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THE EFFECT OF SOYBEAN AND CORN OIL ON THE LIPID COMPOSITION OF *CANDIDA LIPOLYTICA*

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The growth of *Candida lipolytica* on a synthetic medium using corn as well as soybean oil as carbon source has been studied. It has been observed that under the studied conditions 40% corn oil and 60% soybean oil remained unutilized. It has been however, observed that *C. lipolytica* does not assimilate all the substrate oils at levels higher than 20 g/L. The fatty acid composition of the oil recovered from the cells of *C. lipolytica* is fairly similar to that of the substrate oil used as carbon source in the medium.

Key words: *Candida lipolytica*, Substrate, Interesterification, Fermentation, Biotechnology.

Introduction

It is well known that micro-organisms can carry out varied transformations and provide useful models for the study of intricate aspects of lipid biochemistry [1]. Some of the more important industrial reactions accomplished by whole microbial cells or enzymes include oxidation [2], hydrolysis [3], esterification [4], interesterification [5], transesterification [6] and epoxidation [7]. A recent report indicates that fatty acids are being produced from triglycerides by the use of lipase [8].

Fatty products of industrial importance are normally manufactured (from fats and oils) by chemical modifications requiring metallic catalysts and high temperature and pressure. On the contrary application of enzymes or whole microbial cells make it possible to produce some of these products with greater rapidity and better specificity under much milder conditions. The fermentation process of the old usually employs the whole organism, whether plant, animal or microorgaism and the use of single cells and the isolated enzyme systems from these sources is associated with newer technologies (Biotechnology).

Lipid production by microbial fermentation, either by old or new techniques, usually uses carbohydrates as a carbon source. In recent years, however, a number of microbes have been identified which are known to use fats and oils as carbon source and accumulate oil in their cells [9-11]. In addition feeding of fats or fatty acids to selected micro-organism has been attempted to up-grade the oil quality (by desaturating or even saturating the component fatty acids).

As a matter of general interest, therefore, the present study was initiated to define and refine various parameters for the growth of *Candida lipolytica* in a medium where oils acted as source of carbon. The oils used were those from corn and soybean. The study showed that the yeast could grow well on corn as well as soybean oils; not assimilate all the substrate oil at levels higher than 20 g/L and accumulate more lipids with increase in substrate oil concentration. It was also observed that under most favourable conditions 40% corn oil and 60% soybean oil remained unused.

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Materials and Methods

Candida lipolytica culture was maintained on agar slants containing yeast extract (0.3%), malt extract (0.3%), peptone (0.5%) and glucose (1%). The synthetic liquid medium used for the growth of *C. lipolytica* consisted as under:

Asparagine	0.2 g
K_2HPO_4	0.1 g
Mg SO_4	0.05 g
Thiamine hydrochloride	0.5 g
Fe $(NO_3)_2 4H_2O$	0.145 mg
Zn SO_4. $7H_2O$	0.088 mg
Mn SO_4. $4H_2O$	0.31 mg
H_2O	100 ml

The medium (120 ml.) was sterilized in a 300 ml flask by autoclaving at 121°. Refined and bleached soybean oil and corn oil (3%) were added as carbon source in different flasks. A loopful of *C. lipolytica* grown on yeast-malt-agar slants, was transferred to the sterile medium and the flasks and their contents were mechanically shaken on a rotary shaker at 28° (200 rpm) for 5 days.

Extraction of lipids. Yeast cells were harvested by centrifugation at 7000 rpm for 15 mins. in a centrifuge. To remove and measure unassimilated oils, hexane was layered into the supernatant, recovered and evaporated. The intracellular lipids were extracted by grinding and soaking the dried cells in a chloroform: methanol (2:1 v/v) mixture.

Analytical methods. Lipid extracts were monitored by thin layer chromatography. Tri-glycerides were converted to

methyl esters by the method of Brignali, *et al.* [12]. Fatty acid compositions were determined by the GLC techniques (glass column, 1.5 m x 4mm, packed with 20% PEGS on diatomite (80 - 100 mesh); column temperature 200°, carrier gas nitrogen, flow rate 40 ml/min; detector temperature 250°; Pye Unicam 204 series unit) [13]. The identification was carried-out by running a standard mixture of methyl esters under identical conditions and comparing their retention times. Confirmation was made by coinjection. The percentage of compositions were recorded with a Pye Unifam DP 88 computing integrator. Results are given in Table 3.

Results and Discussion

The effect of substrate oil concentration on the accumulation of lipids by *C.lipolytica* is presented in Table 1. It is obvious that the yeast can utilize both corn and soybean oils as carbon source although in different proportions. Feeding the oils as carbon source at levels higher than 20g/L produced poor assimilation and enhanced lipid accumulation. Under most favourable conditions only 60% of corn oil and 40% of soybean oil was assimilated and the rest remained unused. It further showed that *C. lipolytica* does not accumulate sufficient lipids during the growth phase (72 hrs.) when glucose is substituted in the medium as a carbon source by corn or soybean oil.

The pH of the medium is a very sensitive parameter and it was maintained throughout the experiment using phosphate buffer, the results show that its variation from 4.5 to 5.5 did not appreciably influence the fermentation efficiency. However, considerably less lipid accumulation occured at a pH range of 6.0 - 6.5 (Table 2).

The fatty acid composition of the fed oils and those recovered from the yeast cells is fairly similar though with minor changes in some constituent fatty acids (Table 3). For instance palmitic acid is decreased with corn oil and unaltered with soybean, palmitoleic acid is significantly increased with both the substrate oils, stearic acid is unchanged with corn oil but reduced with soybean oil, oleic and linoleic acids are almost unchanged while there is definite decrease in case of linolenic acid when corn and soybean oils were used as substrates for the growth of *C. lipolytica*.

The fatty acid composition of the fed oils and those recovered from the yeast cells is almost identical in case of soybean oil and is slightly changed for corn oil in palmitic, oleic and linoleic acids (Table 3). There is an increase in palmiticoleic acid and decrease in the stearic and linoleic acid contents of the recovered yeast cells oils in comparison to the fatty acids of the fed oils. This observation is contrary to that of Bati *et al.* [14] but in agreement with that of Glatz *et al.* [15] and others [10,11].

The change in the lipid contents and free fatty acid formation during growth of *C. lipolytica* on a medium containing corn and soybean oils as carbon source was followed for 24, 48, 72, 96 and 120 hrs. These results show that the hexane extracts has rather higher proportions of FFA while chloroform: Methanol extract consisted largely of triglycerides. The FFA formation at the studied various time intervals and extracted by hexane is presented graphically and indicates the lipolytic activity of the yeast (Fig. 1). Interestingly the formation of FFA is rather similar both with corn or the soybean oils when used as substrates in the medium. The triglyceride

TABLE 1. EFFECT OF SUBSTRATE OIL ON LIPID ACCUMULATION BY C. *LIPOLYTICA*.

Substrate oil (gm/L)		Yeast Lipids %	
Corn	Soybean	Corn	Soybean
20.0	20.0	25.0	23.4
22.5	22.5	28.2	25.8
25.0	25.0	28.7	26.6
27.5	27.5	29.0	26.5
30.0	30.0	29.0	26.6

TABLE 2. EFFECT OF pH ON LIPID ACCUMULATION BY *C. LIPOLYTICA* AT VARIOUS CULTURE TIMES.

Initial pH	Culture time (hrs.)		0	24	48	72	96	120
4.5	Lipids %	C	3.4	5.6	7.9	16.8	28.0	28.1
		S	3.6	5.0	5.3	14.3	27.4	27.4
5.0	Lipids %	C	4.1	6.2	8.4	18.0	29.0	29.0
		S	3.9	5.4	6.0	16.2	27.5	27.6
5.5	Lipids %	C	5.0	6.0	9.0	17.3	28.5	28.3
		S	4.3	5.9	5.9	15.0	26.8	26.8
6.0	Lipids %	C	4.0	4.0	6.2	16.8	26.0	26.0
		S	3.5	3.5	5.0	14.9	24.9	24.7
6.5	Lipids %	C	4.0	4.2	6.5	15.0	24.1	24.2
		S	3.8	3.7	5.1	14.0	24.0	24.2

C = Corn oil, S = Soybean oil, As carbon source in the medium at g/L level..

TABLE 3. FATTY ACID COMPOSITION OF THE OILS FED TO AND RECOVERED FROM THE CELLS OF *C. LIPOLYTICA*.

Fatty acids	Corn oil		Soybean oil	
	Fed	Recovered	Fed	Recovered
$C_{14:0}$	0.52	0.41	0.35	0.28
$C_{16:0}$	10.0	8.5	11.2	11.0
$C_{16:1}$	0.5	2.03	1.2	6.0
$C_{18:0}$	4.2	3.8	3.2	1.3
$C_{18:1}$	20.6	21.8	24.5	24.5
$C_{18:2}$	56.4	55.6	50.6	49.5
$C_{18:3}$	9.0	7.5	9.0	8.0

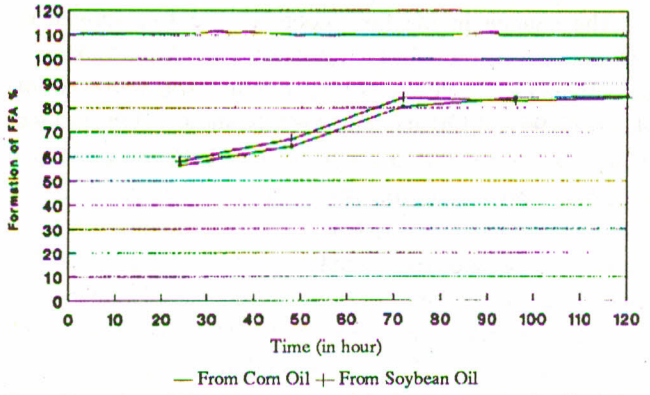

Fig. 1. Formation of FFA (Hexane extract) during the growth of *C. lipolytica*.

contents extracted from the chloroform methanol extract, are however, different and are presumed to be the outcome of the storing capacity of the yeast cells when grown on different carbon sources. The lipase activity of the yeast may be responsible for this phenomenon. The hydrolysis of triglycerides of substrate oil will yield high FFA and its resynthesis during growth phase in the intercellular oil will provide the triglycerides.

On the whole, under the studied fermentaion conditions, *C. lipolytica* tends to deposit the fatty acids in its triglycrides similar to those present in the oil on which it is fed. This is a reasonable outcome, for if the organism uses the accumulated oil as a reserve fuel, the fatty acid composition is probably of little consequence. In order to obtain oils of altered compositions, changed fermentation conditions can be studied, which may induce *C. lipolytica* to radically change the stored oil as it has a potent lipase [16]. Alternatively, however, other microorganisms, fastidious about the oil they deposit can also be studied for the same purpose. Thus there may exist, though limited, a possibility of biomodification of fats and oils through fermentation/biotechnology techniques using the right microbes and fermentation conditions.

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