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THE EFFECT OF SURFACTANTS ON THE PHYTASE PRODUCTION AND THE REDUCTION OF THE PHYTIC ACID CONTENT IN CANOLA MEAL BY *ASPERGILLUS CARBONARIUS* DURING A SOLID STATE FERMENTATION PROCESS

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Summary

During the growth of A. carbonarius, the rates of biomass growth, phytase production and phytic acid content reduction in canola meal media during solid state fermentation were higher in the presence of Na-oleate or Tween-80 than in the control medium which was not supplemented with these surfactants. Addition of Triton X-100 had a negative effect on the studied processes.

The optimum concentration of Na-oleate in solid state culture media was 1%.

Introduction

In 1991/1992 Canada produced more than 4.2 x 10^6 metric tons of canola seeds (Statistics Canada, 1993). The seeds are used for oil production. Canola meal is a by-product of this production. It contains 37-40% protein and is used as animal feed (Clandinin, 1986). The occurrence of 4-6% phytic acid (myo-inositol hexaphosphoric acid) in canola meal reduces its nutritional value; it binds to multivalent cations, such as Zn^{2+}, Ca^{2+} and Fe^{3+}, and so their bioavailability is decreased. Inhibition of enzymes such as α-amylase (Sharma et al., 1978), trypsin, tyrosinase and pepsin (Graff, 1986) by phytic acid has also been reported.

It is necessary to hydrolyze phytic acid or reduce its content in canola meal in order to increase the nutritional value of this commodity. It has been demonstrated that phytase (E.C.3.1.3.8) hydrolyzes phytic acid and thus eliminates the metal chelating capability (Courtois and Joseph, 1947; Courtois and Perez, 1948; Sudarmadji and Markakis, 1977). It is known that plants as beans (Chang et al., 1977), cotton seeds, soybean (Han et al., 1987), wheat (Peers, 1968), and mung beans (Mandal et al., 1972) contain endogenous phytase which, under certain circumstances, reacts with their phytic acid and reduces its content in these materials.

There are also plants, such as canola, that do not contain this enzyme. In this case other possibilities for the reduction of the phytic acid content should be considered.

Chemical methods have been used to reduce phytic acid in canola meal but a partial loss of nutritional constituents such as proteins and minerals has been often noticed when these techniques were applied (Gillberg and Tornell, 1976; Ford et al., 1978; Alli and Houde, 1987).

Enzymatic methods can also be applied for this purpose: e.g. if an external phytase is added to canola meal it can hydrolyze its phytic acid content.

Many microorganisms are able to produce phytase (Shieh and Ware, 1968). Some of them, such as *Rhizopus oligosporus* NRRL 2990, *Aspergillus niger* NRC 5765, *A. carbonarius* NRC 401121, *A. ficuum* and *Saccharomyces crevisiae*, were used in a solid state fermentation process for the reduction of phytic acid content in canola meal (Nair and Duvnjak, 1991).

It has been known that many factors can affect enzyme production. Among them is the addition of surfactants to the media (Rees and Maguire, 1968; Amtual et al., 1988; Han and Gallagher, 1987).

Taking into account that data on the production of phytase by *A. carbonarius* are scarce, the aim of this work was to study the effect of surfactants on the growth of *A. carbonarius* NRC 401121, production of phytase and reduction of the phytic acid content in canola meal during solid state fermentation (SSF).

Material and Methods

Microorganism, media and conditions of growth. A. carbonarius NRC 401121 was used in this study. The spores of this microorganism were prepared in a Roux bottle on a solid medium composed of 4.5% malt agar, 0.5% glucose, 0.5% yeast extract, and distilled water (all % are w/v). The medium was sterilized at 115°C for 15 min, inoculated after cooling and incubated at 30°C until sporulation. The spores were suspended in sterile distilled water and kept at 4°C for further use.

Vegetative inoculum for SSF was prepared in a medium composed of 0.8% nutrient broth, 0.5% yeast extract and distilled water. Erlenmeyer flasks (250 ml) with 100 ml of the medium in each were sterilized at 115°C for 15 min, cooled and inoculated with 1 ml spore suspension. Incubation was carried in a rotary shaker at 30°C for 60 h. Since the microorganism grew in the liquid medium in the form of pellets, the biomass was homogenized in a blender for 45 seconds prior to its utilization for inoculation.

Solid media composed of 50 g of canola meal, 40 ml of distilled water and either 0.5% of sodium oleate, or Tween-80, or Triton X-100, or various amounts of Na-oleate were put in a 500 ml Erlenmeyer flasks and sterilized at 121°C for 45 min. After cooling, the media were inoculated with 20 ml of homogenized inoculum and incubated at 30°C. The changes in the biomass (expressed in terms of its glucosamine content) and enzyme productions and phytic acid content reduction were followed during a SSF process.

Analysis of samples

Phytic acid. Phytic acid was extracted approximately from 2 g of wet solid state culture (SSC) samples by using 33 ml of 2.4% HCl under continuous shaking (200 rpm) for 1 h. After extraction, the suspension was centrifuged (6000 x g, 15 min), the supernatant collected and phytic acid measured by the Haug and Lantzch (1983).

Enzyme activity. Enzyme activity was determined in crude enzyme preparations extracted from SSC using 2% aqueous solution of $CaCl_2.2H_2O$ and shaking the suspension for 1 h on a rotary shaker at 200 rpm. The wet SSC sample to extractant ratio was 1:5 (w:v). At the end of the extraction, the suspension was squeezed through a double layer of cheese cloth and centrifuged (20000 x g, 10 min). The clear supernatant was designated as a crude enzyme preparation. Phytase activity was assayed using sodium phytate as the substrate and measuring spectrophotometrically the inorganic phosphorus released as described by Harland and Harland (1980).

The reaction mixture consisted of 5 ml of 0.2 M acetate buffer (pH 4.7), 1 ml of 2.25 mM phytic acid and 0.1 ml of crude enzyme. The reactions were carried out in duplicate at 53°C for 10 min, and were stopped by adding 5 ml of 10% trichloroacetic acid.

One unit of enzyme activity is defined as the amount of enzyme required to release 1 mg of inorganic phosphorus from 1 ml of 2.25 mM phytic acid solution per hour at the given temperature and pH.

Biomass in solid culture. Glucosamine content of biomass was taken as an equivalent value for the biomass amount and growth. Glucosamine, after being released from biomass by hydrolysis according to Sakurai et al. 1976, was measured by the Blix method (1948).

It has been known that the quantification of biomass in SSC is rather difficult and the methods for it suffer of certain drawbacks. Although the applied method in this work is not also ideal, it is able to give reproducible results if fermentations are carried out under standardized conditions (Desgranges et al., 1991).

Results and Discussion

Effects of sodium oleate, Tween-80 and Triton X-100, on the biomass and phytase productions and phytic acid content reduction in canola meal were examined in this study. The solid state cultures with sodium oleate and Tween-80 produced more biomass (expressed in terms of glucosamine content) and phytase than the control which was not supplemented with surfactants (Fig. 1a and b). Triton X-100 had a negative effect on these processes.

The same surfactants were tested in a liquid medium for the production of phytase by *A. ficuum* (Hahn and Gallagher, 1987). An increase in the enzyme level of 1.3, 1.7 and 4.8 times was noticed when 0.5% of Triton X-100, Tween-80 and Na-oleate was added to the medium, respectively. The authors reported that these surfactants also affected the production of phosphatase by the same microorganism; in this case Triton X-100 was the most efficient and increased the enzyme level by 3.9 times, while the increase caused by Na-oleate was only 1.3 times higher than in the control.

Many other researchers studied the effect of surfactant on the production of enzymes. Rees and Maguire (1968) found that the addition of Tween 80, sucrose monopalmitate, nonionic surfactants, to fungal cultures increased the yields of cellulase, xylanase, amylase, benzoyl esterase, sucrase and purine nucleosidase.

The effect of Tween-80 on the production of extracellular carboxymethyl cellulase (CMCase) and avicelase by *Cellulomonas flavigena* in submerged medium has been investigated

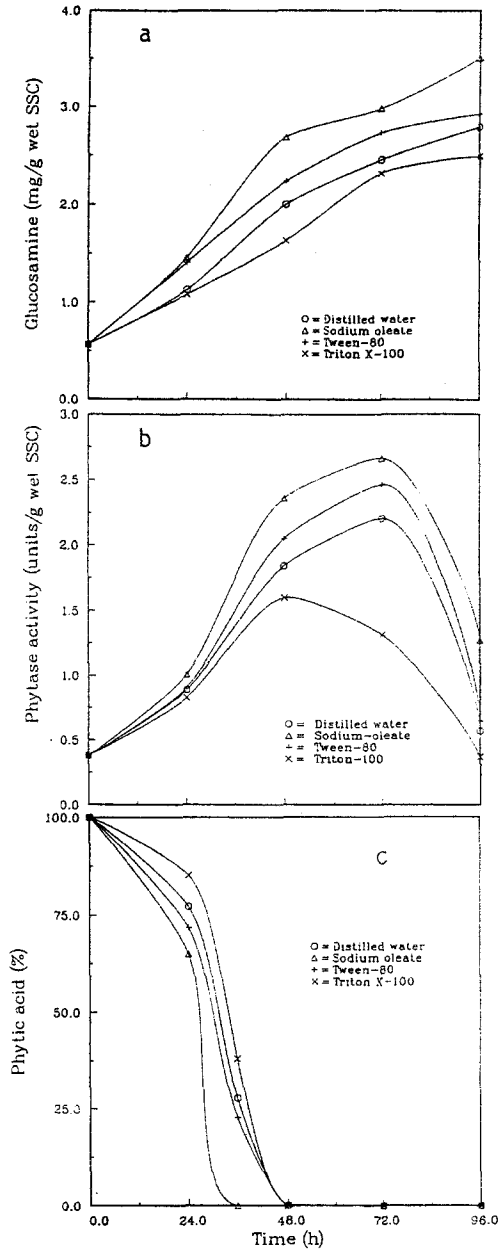

Fig. 1 Effect of surfactants on biomass growth (expressed in terms of its glucosamine content), phytase production and phytic acid content reduction in canola meal.

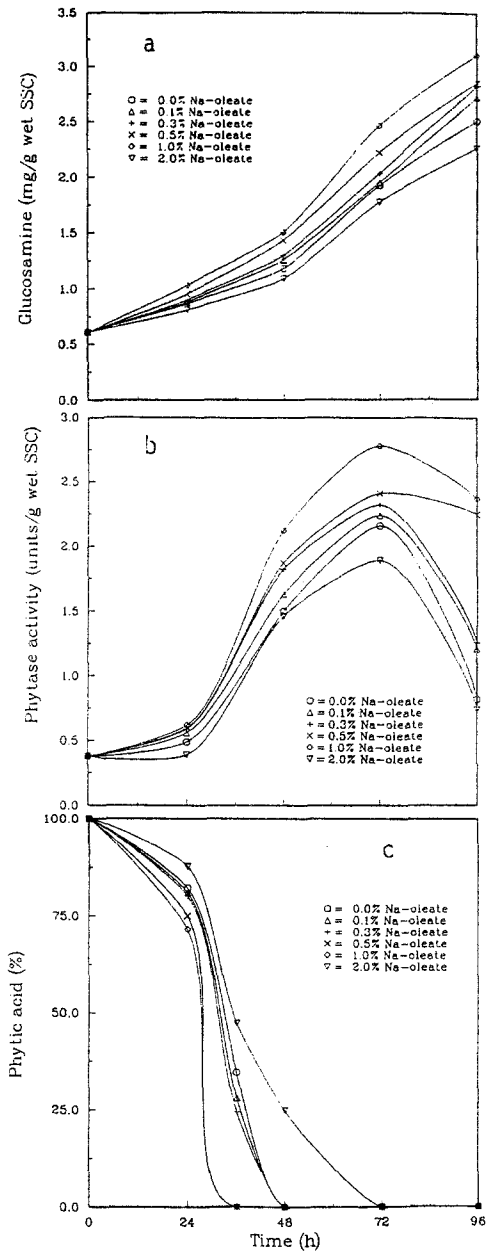

Fig. 2 Effect of Na-oleate concentration on biomass growth (expressed in terms of its glucosamine content), phytase production and phytic acid content reduction in canola meal.

by Amtual et al. (1988). They found that this surfactant in a concentration of 0.1% in the liquid medium resulted in more than a two-fold increase in activities of both CMCase and avicelase in the culture supernatant.

These authors suggested that the surfactants altered the cell permeability which resulted in a higher release of these enzymes.

Taking into account that the enzyme levels in this study were affected by the surfactants, it was logical to expect that the phytic acid content would also be influenced. The results show that the highest and the lowest rates of phytic acid content reduction were observed in the systems containing sodium oleate and Triton X-100, respectively (Fig. 1c).

Since Na-oleate gave the best results, the effect of concentration of this surfactant on the above processes was examined. The biomass growth rate and its total amount increased with an increase in the Na-oleate concentration and attained its peak in the medium containing 1% of the surfactant (Fig. 2a). When the concentration of Na-oleate was 2% the rate of biomass production and the amount of biomass produced were below the values obtained for the control.

The profile of enzyme activity (Fig. 2b) was consistent with the result for biomass. In this case, 1% Na-oleate was also the optimum concentration.

Phytic acid content reduction in the media supplemented with various amounts of Na-oleate is shown in Fig. 2c. Phytic acid was completely hydrolyzed after 36 h of fermentation in the systems containing 0.5% and 1% Na-oleate. The results also show that the slowest rate of phytic acid content reduction was observed in the system containing 2% Na-oleate. It required 72 h to reduce phytic acid content to zero.

Conclusion

The results from this study showed that the rates of biomass (expressed in terms of its glucosamine content) and phytase productions and the maximum amounts of these two products were higher in the solid state cultures with *A. carbonarius* that were supplemented with 0.5% Na-oleate or 0.5% Tween-80 than in the control which did not contain surfactants.

The culture with 0.5% Triton X-100 gave lower biomass and phytase values than the control. Compared with the control, Na-oleate and Tween-80 increased and Triton X-100 decreased the rates of phytic acid content reduction in canola meal.

Na-oleate in concentration of 1% in the medium gave better results than the media containing 2% or below 1% of this surfactant.

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