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UTILIZING OF POTATO STARCH RESIDUE STREAM TO PRODUCE FATTY ACIDS AND OTHER PRODUCTS BY *Saccharomyces cerevisiae* (Y- 1646)

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ABSTRACT

Some fatty acids and other important products can be obtained from potato washing residue stream produced during Chips manufacturing, which hydrolyze by hot acid H_2SO_4 1% v/v 60 min under semianerobic conditions as economical source by *Saccharomyces cerevisiae* (y- 1646). *Saccharomyces cerevisiae* was able to utilize from this residual and production an acceptable amount of fatty acid and other important products. 2, 3 –dihydroxypropyl ester was found in higher levels (568.1 gl^{-1}) and was followed by hexadecanoic acid (221.4 gl^{-1}), and oleic acid (omega 9) 2.1 gl^{-1} at 35 °C after 36 h from addition of $ZnCl_2$ (0.4 gl^{-1}). Study recommends intensive investigation in this research point to enhance the production of that extremely important compound.

Keywords: residue stream, fatty acid, fermentation, *Saccharomyces cerevisiae*.

INTRODUCTION

Certain *Saccharomyces cerevisiae* strains are able to supply nutrients i.e. peptides, vitamins, organic acids and cofactors (Nisbet and Martin, 1994 , Rossi *et al.*, 1995 and Chaucheyras *et al.*, 1996). Yeasts play an important role in food chains, and carbon, nitrogen and sulphur cycles. Polyunsaturated fatty acids (PUFAs) are commercially obtained primarily from seeds, marine animals, microalgae and microheterotrophs. Over the past few years, progress has been made in engineering *S. cerevisiae* for fatty acid production. More specifically, in *S. cerevisiae*, only saturated (mainly 16:0 and 18:0) and mono-saturated (18:1ω9) fatty acids, are synthesized de novo (Daum and Vance 1997, Martin *et al*, 2002). Formation of PUFAs requires the expression of heterogeneous genes to introduce additional double bonds (eg. Δ6-, Δ12-desaturases) and produce enzymes for chain elongation (elongases) (Veen and Lang 2004, Dyal and Narine 2005). PUFA have been known and recognized by health professionals for several years for their benefits throughout all phases of life. So development of a safe and secure source of vegetarian PUFA based on a renewable and economical source. On the other hand, increasing energy costs and environmental concerns have emphasized the need to produce sustainable renewable fuels and chemicals. Major efforts to this end are focused on the microbial production of high-energy fuels by cost-effective 'consolidated bioprocesses'. Fatty acids are composed of long alkyl chains and represent nature's 'petroleum', being a primary metabolite used by cells for both chemical and energy storage functions. These energy-rich molecules are today isolated from plant and animal oils for a diverse set of products ranging from fuels to oleochemicals. A more scalable, controllable and economic route to this important class of chemicals would be through the microbial conversion of renewable

feedstocks, such as biomass-derived carbohydrates. Furthermore, we show engineering of the biodiesel-producing cells to express hemicellulases, a step towards producing these compounds directly from hemicellulose, a major component of plant-derived biomass. To overcome these limitations, several studies have focused on enzymatic transesterification using lipase with whole cell biocatalysts technology and biodiesel production from microalgae. In addition, engineered host fermentation by using *Escherichia coli* and *Saccharomyces cerevisiae* containing heterologous genes has also been shown to be involved in the production of FAEEs (Sakuragi *et al.*, 2011). The aim of study used factories wastes like the potato water stream produced during Chips manufacturing to produce fatty acids and important product by *Saccharomyces cerevisiae* which can be used in many areas.

MATERIALS AND METHODS

Yeast Strain

The yeast used was *Saccharomyces cerevisiae* y-1646 from South Africa (Department of Microbiological, Biochemical and Food Biotechnology, Faculty of Natural and Agricultural Sciences, University of the Free State). Yeast strain has propagated and stored on yeast extract- malt extract (YM) slants (3 gl^{-1} of yeast extract, 3 gl^{-1} of malt extract, 5 gl^{-1} of peptone and 10 gl^{-1} of glucose) at 4 °C. Active cultures for inoculation were prepared by growing the yeast in YM broth on a rotary shaker at 150 rpm for 16 h at 25 °C (initial pH 3.8 - 4.5) .

Potato Starch Residue Stream

The potato starch residue stream was collected from a Chpis' Factory for Food Industries S. A. E. Assiut, Egypt. Samples were transferred to the laboratory in an ice box, and then kept frozen until use. Concentration of starch in the residue stream samples was 10–20 gl^{-1}.

Acid Hydrolysis

The starch contained in potato residue stream was hydrolyze by hot acid (H_2SO_4 1% v/v) at 100% for 60 min. Samples were incubated at room temperature for 15 min with hand shaking intervals. Glucose resulted from hydrolysis was estimated using method described by Nelson-Somogyi method (Somogyi, 1952). The resulted hydrolyzed starch solution was neutralized with NaOH and prepared as growth or fermentation medium for the yeast.

Fermentations Semi-anaerobic Conditions

Repeated culture was carried out in triplicate using a medium contained potato starch residue stream which was hydrolyzed with 1 %, v/v H_2SO_4 at pH 7.0 to estimate fatty acids, alcohols and other products by yeast strain smi-anerobically. The prepared medium was sterilized at 121°C for 20 min. Experiments were initiated by transferring prepared cell suspension with 10 ml (1.2×10^6 cell/ml) into 150 ml of the medium in 250 ml Erlenmeyer flasks, then shaken in the incubator at 150 rpm at 35°C for 36 h. The experiment was under semi-anaerobic conditions by replacing conical flasks with 250 bottles fitted with plugs and incubated under the same afore mentioned conditions. Incubation of the plated medium was carried out at

35°C for 36 h, $ZnCl_2$ (0.4 gl^{-1}) were added separately to the hydrolysed potato starch with H_2SO_4 (1% v/v) which was the best temperature, time and concentration of *Saccharomyces cerevisiae* (y-1646) according to (Hashem and Darwish, 2010).

Analytical Method

The estimation of starch was carried out using the iodine colorimetric method as described by (Tomas and Chamberlain, 1980). Reducing sugars were estimated by the dinitrosalicylic acid method using glucose as the standard (Miller, 1959).

Determination, fractionation and identification of fatty acids, alcohols and other products in hydrolyzed potato washing residue stream were estimated by Gas Chromatography/Mass Spectrometry (GC/MS). A Hewlett-Packard (HP) system 6890 series gas chromatograph coupled with a HP model 5975B quadruples mass spectrometer; cross-linked 5% phenyl methyl siloxane capillary column (HP-5MS, 30m x 0.25mm id x 0.25µm film thickness). GC operating conditions were as follows: initial temperature 40°C (1 min hold), increased at 20°C min-1 to 210°C, then increased at 1.5°C min-1 to 215°C (4 min hold); injector temperature 240°C; carrier gas Helium (99.999%), flow-rate 1.3 ml^{-1} min; ion source temperature 270°C; operated in the split less mode; purge off time 1 min; injection volume 1 µl nominal. MS operating conditions were: solvent delay 6 min; electron-impact (EI) mode ionization voltage 70 eV using selected ion monitoring (SIM); dwell time for each ion 100 ms. Data acquisition and processing were provided by a Vectra VL 5/90 Series 3 Computer equipped with a HP G1030A ChemStation data system (EPA, (1998) methods 8270D, 8141B).

RESULTS AND DISCUSSION

Production Fatty Acid by *Saccharomyces cerevisiae*.

Microorganisms have often been considered for the production of oils and fats as an alternative to agricultural and animal sources. However, owing to their resemblance to plant oils and animal fats, microbial oils will inevitably have to compete with traditional lipid products if they are to be produced commercially. Although amazing diversity of fatty acid structures occurs in the microbial kingdom, many of the minor fatty acids have potential uses but are not available in sufficiently large quantities (Certik and Shimizu, 1999). Figure (1) shows that the content of fatty acids and other products by *Saccharomyces cerevisiae* y - 1646 on hydrolyzed potato starch residue stream under semi-anaerobic conditions at 35 °C after 36 h by GC/MS . *Saccharomyces cerevisiae* were recorded a large amount (568.1 g l^{-1}) of 2,3 –dihydroxypropyl ester, and (227.8 g l^{-1}) hexadecanoic acid (palmitic acid) (Fig.2) palmitic acid is the most prominent saturated fatty acid occurring in fish oils, in the milk and storage fat of many mammals, and in vegetable fats. Octanoic acid is a by-product of ethanolic fermentation by yeasts. Like other lipophilic weak acids, octanoic acid is used as an antimicrobial food additive (Viegas and Correia 1995) in this study *Saccharomyces cerevisiae was* recorded (26.4 g l^{-1}) during grows in hydrolyzed potato washing residue stream (Fig.3). On the other hand, oleic acid (18:1c ω9) is accumulated in

acceptable amount 8.5 g l^{-1} in hydrolyzed potato washing residue stream (Fig.3), which is a major ingredient in medications used for obstructing the progression of Adrenoleukodystrophy (ALD), which is a fatal disease that affects the brain and adrenal glands. The primary saturated fatty acids are palmitic acid followed by stearic acid. Stearic acid (18:0) is a minor component in most vegetable fats, and its trivial name derives from the fact that it is a major component in the tallow of ruminants. *Saccharomyces cerevisiae* was produced 14.4 g l^{-1} during period of fermented hydrolyzed potato washing residue stream of Stearic acid, respectively (Fig.3). A significant exception to this generalization is that the long-chain saturated stearic (octadecanoic) acid, does not contribute to the elevation of LDL cholesterol (Technical, 2006). Composition of the growth medium affected significantly the fatty acid profile, so growing *Saccharomyces cervisiae* in the hydrolyzed potato washing residue stream may be attributed these types and amount of fatty acid.

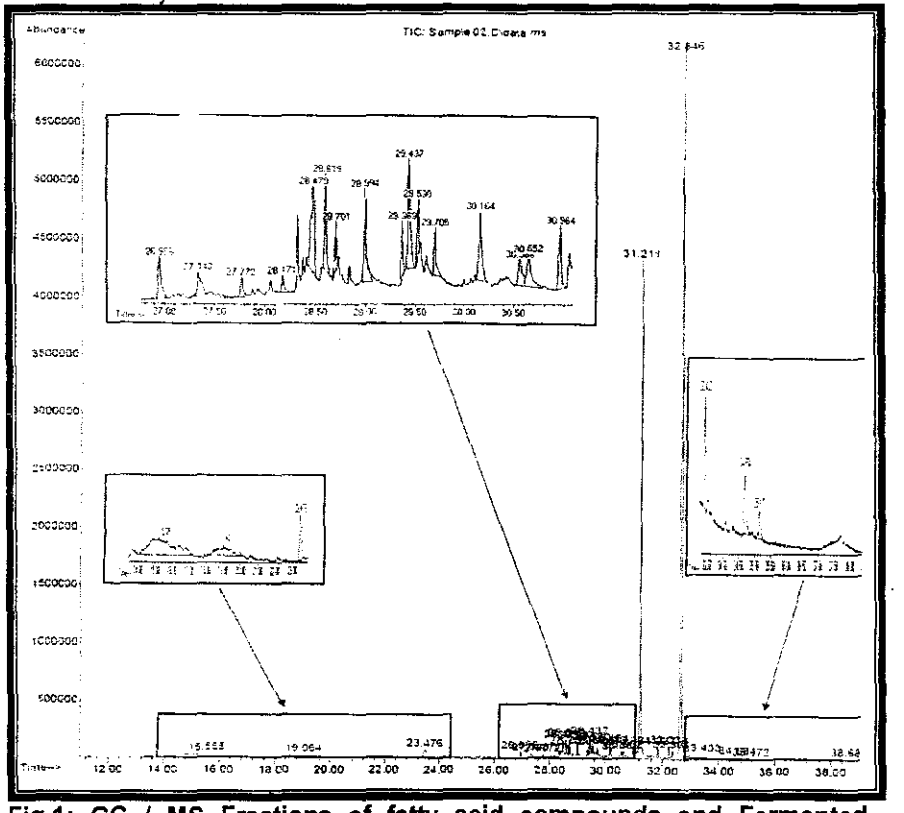

Fig.1: GC / MS Fractions of fatty acid compounds and Fermented hydrolyzed potato washing residue stream by *Saccharomyces cervisia* y - 1646.

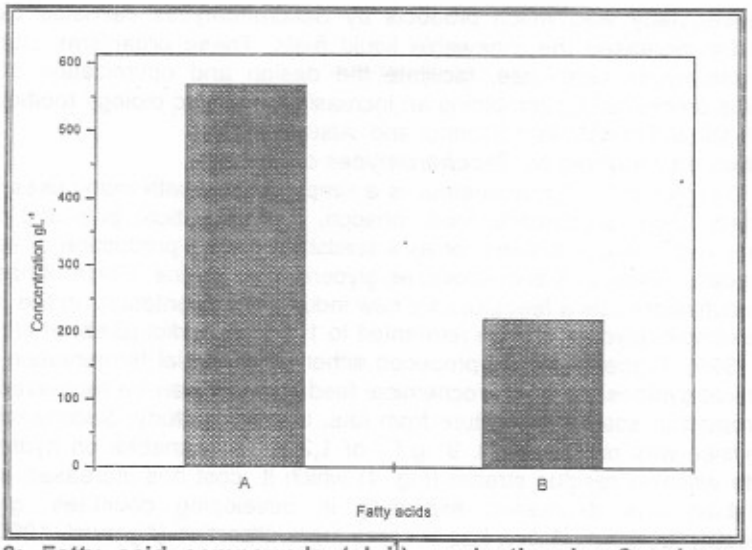

Fig. 2: Fatty acid compounds (gl $^{-1}$) production by *Saccharomyces cerevisiae* y- 1646 on hydrolyzed potato starch residue stream under semi-anaerobic conditions at 35 °C after 36 h by GC/MS. [(A) 2,3 –dihydroxypropyl ester; (B) hexadecanoic acid (palmitic acid)].

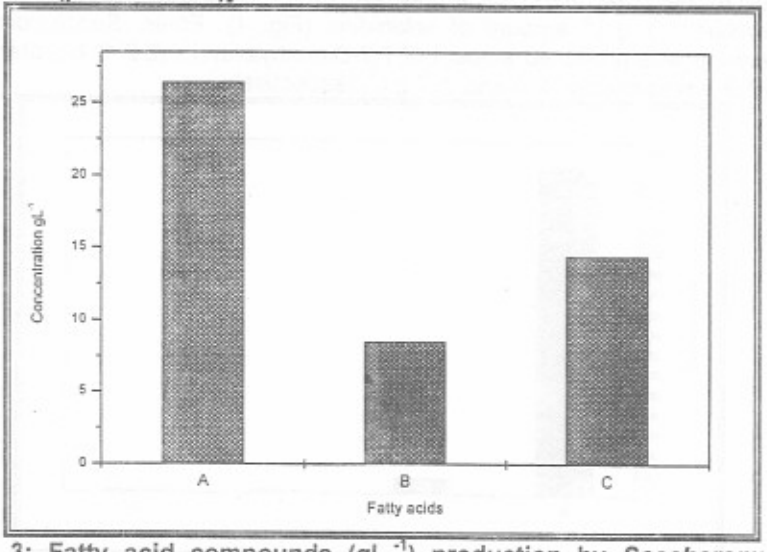

Fig. 3: Fatty acid compounds (gl $^{-1}$) production by *Saccharomyces cerevisiae* y- 1646 on hydrolyzed potato starch residue stream under semi-anaerobic conditions at 35 °C after 36 h by GC/MS. [(A) octanoic acid; (B) oleic acid (omege-nine); (C) stearic acid].

Moreover, fatty acid which products by *Saccharomyces cervisiae* can be used for increased the renewable liquid fuels. These organisms, such as *Saccharomyces cerevisiae*, facilitate the design and optimization of new biofuels processes by combining an increasing synthetic biology toolbox with a well-studied metabolism (Connor and Atsumi, 2010).

Production Alcohols by *Saccharomyces cerevisiae*

Glycerol, a 1,2,3-propanetriol, is a simple alcohol with many uses in the cosmetic, paint, automotive, food, tobacco, pharmaceutical, pulp and paper, leather and textile industries or as a feedstock for the production of various chemicals. Glycerol is also known as glycerin or glycerine. Glycerol has also been considered as a feedstock for new industrial fermentations in the future. For example, glycerol can be fermented to 1,3 propanediol (Biebl *et al.*, 1998 and 1999). Glycerol can be produced either by microbial fermentation or by chemical synthesis from petrochemical feedstocks or can be recovered as a by-product of soap manufacture from fats. In current study, *Saccharomyces cerevisiae* was produced 80. 9 g l^{-1} of 1,2,3 – propanetriol on hydrolyzed potato washing residue stream (Fig. 4) which it cost has increased and its availability has decreased especially in developing countries, glycerol production by fermentation has become more attractive (Agarwal, 1990; and Wilke, 1999). Solanidine (Solanid-5-en-3-ol) is a steroidal aglycon of potato (*Solanum tuberosum L.*) glycoalkaloids are a very important precursor for the synthesis of hormones and some pharmacologically active compounds, Nikolic and Stankovic (2003) was calculated the yield of solanidine of potato vines to be 0.24 g/100 g. In present study recorded *Saccharomyces cerevisiae* 1.1 g l^{-1} amount of solanidine (Fig. 4). Ether, *Saccharomyces cerevisiae* was produced amount of 7,7-Dimethylbicyclo (2.2.1) hepaten-1-ol and 1,3 – propanetriol 4. 0 and 0. 7 g l^{-1}, respectively.

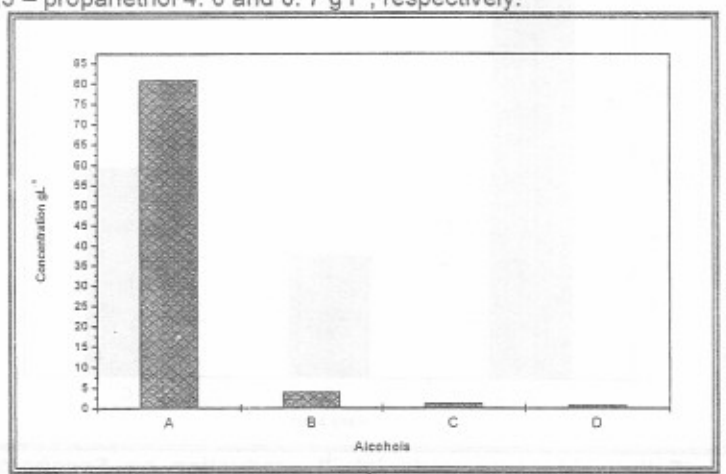

Fig. 4: Alcohols compounds (gl^{-1}) production by *Saccharomyces cerevisiae* y- 1646 on hydrolyzed potato starch residue stream under semi-anaerobic conditions at 35 °C after 36 h by GC/MS. [(A) 1,2,3 - propanetriol; (B) 7,7-Dimethylbicyclo(2.2.1)hepaten-1-ol; (C) Solanidine; (D) 1,3 - propanetriol].

Production amine and other products by *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae was recorded in this study 17.3 g l^{-1} of Levoglucosan during grows in hydrolyzed potato washing residue stream. Ether, *Saccharomyces cerevisiae* produced acceptable amount of 3-methylindole, scatole and Hexahydro-3-(phenylmethyl) were 7.1, 4.2 and 4.2 g l^{-1}, respectively (Fig. 5).

Levoglucosan 1,6-anhydro-β-D-glucopyranose is known to be an important intermediate in the pyrolysis of cellulose, but little is known about its subsequent thermal degradation. Levoglucosan is formed in high yields (up to 60 %) by the pyrolytic unzipping of cellulose (Nimlos and Evans, 2002). Microalgal growth was enhanced by the addition of levoglucosan to the culture medium (Luyen *et al.*, 2007).

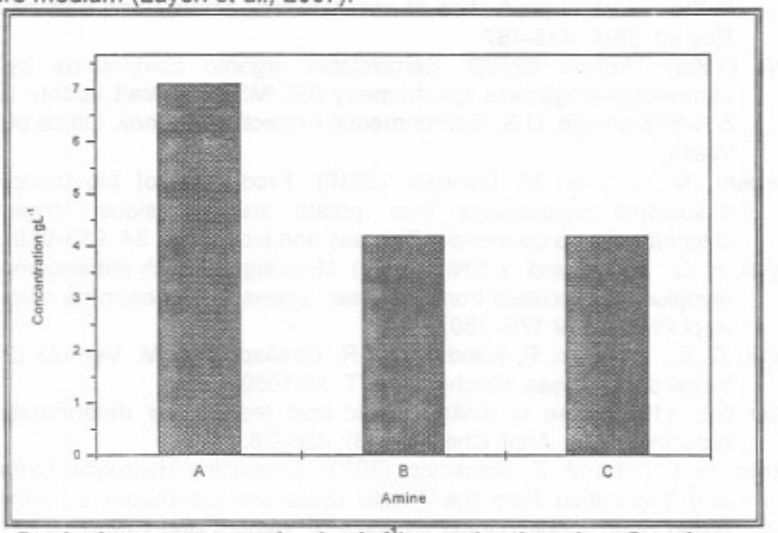

Fig. 5: Amine compounds (g l^{-1}) production by *Saccharomyces cerevisiae* y- 1646 on hydrolyzed potato starch residue stream under semi-anaerobic conditions at 35 °C after 36 h by GC/MS [(A) 3-Methylindole; (B) scatole; (C) Hexahydro-3-(phenylmethyl)].

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الاستفادة من النشا الناتج من مخلف ماء البطاطس لإنتاج أحامض دهنية ومنتجات أخرى بواسطة ال *Saccharomyces cerevisiae* y - 1646

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يمكن الحصول على بعض الاحماض الدهنية والمركبات الهامة من النشا الناتج من عملية غسيل البطاطس أثناء صناعة الشبسى(كمصدر اقتصادى) باستخدام الـــ (y- 1646) . *Saccharomyces cerevisiae* فهى قادرة على الاستفادة من النشا الناتج من عملية غسيل البطاطس أثناء صناعة الشبسى المعالج بالحمض تحت الظروف شحيحة الهواء على ٣٥ ° م لمدة ٣٦ ساعة لإنتاج كميات مقبولة من الاحماض الدهنية ومركبات اخرى لها اهمية أقتصادية .ومن المركبات التى سجلت اعلى قيمة انتاج ال 2, 3 –dihydroxypropyl ester (٥٦٨.١ ملجم / لتر) ثم Hexadecanoic acid(٢٢١.٤ ملجم / لتر، ثم حمض الاوليك (اومجا ٩) فقد سجل (٢.١ جم / لتر) .وتوصى الدراسة بتكثيف البحث في هذه النقطة لزيادة إنتاج هذة المركبات الهامة.

قام بتحكيم البحث

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أ.د / عبد الجواد محمد الشواف	معهد الكفاية الإنتاجية بالزقازيق