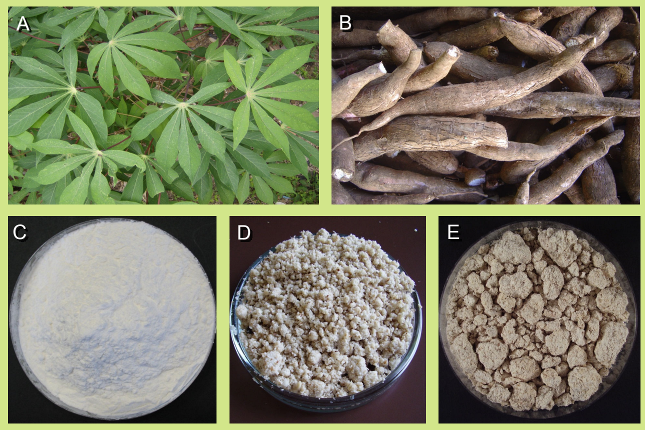

Figure 1: Tropical plant cassava (*Manihot utilissima* Pohl, *M. esculenta* Crantz) (A) has tuber root (B) which is the source of commercial tapioca starch (C). After starch extraction, a wet residue called cassava bagasse is left (D) and dried under the sun (E). This starch and fibre rich organic material was used in this studies as carbon source for solid state fermentation using selected *Rhizopus* strains (Figure 2).

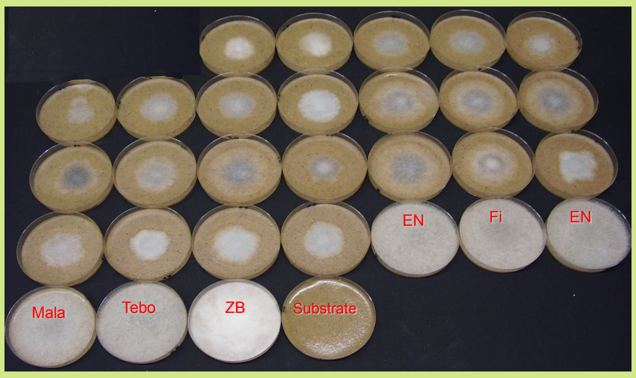

Figure 2: Selection of 28 *Rhizopus* strains on cassava bagasse mash plates containing gelatinized cassava bagasse (15%) as the sole C-source, supplemented with modified mineral base E-N (Owens and Keddie 1969) and 1% $(NH_4)_2SO_4$ as N-source, at 30°C for 44 hours resulted in 5 best growing strains, namely EN, Fi, Mala, Tebo and ZB.

RESULTS & DISCUSSION:

The fermented cassava bagasse obtained here is shown in Figure 3, with brown substrate bound tightly by cottony mycelia. The true protein content ranged from of 6.6 – 9.2 g/100g dry weight (DW) (Figure 4), which is comparable to previous reported results of 10-12% (Raimbault 1998). The fungal bioconversion process was able to reduce the carbohydrate content of the cassava bagasse from initially 83% to around 30%.

The water soluble vitamin profiles of the fermented cassava bagasse indicated that the selected *Rhizopus* strains are able to synthesize these vitamins which they require for their growth (Figure 5 and Table 1).

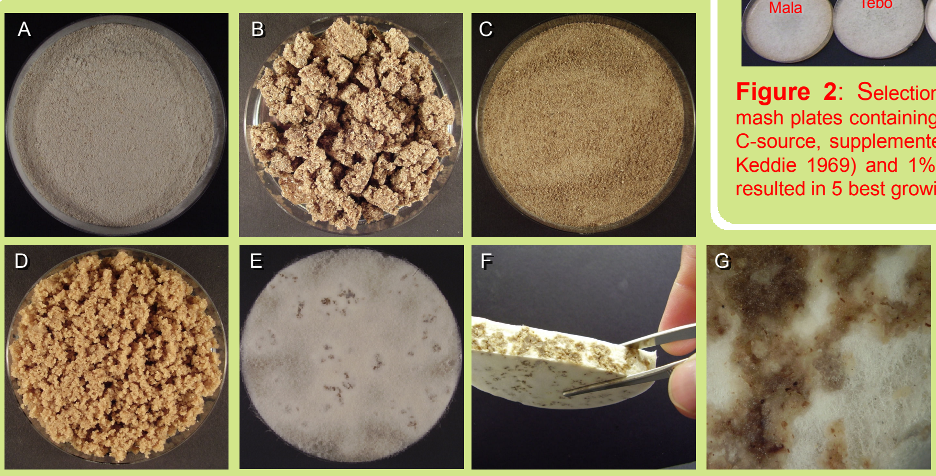

Figure 3: Sun dried cassava bagasse was pulverized (A), 60% moisturized, and pregelatinized by 15 minutes autoclaving at 121°C and drying overnight at 70°C. The resulting flake (B) was mechanically ground (C) and moisturized with salt solution (D). After 5 days fermentation in 9 cm Petri dishes with the selected *Rhizopus* strains (Figure 2) a cassava bagasse cake (E, F) was obtained. Dense white mycelial mass is seen to fill the space between the substrate (G).

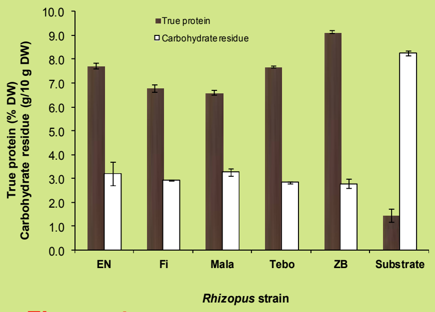

Figure 4: The contents of true protein and carbohydrate residue of cassava bagasse fermented with *Rhizopus* spp.

Table 1: Concentration of thiamine and folic acid produced by the selected *Rhizopus* spp. after 5 days fermentation on cassava bagasse.

Strain	Thiamine	Folic acid
	µg/100g DW	µg/100g DW
Substrate	11.94	18.07
EN	62.12	59.41
Fi	68.78	17.22
Mala	69.25	23.89
Tebo	54.79	34.37
ZB	133.13	18.57

Figure 5: Riboflavin, vitamin B_6, niacin and biotin contents of the unfermented cassava bagasse (CB-0) and cassava bagasse fermented with *Rhizopus oryzae* EN (CB-EN), Fi (CB-Fi), Mala (CB-Mala), ZB (CB-ZB) and *R. oligosporus* Tebo (CB-Tebo) in comparison with those of some feedstuffs stated in literature (Combs 2008). Zero indicated no data was given by the literature.

Compared to some feedstuffs (Figure 5), the fermented cassava bagasse contains a higher concentration of biotin. The profiles of riboflavin and vitamin B_6 are very similar to those of fish meal (herring).

These results indicated that bioconversion of agroindustrial waste such as cassava bagasse using *Rhizopus* spp. can improve the water soluble vitamin contents comparable to those of feedstuffs.

REFERENCES:

Combs GF Jr (2008) Vitamin Contents of Feedstuffs. In: The vitamins: fundamental aspects in nutrition and health, 3rd ed, Academic Press, California, pp 562-564

Raimbault M (1998) General and microbiological aspects of solid substrate fermentation. Electron J Biotechnol 1:26-27

Owns JD, Keddie RM (1969) The nitrogen nutrition of soil and herbage coryneform bacteria. J Appl Bacteriol 32:338-347

Acknowledgement:

Special thanks are due to Deutscher Akademischer Austauschdienst (DAAD), Germany, for financial support of Catur Sriherwanto to carry out the research. The authors also thank David Monniaux and Wilfredo Rodríguez for making the photos of cassava plant and tuber root, respectively, freely available on Wikimedia websites.

Presented at the 14th European Congress on Biotechnology, 13-16 September 2009, Barcelona, Spain.