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ABSTRACT

Rhodotorula glutinis has been one of the top candidates among oleaginous yeast for several microbial lipid applications, such as biofuel production. In this project the effect of sesamin on the fatty acid profile was investigated by culturing *R.glutinis* J195 with low nitrogen and high carbon cultures with DMSO as the solvent of sesamin, firstly in baffled flasks and thereafter in fermenters. Glucose consumption, biomass accumulation, lipid content and lipid composition were determined. Sesamin had effect on the fatty acid profile by lowering the amount of several fatty acids as well as increasing others. There was neither decrease in biomass accumulation nor change in the pattern of glucose consumption by addition of sesamin. The most encouraging result was the slight increase of docosahexaenoic acid (22:6n-3),which is a polyunsaturated fatty acid that is regarded as one of the health beneficial ω3 fatty acids found in fish. However the results are obscure due to different sample treatments and impure lipid samples as well as that the fatty acid standard seemingly had oxidised.

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Rhodotorula glutinis

The oleaginous yeast *Rhodotorula glutinis* is a so called red yeast. *R. glutinis* is a budding, asexual yeast that belongs to the phylum basidiomycota, in the earliest diverged subphylum of basidiomycota Pucciniomycotina, in the order of Sporoidiobolales (Coelho et al., 2011). The genus itself is considered ubiquitous and can be found both in extreme environments as well as more hospitable locations. Examples of such extreme settings where *Rhodotorula* species can be found are the air, the deeper levels of the sea as well as samples from the Dead Sea, known for its extreme saline environment (Querol and Fleet, 2006; Wirth and Goldani, 2012; Butinar et al., 2005). Since the genera are ubiquitous, it is found as well in the soil, our foods and even on our skin (Wirth and Goldani, 2012; Vaughn et al., 1969; Querol and Fleet, 2006; Strausbaugh et al., 1996).

R. glutinis has been reported as an opportunistic pathogen that can cause Rhodotorulosis and other infections (Wirth and Goldani, 2012; Lanzafame et al., 2001; Hsueh et al, 2003), which has been mainly seen in patients with either lowered immune system or who have had a central venous catheter (Wirth and Goldani, 2012). This in turn may be explained by the apparent high affinity *Rhodotorula* species has for plastic, which is documented by its abundant isolation from various plastic items such as catheters, dialysis equipment, toothbrushes, shower curtains and mouthguards (Wirth and Goldani, 2012; Glass et al., 2011).

Rhodotorula glutinis has several interesting biochemical properties that can be utilized in the industry, such as its enormous potential for lipid accumulation which can be up to more than 70% of its dry weight, and contains both saturated and unsaturated fatty acids (FA) (Ratledge and Tan, 1989). A previous fatty acyl composition has been reported by Ratledge and Tan (1989) where it has been shown in that particular experiment that 68% of its FA are C18 FA of which 9% are 18:0 (Stearic acid), 40% are 18:1, 16% are 18:2 and 3% are 18:3 FA. The remaining 32% are 30% 16:0 (Palmitic acid) and 2% 14:0 (Myristic acid) with traces of 16:1 FA. Due to its enormous lipid production *R. glutinis* has been considered to be used in biofuel production (Dai et al., 2007; Cheirslip et al., 2011; Meng et al., 2006). In the food industry *Rhodotorula* spp. are used as sources of lipases (Querol and Fleet, 2006).

Also their high production of carotenoids is of commercial interest as food colorants and antioxidants. Carotenoids had an estimated market value of $1.2 billion in 2010 and are expected to reach $1.4 billion in 2018 (Mata-Gómez et al., 2014). The production has been reported to be up to

1.2 mg/g β-carotene (dry weight), which has been suggested as appropriate for industrial production (Marova et al., 2012).

Sesamin

Sesamin is a compound found in sesame seeds from *Sesamum indicum* or the bark of Fagara plants. It is part of the lignan family that act as antioxidants and phytoestrogens and is poorly soluble in water (Arita et al. 2012). It has been shown that sesamin is a specific inhibitor of the enzyme Δ5-desaturase in both filamentous fungi and rat liver, that catalyses the synthesis of arachidonic acid (20:4n-6) from dihomo-γ-linolenic acid (20:3n-6) (Shimizu et al., 1991). Other properties are inhibition of cholesterol absorption and synthesis (Hirose et al., 1991), lipid lowering and blood pressure lowering properties (Tsuruoka et al., 2005). Sesamin has been shown to up-regulate the gene-expression of several enzymes involved in the β-oxidation of fatty acids (Seon Lim et al., 2007), as well as regulating xenobiotics and alcohol at an mRNA level in rats (Tsuruoka et al., 2005). The cause of the lipid lowering effect has been suggested by Ashakumary et al. (1999) to be because of changes in the fatty acid metabolism in the liver. In the consumers industry sesamin is used as a fat reducing or anti-aging compound (Arita et al., 2012).

Possible use of modified fatty acid profile in *R. glutinis*

Fish oils are in high demand, however there is a shortage of it and therefore it has been tried to feed the fish with vegetable oils (Trattner et al., 2008b). Another problem is also that fish species that are carnivorous such as salmon, brought up by aquaculture are fed with fish, thus putting strain on the ecosystem (FAO, 2008). Freshwater fish are able to convert C18:2n-6 (linoleic acid) and C18:3n-3 (linolenic acid) from vegetable oils into long chain polyunsaturated fatty acids, such as 20:4n-6 (arachidonic acid), 20:5n-3 (eicosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid) (Wagner et al., 2014). The fish, however, produces less long chain polyunsaturated fatty acids when fed vegetable oils, which is disadvantageous when regarding consumers health (Trattner et al., 2008 b). It has been tried earlier to use sesamin directly in the feed of rainbow trout (*Oncorhynchus mykiss*) with vegetable oils to try to alter the FA profile, where there was a 37% increase of 22:6n-3 was observed in the white muscle tissue (Trattner et al., 2008a). Since *R. glutinis* grows fast, is rich in carotenoids and C18 fatty acids, it is well suited to be used as fish feed for e.g. salmonids, especially if the fatty acid profile can be modified so that the FA profile have a higher ratio of C18:2 and C18:3 or longer polyunsaturated fatty acids. The aim of this study was to examine if adding sesamin to a culture of *R. glutinis* will modify the FA profile of *R. glutinis*.

Yeast strain

The yeast used in this study was *Rhodotorula glutinis* J195 (strain collection at the Department of Microbiology, SLU, Uppsala). The yeast was grown on YM-agar (see below) for 48 h at 25ºC and then stored at +2 ºC.

Media

YM- agar

YM-agar (Yeast mould- agar), containing yeast extract (YE) 3g/L (Bacto™ Yeast extract, Becton, Dickinson and Company (BD), USA), malt extract 3g/L(Bacto™ malt extract, BD, USA), peptone 5g/L(Bacto™ peptone, BD, USA), glucose 10g/L (Anhydrous glucose, FORMEDIUM LTD, England) and agar 16g/L (Bacto™ Agar, BD, USA). For the pre-cultures of *R. glutinis* YM-medium was used (YE 3g/L, malt extract 3g/L, peptone 5g/L and glucose 10 g/L).

YNB-mixture

10X YNB mixture was composed as follows 10X Difco™ Yeast Nitrogen base w/o amino acids (BD, USA), 7.5 g/L YE and 20 g/L ammonium sulphate (Merck KGaA, Germany) was prepared and sterile filtered with a 25 mm w/0.2 µm polypropylene filter (VWR international, USA).

Potassium dihydrogen phosphate buffer

A 10X potassium dihydrogen phosphate buffer was prepared by following a recipe for a pH of 6 in 200 mL; 100 mL 0.1 M KH_2PO_4, 11.2 mL of 0.1M NaOH and 88.8 mL deionized water and was then autoclaved at 120 °C for 20 minutes. A 10X potassium dihydrogen phosphate buffer was prepared by following a recipe for a pH of 6 in 200 mL; 100 mL 0.1 M KH_2PO_4(Merck KGaA, Germany), 11.2 mL of 0.1M NaOH (Sigma-Aldrich, USA) and 88.8 mL deionized water. The 10X buffer was done by multiplying the molar contents by 10 to 1 M to create a 100 mL 10X buffer with a measured pH of 5.84 (20 mL trial) and 5.62 (100 mL trial) that was then autoclaved at 120 °C for 20 minutes.

20 and 100 ml culture media

The 20 mL media were prepared as following: The sesamin medium containing 2% Vol. dimethyl sulfoxide 99% (DMSO) (Merck KGaA, Germany), 70 g/L glucose and sesamin 98% (KEB Biotech, China) was prepared with three different concentrations (0.002 g/L, 0.02 g/L and 0.2 g/L sesamin) and two controls (one with DMSO/glucose and one with only glucose). The sesamin was weighed on a four decimal scale for the two latter concentrations the smallest concentration was made by weighing 0.0020 g of sesamin and then dissolved with 2 mL DMSO to create a 10X solution,

thereafter 200 µl were taken and diluted with 1.8 mL DMSO. Next the cultures were diluted to 80 mL (20 mL trial) with deionized water and autoclaved at 120 °C for 20 minutes. The 0.2 g/L sesamin medium showed a milky appearance; most likely had the sesamin precipitated, but was still used to see if there was any effect on the yeast. The culture media was prepared by adding 10 mL 10X buffer and 10 mL 10 X YNB solution to each of the five solutions reaching a final volume of 100 mL and thereafter 20 mL was transferred to each of its designated duplicates. The three 100 mL cultures (glucose control, DMSO control and sesamin culture) were prepared in the same manner as the 20 mL cultures but scaled up to 250 mL of total culture, and then 100 mL were transferred to its separate duplicate.

Preculture

500 mL baffled shake flasks containing 100 mL YM-medium were inoculated with *R. glutinis* cells and incubated on a shaker table at 125 rpm for approximately 48 hours. The OD of the pre-cultures was measured and the corresponding volume to reach a start OD of 1 in the main cultures was harvested. The cells were collected in sterile Eppendorf tubes centrifuged for 5 min at 4500 g and the supernatant was discarded, then the cells were resuspended in 9 g/L NaCl and centrifuged once more. The cells were suspended in 100 µl (20 mL trial), 500 µl (100 mL trial) and 2 mL (fermenter trial) NaCl solution and added to each of the baffled flasks and put on a shaker table at 100 rpm or added to the fermenters.

Test culture 20 mL

The test cultures of 20 mL in 100 mL baffled shake flasks containing either the control medium or media with different concentrations of sesamin was inoculated by cells from the pre-culture to a start OD_{600} of approx. 1.The pH of the culture media ranged between 5.71 and 5.78. OD_{600} was measured during the cultivation, 2-3 times per day. During the cultivation the glucose level in the media was monitored by Medi-Test Glucose (Macherey-Nagel, Germany) strips. Cells from the cultures was investigated in a microscope to see eventual differences in amount/ sizes of lipid bodies It was decided to use the 0.02 g/L sesamin culture since no visual differences in the lipid accumulation could be seen when comparing to the higher and lower concentration in the microscope.

Test culture 100 mL

Preculture was prepared as described above. The culture media were also prepared as described but the total volume was 100 mL and the baffled shake flasks 500 mL. The pH of the three media (sesamin 0.02 g/L, DMSO control and glucose control) measured and all ranged between 5.69 and 5.74. The cells used to inoculate the cultures were washed as described before but were resuspended in 500 µl of 9 g/L NaCl. The OD_{600} was measured during the cultivation-and samples for dry weight

determination (see below) and high performance liquid chromatography (HPLC) were taken. One to two millilitres of each culture were transferred and centrifuged at 1523 G for 5 min. The supernatant was thereafter filtered with a 25 mm w/0.2 µm polypropylene filter (VWR international, USA) and frozen at -20 °C.

Fermentation 300 mL cultures

Completely assembled Infors-HT Multifors 2, equipped with pH electrode (Mettler Toledo, USA), pO_2 sensor (InPro® 6800 series, Mettler Toledo), 0,45 µm filters (Acrodisc®, Pall, USA) were autoclaved at 120 °C for an hour with their designated media excluding YNB solution. NaOH 5 M (Sigma-Aldrich, USA) was used as base and polypropylene glycol 2'000 (Sigma Aldrich, USA) as anti-foam; no acid was used. The media were the same as in the 100 mL cultures scaled up to 300 mL excluding the buffer; the first containing glucose (70 g/L) and YNB mixture (YNB 1x, YE 0.75 g/L and 2 g/L ammonium sulphate), the second glucose YNB mixture and DMSO 2 vol %, and lastly the third with glucose YNB mixture, DMSO 2 vol% and 0.02 g/L sesamin. During majority of the fermentation process the pO_2 was stable at between 20-25% except for the first hours of fermentation (≈80 %) and when the sugar was consumed (≈65%). The fermentations were done in duplicates, though it should be mentioned that the glucose control and the DMSO control were singulars due to contaminations during the fermentation process.

Sampling was done according to the Infors-HT Multifors manual generally three times a day until the sugar content had reached zero, which was approximately after 72 hours. The OD_{600} was measured at each sampling as well as taking glucose samples for HPLC done in the same manner as the 100 mL cultures. Glucose strips were used after 48 hours and forward to roughly control the glucose content. The biomass accumulation was sampled once a day and when lipid samples were collected. Lipid samples were collected approximately after 48 hours of each culture, where 10 mL were collected in triplicates. These were washed three times with deionized water, frozen at -20 °C and freeze-dried in the same manner as described below, excluding the glucose control which was simply spun down, had the supernatant discarded and freeze dried. It should also be noted that the DMSO control was at first centrifuged and frozen, but was then thawed and washed before freeze-drying.

HPLC

The frozen samples were thawed and diluted ten times with 0.2 µm filtered (VWR international, USA) deionized water in HPLC vials to 1 mL. Glucose standards were prepared by filtering the glucose solution through 0.2 µm filters and diluting the solution to concentrations ranging from 0.1 g/L to 10 g/L with at least two points in between. A Rezex-ROA-Organic Acid H^+ 330 x 7.80 mm (Skandinaviska Genetech AB) column was heated up with a 0.3 mL/min flow and set to 0.6 mL/min

with 0,005M H_2SO_4 as the fluid phase in a Agilent technologies 1100 series (Agilent technologies Inc., USA) equipped with an automatic injector and quaternary pumps. When the temperature had reached 60 °C and a pressure of 40 Bar the samples were analysed.

Dry weight determination

Samples for dry weight determination and lipid analyses were taken in triplicates of 2 mL of each culture transferred to pre-weighed, pre-dried 2 mL Eppendorf tubes. The cell suspensions were centrifuged at 16089 g for 2 min and were cleaned three times with deionized water with centrifugation steps in between. Thereafter was the cell pellets were dried at 105 °C overnight. After the samples were cooled in an desiccator and weighed and the dry weight of the sample was calculated.

Freeze drying

Before lipid extraction the samples were freeze-dried. 10 mL was taken in triplicates of each culture and pipetted into a 50 mL falcon tube. The cell suspension was centrifuged at 5000 g for 20 min and the supernatant was discarded. Next the samples were cleaned in three cycles with deionized water, centrifuged and in-between each cycle the supernatant was discarded. The pellets were thereafter put into the freezer at -20 °C for a few hours. When the pellets had all frozen the lids were removed, covered with tissue paper and holes for each tube were manually made. The Falcon tubes were afterwards put into a vacuum drier (Edwards Modulyo, United Kingdom) with a cooled platform at -45 °C. The pressure was set at 0,133 mBar and the samples were freeze-dried overnight. The freeze-dried samples were then moved to a freezer and stored at -20 °C until lipid extraction

Lipid extraction

Method optimisation was performed by using a modified protocol of the work of Folch et al. (1957) which was tested with and without cell membrane disruption by using 1M HCl (Merck KGaA, Germany).

<u>Lipid extraction protocol with/without HCL</u>

100-200 mg of dry yeast (if there is a high lipid content in the cells less sample was needed) of each sample were weighed on a four decimal scale and transferred into 10 mL glass tubes with screw caps. Thereafter 1 mL of cold deionized water was added to the cells and left to soak for 15 minutes at room temperature. After soaking, was 3.75 mL of 1:2 chloroform (Merck, KGaA, Germany):methanol (Merck, KGaA, Germany) mixture were added to the cells (1 mL of 1 M HCl is to be added if using HCl as well) and vortexed for 30 seconds, the tubes were then put into a sonicator for an hour and were vortexed for 1 minute every 15 minutes during this hour. The samples were after sonication put into a shaker for 15 minutes.

Additional 1.25 mL chloroform was added to each tube and the samples were vortexed vigorously for 1 minute. To make the separation more effective (Folch et al., 1957) 1.25 mL 8 g/L KCl (Merck KGaA, Germany) was added (HCl use 0.25 mL of 8 g/L KCl instead) and the tubes were vortexed briefly. The tubes were centrifuged thereafter at 1006.2 g for 3 minutes at room temperature. The lipids were extracted from the lower chloroform phase by using a Pasteur pipette and transferred into pre-weighed tubes. To ensure a high yield as possible a second extraction was done by adding additional 1.88 mL chloroform to the water phase, vortex for 1 minute and centrifuge once more at 1006.2 g for 5 minutes. The chloroform phase was extracted and transferred to the tubes containing the extracted sample. The chloroform was evaporated using N_2 gas and the samples were weighed.

Methylation of lipids

The method is based on Lars Åke Appelqvist's (1968) method, where one sample of each triplicate from the four cultures (C, D, S_1 and S_2) was prepared for GC analysis by methylating the samples. The samples were dissolved in 0.5 mL hexane (Merck KGaA, Germany) and then 2 mL of SeccoSolv® dried methanol were added, the samples were thereafter put in a heating block at 65 °C for 10 minutes. The tubes were vortexed briefly and 3 mL of Boron triflouride 20% in methanol complex (Merck KGaA, Germany) was added and put once more on the heating block for additional 10 minutes. Then the tubes were cooled under running water and had 2 mL of 200 g/L NaCl and 2 mL hexane added to the tubes. The tubes were vortexed vigorously, centrifuged at 1006.2 g for 3 minutes and the upper hexane layer was extracted using a Pasteur pipette to new glass tubes. A second extraction was performed by adding 1 mL hexane, vortex, centrifuging once more and extracting the upper layer. Evaporation of the samples was done using N_2 gas. When the samples were dried they were dissolved in 1 mL hexane and diluted to 100 µl with the lipid concentration 1.5 mg/mL in GC vials. The remaining sample was stored in the glass tubes at -20 °C.

Lipid analysis and composition by GC

The lipid samples were analysed in a gas chromatograph (Agilent Technologies) with a flame ionization detector and split injector, using a BPX 70 GC capillary column 100m X 0.22 mm with the internal diameter BPX70;0,25 µm (SGE international private LTD). The standard peaks (Standard 68A, Nu-Check Prep, Inc.) were identified manually and compared to the samples. Because of some of the standard peaks showed a different area% than supposed to, each peak was recalibrated by calculating the response factor. 98-99 of the area% was used, however when recalibrating by using the response factor the total lipid content was shown as values between 55-62%.

20 mL cultures

To first test which concentration of sesamin would be used for further scaling up as well as initial effect of sesamin were five duplicate cultures prepared. A glucose control, a DMSO control and three different sesamin concentrations were made in 100 ml baffled bottles. The cultures were checked microscopically and well as growth of each were monitored by measuring OD_{600}. Differences in OD_{600} were observed (Fig. 1), the OD_{600} for both glucose control (C) cultures were higher most of the time than the rest during the procedure. The DMSO control (D) and the sesamin (L/M/H) cultures had similar curves, indicating that DMSO at 2 vol. % may inhibit the growth of *R. glutinis*. However pipetting mistakes may have been done in the latter stages of the measurements.

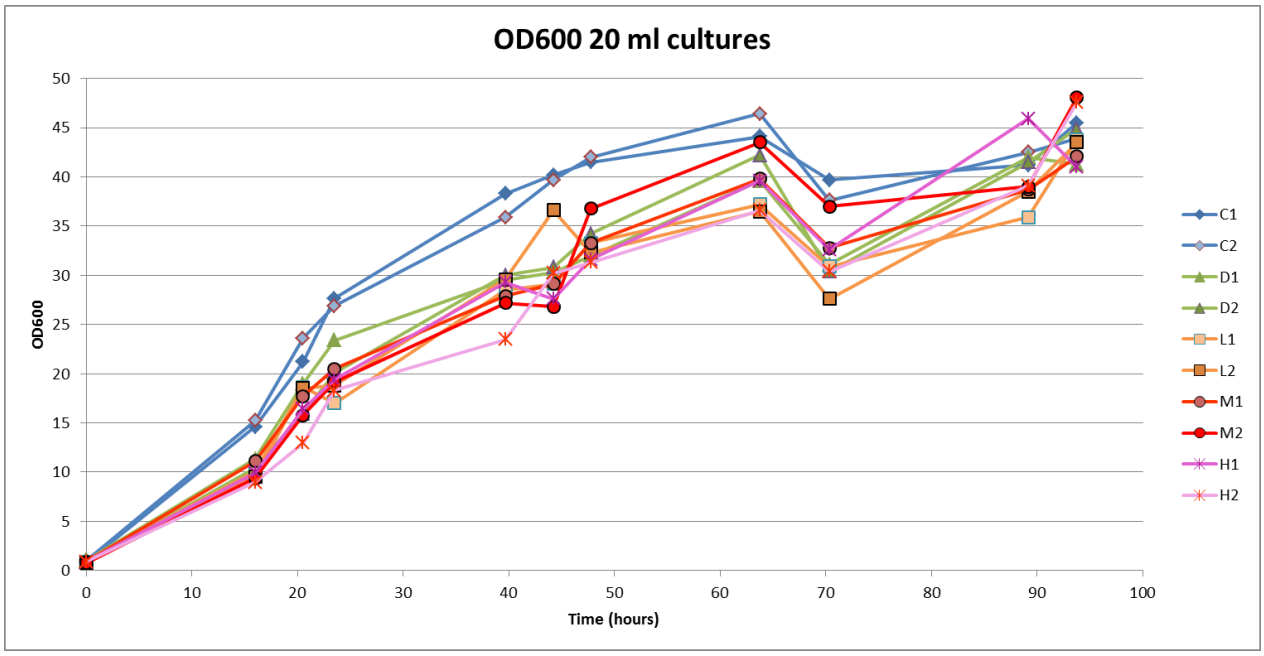

Figure 1 Growth of *R. glutinis* in the 20 ml cultures in the absence or presence of sesamin and the solvent DMSO. The figure shows the OD_{600} of the 20 mL cultures where C1/C2 are the glucose controls, D1/D2 the DMSO controls, L1/L2 the low sesamin concentration, M1/M2, the intermediate sesamin concentration and H1/H2 the high sesamin concentration.

100 mL cultures

The intermediate sesamin concentration (0.02 g/L sesamin) was chosen and scaled up with the two controls to 100 ml cultures in 500 ml baffled bottles. Glucose consumption and growth were measured by HPLC and OD_{600}. The OD_{600} measurements (Fig. 2) showed a somewhat different trend when scaling up. The D cultures still showed slower growth than the C controls; however the sesamin (S) cultures showed a higher density than the D control. Glucose consumption was lower in D than in the other samples which had somewhat similar values (Fig. 3).

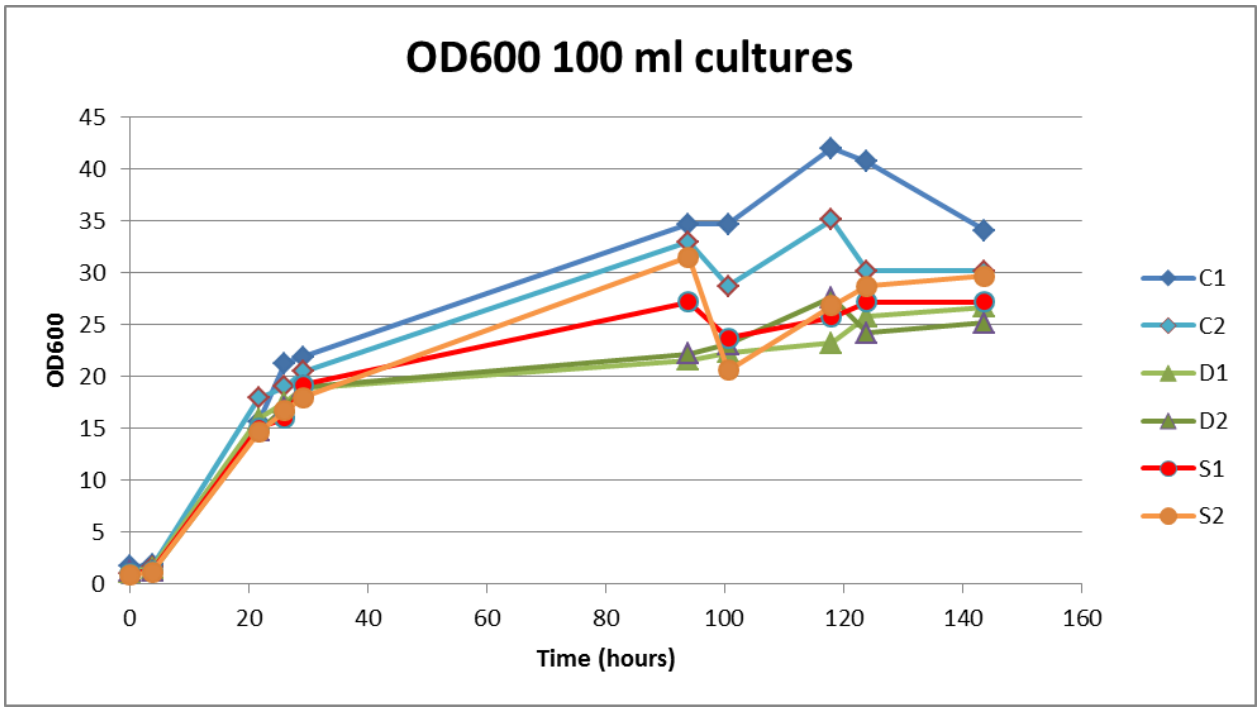

Figure 2 Growth of *R. glutinis* in the 100 ml cultures in absence or presence of sesamin and the solvent DMSO. Displaying the 100 mL cultures OD_{600} measurements where C1/C2 are the glucose controls, D1/D2 the DMSO controls and S1/S2 the sesamin cultures. The controls had marginally higher values compared to the sesamin samples, The DMSO cultures showed a lower final OD_{600} value compared to the sesamin cultures.

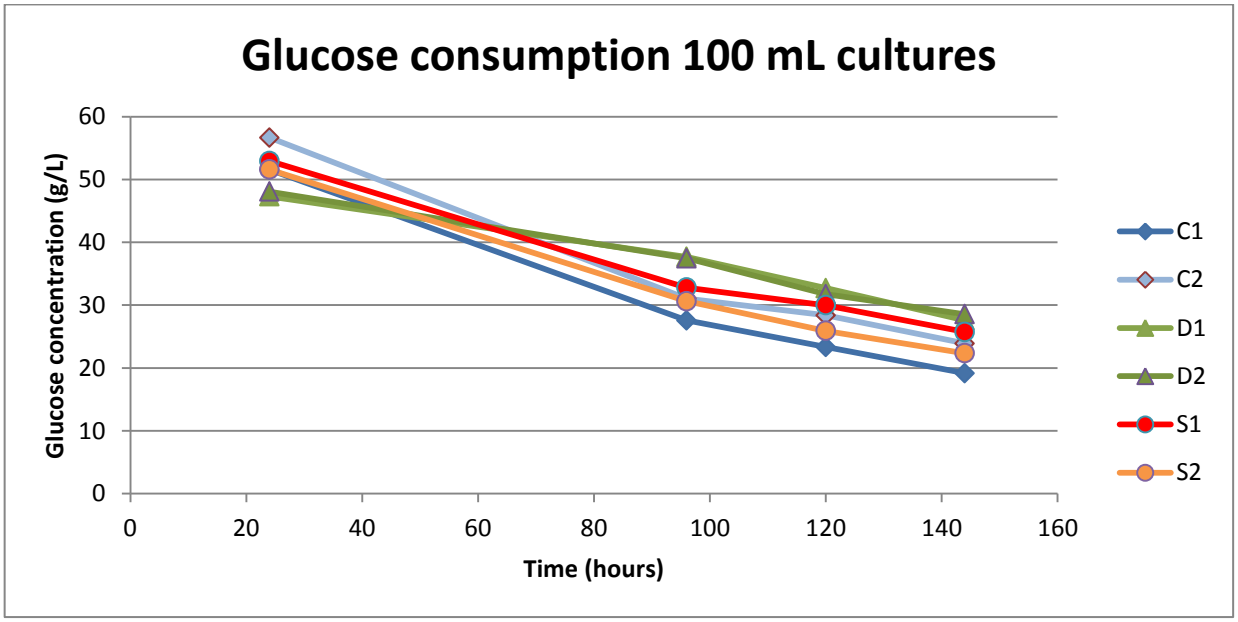

Figure 3 Glucose consumption of *R. glutinis* in the 100 ml cultures in the absence or presence of sesamin and the solvent DMSO. Showing the glucose consumption of the different cultures, C1/C2 are the glucose controls, D1/D2 are the DMSO controls and S1/S2 are the sesamin cultures. D1 and D2 showed lower consumption compared to the other cultures where there rate was more similar.

Fermenter cultures

The 100 ml experiment was scaled up to 300 ml in fermenters, with controlled air flow and pH for maximum growth. OD_{600}, glucose consumption and biomass accumulation were examined The values of the glucose control and the DMSO control in the OD_{600} (Fig. 4) had erroneous shown as lower values due to that in the latter cell transfers the cultures were highly viscous and because of this, cells were left in the pipette tip. The cells in this case were transferred first without mixing with NaCl solution into the Eppendorf tube. In contrast, in the sesamin cultures the cells were transferred to tubes containing NaCl solution and thoroughly mixed, ensuring that no cells were left in the tip. The glucose consumption (Fig. 5) was only slightly different between the glucose control compared to the DMSO control and sesamin culture, indicating further based on the previous cultures that the growth was somewhat affected by DMSO. In the biomass accumulation measurements (Fig. 6), the glucose treatment was once again slightly deviating from the other treatments after 24 hours, however the end result was nearly the same.

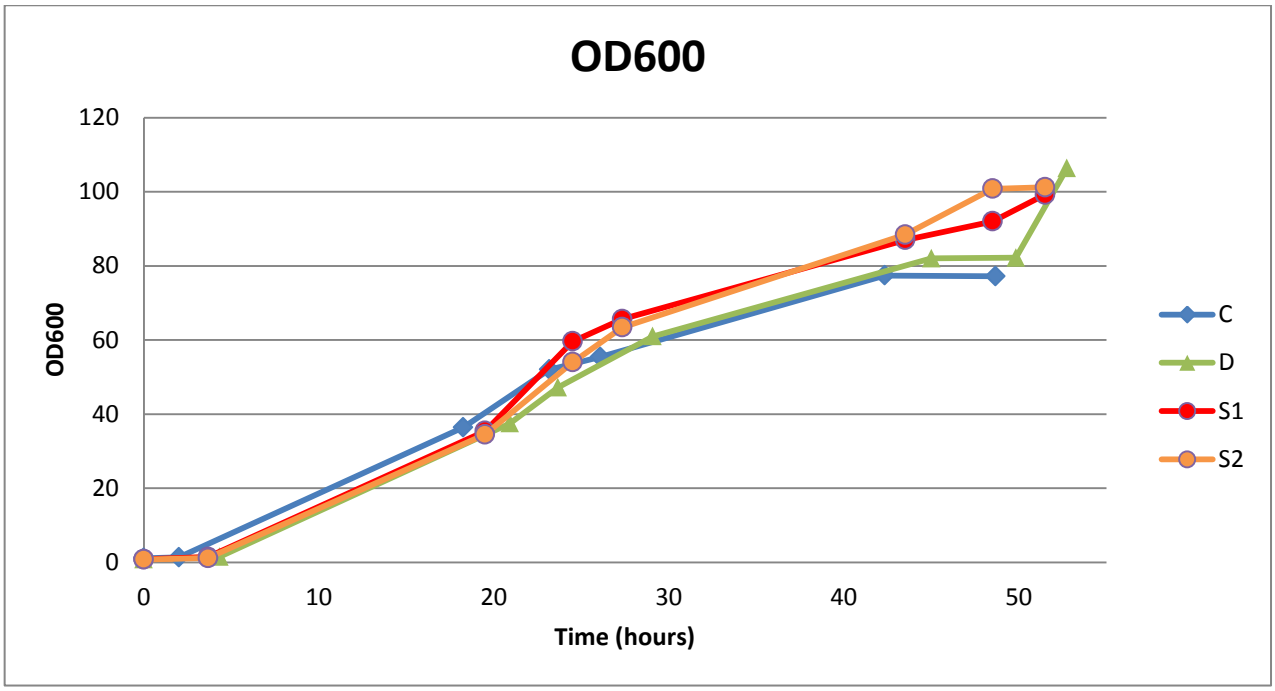

Figure 4 Growth of *R. glutinis* in the fermenter cultures in absence or presence of sesamin and the solvent DMSO. Portraying the OD_{600} measurements of the different cultures, C is the glucose control, D the DMSO control and S1/S2 the sesamin cultures. The measurements had erroneous due to that the sesamin cultures were diluted differently than the C and D culture, which can be seen in the last measurement of D, where the D culture was diluted the same way as the sesamin cultures.

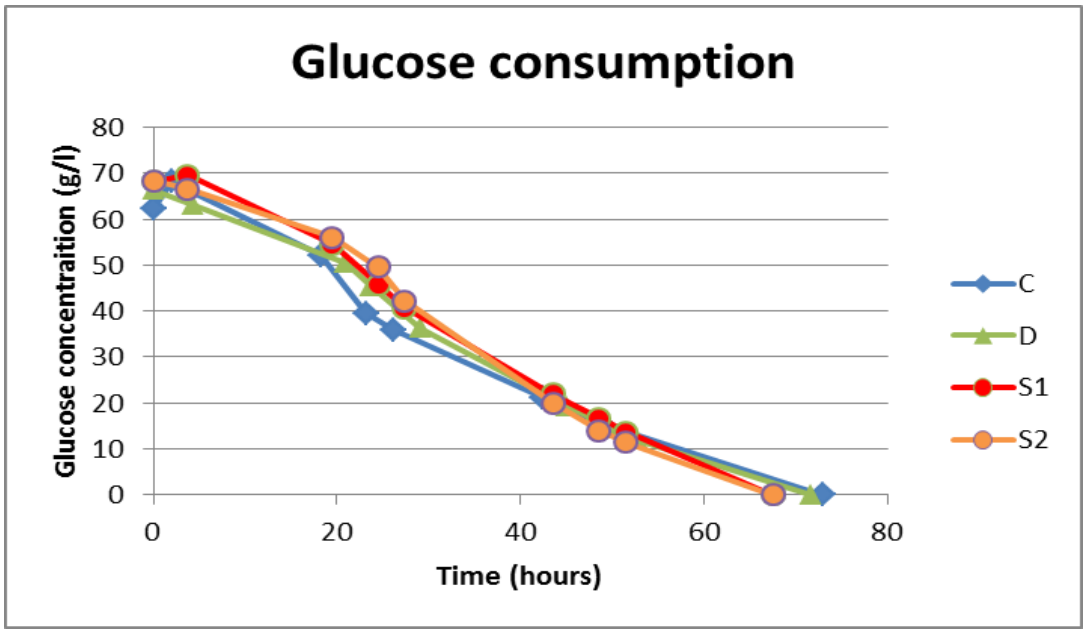

Figure 5 Glucose consumption of *R. glutinis* in the fermenter cultures in the absence or presence of sesamin and the solvent DMSO. Showing the glucose consumption from the fermenter cultures, C is the glucose control, D the DMSO control and S1/S2 the sesamin cultures. The consumption patterns were highly similar between all cultures; however the DMSO control and the sesamin showed slightly slower rates than the glucose control.

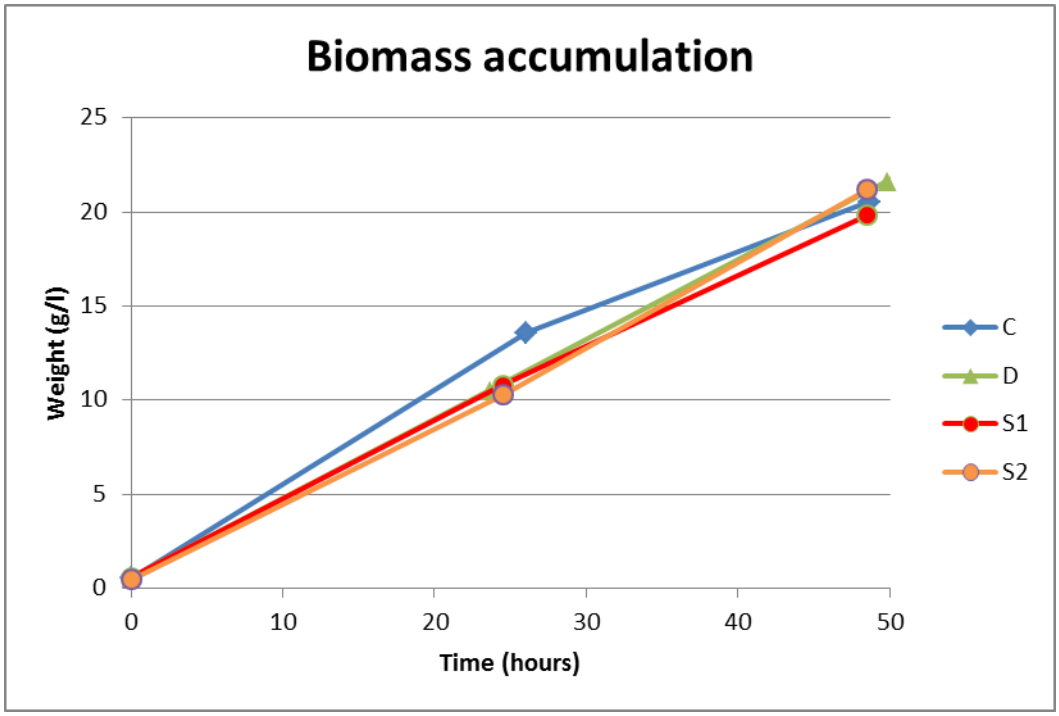

Figure 6 Biomass accumulation of *R. glutinis* in the fermenter cultures in the absence or presence of sesamin and the solvent DMSO. The figure displays the biomass accumulation in the different treatments. The glucose control showed a non-linear curve compared to the other treatments. The remaining cultures showed the same growth pattern.

Approximate starting weight of the glucose control was determined by using the staring values of the other cultures (since this will not differ greatly even if the media differ somewhat) and the latter weight values of the control. These were combined with the OD_{600} values to create a correlation between OD_{600} and weight, which resulted in an R-value of 0.9972. Thus could the trendline equation be used to calculate the approximate starting weight of the glucose control which should be around 0,515 g/L if the OD_{600} was correctly measured.

Lipid extraction

The modified method from Folch et al. (1957) without HCl was chosen since there was no difference in lipid yield per gram dried yeast where approximately 20% of the dry weight was lipids in both extraction methods when using a contaminated glucose control sample.

Lipid content

As seen there were differences in the lipid contents of the different cultures (Table 1), the mean of these, excluding the samples where some of the lipids were lost, were; C 17.35 %, D 48.86%, S1 23.21% and S2 25.66%. The yield of lipids was the double compared to the others in the D sample and the S samples had slightly higher lipid yield (≈6%) than the C culture.

Table 1 Lipid content (%) from each triplicate of the four fermenter cultures, an asterisk indicates sample loss during extraction.

Sample/ replicate	C (%)	D (%)	S1 (%)	S2 (%)
1	15.70	39.62*	24.10	25.56
2	13.07*	49.95	12.33*	24.78
3	19.00	47.76	22.31	26.65

Lipid composition

There were several changes that could be seen in the composition of fatty acids both after addition of DMSO as well as addition of sesamin (Table 2). Seemingly was 18:0, 18:2n-6, 18:3n-3, 20:3n-6 and 24:0 affected by the addition of DMSO and 14:0, 16:0, 16:1, 18:1, 18:3-n3, 20:0, 20:3n-6 and 22:6n-3 affected by sesamin. The proportions of the FA 18:0, 18:2n-6, 18:3n-3, 20:0 and 24:0 were lowered by DMSO, whereas 18:1 was increased by DMSO. The effect of sesamin in the FA 14:0, 16:0, 16:1 and 20:3n-6 was a reduction of the fatty acid content observed, whereas in 18:0, 18:3n-3, 20:0 and 22:6n-3 there was either a normalization compared to the glucose control levels or increase of the fatty acid in general. The overall lipid content seemed to be the same for the sesamin and the glucose control, while the DMSO control had somewhat lower percentage.

Table 2 Showing the fatty acid content of *R. glutinis* under influence of three treatments. All fatty acids included in the standard are shown in the left column, in the right top row is C is the glucose control, D the DMSO control and S1/S2 the sesamin cultures.

Fatty acid	C (%)	D (%)	S1 (%)	S2 (%)
14:0	0.232	0.304	0.035	0.007
14:1	0	0	0	0
16:0	11.137	11.601	8.810	8.021
16:1	0.255	0.177	0.024	0
18:0	12.117	5.005	9.632	12.611
18:1	15.778	24.640	20.765	19.449
18:2n-6	7.272	5.637	5.722	5.153
18:3n-3	11.044	5.885	11.541	15.703
20:0	0.167	0	0.148	0.168
20:1	2.494	1.294	2.086	1.428
20:2n-6	0	0	0	0
20:3n-6	0.300	0.079	0	0
20:4n-6	0	0	0	0
20:3n-3	0	0	0	0
22:0	0	0	0	0
22:1	0	0	0	0
24:0	2.107	0.938	0.926	0.396
24:1	0	0	0	0
22:6n-3	0.004	0.004	0.034	0.016
Total	62.907	55.560	59.723	62.951

This study is to the author's knowledge one of the first to investigate the effect of sesamin on the FA profile of *R. glutinis*. The results of addition of sesamin were consistent with previous results in other works in terms of overall lowered fatty acid content in both saturated and unsaturated FA (Arita et al., 2012; Tsuruoka et al., 2005). Among such was the decrease of 20:3n-6 in this experiment as well as in the filamentous fungi *Mortierella alpina* that has been previously reported by Jareonkitmongkol et al. (1993). The small increase in docosahexaenoic acid (22:6n-3), which is one of the fatty acids that is supposed to be beneficial for human health was also consistent with previous studies in Rainbow trout treated with sesamin (Trattner et al., 2008a; Trattner et al., 2008 b). If sesamin raises the production of 22:6n-3 in *R. glutinis*, this could mean that fish could be fed with yeast that contains long chain polyunsaturated fatty acids, reducing the amount of fish oil fed to fish grown in aquaculture. However in previous studies of *R. glutinis* where the yeast had gone through a treatment with low nitrogen high carbon media like in this study, the relative amount of polyunsaturated FA such as 18:3n-3 were lowered, even though the lipid content went up (Granger et al., 1992). This might suggest that if the nitrogen content is increased and thus the ratio is changed to a more even one, there will be an increase of polyunsaturated FA, but not as much lipids content instead.

To increase the lipid content and have a more preferential FA profile, different types of media should be investigated for maximum yield of polyunsaturated FA. In Easterling et al. (2009), *R. glutinis* were grown with glycerol, xylose and dextrose in different combinations. In their study they found that growth on glycerol increased the amount of C18:2 FA as well as C18:3FA compared to when using xylose or dextrose when grown at 35°C. If glycerol was used instead of glucose the lipid content might rise, since Easterling et al. (2009) found a lipid content of approximately 38% opposed to the approximate 20% that was achieved with glucose as the carbon source in this experiment without sesamin. In their results they also showed that the saturated FA represented 46% of the lipid content and the remaining mono- or polyunsaturated fatty acids, compared to the results in this study, which was approximately 40% saturated FA in the glucose control.

The results from this study are however hard to interpret due to many reasons, one of being that it was not possible in the frame of this work to redo the glucose control or the DMSO control due to time constraints and contaminations, thus only having singulars. Moreover the lipid samples were not completely clean, which could be seen as a raised baseline in the GC chromatogram. Another reason that the results can be merely seen as a guideline and not as concrete results is that the standard had most likely oxidised, giving off the wrong proportions as well as additional peaks that were not

identifiable. Therefore it is highly likely that 22:6n-3 and other fatty acids may have been wrongly identified. Also the applied GC column was not optimal for the method of the lipid analysis that was performed, an improvement (if the experiment should be redone), would be to use a 50 m long column instead of a 100 m column. Starting values for the glucose measurements in the 100 mL cultures and a start weight for the control culture in the fermenter experiment were not present in the study which they should have been.

The most pressing reason to redo this experiment is the difference in treatment of the lipid extraction, since no real conclusions can be made since at least the DMSO control was too divergent in its results, especially in the lipid content. An explanation as to why the lipid content was the double of the others may be because of the freezing-thawing-freezing and freeze-drying treatment the DMSO control was put through. The freezing itself causes damage in the cell wall by ice crystal formation as well as osmotic stress during slow freezing processes and thawing can cause oxidative stress in the remaining live cells modulating the fatty acids in the membrane (Rodríguez-Vargas et al., 2007). This can have caused a higher amount of disruption in the membrane resulting in a higher amount of lipids in the chloroform layer, since when tried with doing either freeze-thawing or freeze-drying in *S. cerevisiae* both freeze-thawing and freeze-drying liberates phospholipids from the membrane to the same degree (Souzu, 1973). Also if care is not taken enzymatic phospholipid degradation may occur in both kinds of treatments (Souzu, 1973). The control should also have been washed with water before freeze-drying, since false results in the lipid yield can occur without (Rattray et al., 1975).

Conclusions

Sesamin may affect the production of long chain polyunsaturated fatty acids in *R. glutinis*, however the experiments have to be redone to confirm this. If the results are correct however, further research should ensue to examine the optimal carbon source, solvent for sesamin in fluid cultures as well as the optimal sesamin concentration for maximum effect of 22:6n-3 production.

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