Substrates for the sustainable production of mannosylerythritol lipids: biological oils vs nanofiltrated lignocellulosic hydrolysates

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This work aims the sustainable production of biosurfactants, mannosylerythritol lipids (MEL), exploring the use of different carbon sources by non-conventional yeasts, *Pseudozyma* spp. Two main sources were assessed: 1) biological oils (poultry oil, waste frying oil, crude soybean oil, and crude rapeseed oil) and 2) lignocellulosic biomass (wheat straw). This thesis demonstrates the capacity of *Pseudozyma* spp. in producing MEL from biological oils and from sugars in the presence hemicellulosic hydrolysates, inhere evaluated for the first time. The highest MEL titres were achieved with oils as substrate after 14 days of bioconversion: refined soybean oil (18.3 gMEL/l), crude soybean oil (13.7 gMEL/l), poultry oil (12.9 gMEL/l), crude rapeseed oil (11.5 gMEL/l), and waste frying oils (8.3 gMEL/l). The best MEL titre using hemicellulosic hydrolysates supplemented with glucose (1.47 gMEL/l) was achieved, after detoxification by nanofiltration. The nanofiltration with a polyamide membrane (NF-PA2) removed 80% of 2-furfuraldehyde, 56% of formic acid, and 43% of acetic acid were removed with a low monosaccharides loss, (e.g. 17% of D-xylose). MEL recovery was achieved by, liquid-liquid extraction with ethyl acetate attaining nearly 80% purity and 82% yield.

*Keywords*: Mannosylerythritol lipids, Lignocellulosic biomass, Nanofiltration, Detoxification

1. Introduction

Nowadays, there is an increasing interest in biosurfactants [1,2], as in “clean” renewable (bio)energies and other bio-based chemicals, supporting a future-oriented bioeconomy policy. Biosurfactants are relatively nontoxic and biodegradable compared to synthetic ones. The mannosylerythritol lipids (MEL) are a family of biosurfactants which contain 4-O-D-mannopyranosyl-meso-erythritol as the hydrophilic group and usually two fatty acids and/or an acetyl groups as the hydrophobic moiety. Among their applications are its potential antitumor and differentiation-inducing activity against many cancer cell lines for stem cells research [1], its antiagglomeration properties on ice-water slurries [3], or it increases the effectiveness of secondary refrigerants [4].

*Pseudozyma* sp. has demonstrated its capacity to produce MEL from different substrates, for instance pentoses, glucose, vegetable oils and lignocellulosic materials [5–7]. MEL titres are higher when produced from oils compared to other
carbon sources, such as glucose [1,8–10]. However, this feedstock competes with substrates that are used for food and requires big land extensions for the crops [11]. Furthermore, its downstream processing is solvent-intensive [12,13]. Due to the inconvenient of using edible oils, the use of the alternative substrates for MEL production has been suggested. The use of agro-industrial wastes is one of the attractive strategies for sustainable biosurfactants production [14,15]. However, up till now the use of these materials has not been extensively assessed, in part because of the harsh biomass deconstruction that has the purpose to release fermentable sugars from cellulose an/or hemicellulose. Biomass pretreatment produces several by-products potentially inhibitory for microorganisms: furfural, acids (acetic, formic) and phenolic compounds [6,14,16]. Hence, a detoxification step is often suggested [17]. Many detoxification processes have been described in the literature [17]. Nonetheless, due to the complex mixture of compounds, the combination of different detoxification methods might be required to achieve an acceptable reduction of inhibitors in the hemicellulosic hydrolysates.

In the present study, is suggested the use of nanofiltration (NF) membranes as an alternative upstream procedure to reject fermentable sugars with the permeation of yeast inhibitors. This detoxification procedure aims to replace multistep detoxification processes before the bioconversion step.

Secondly, two sets of substrates were assessed towards sustainable MEL production: 1) biological oils (poultry oil, waste frying oil, crude soybean oil, and crude rapeseed oil) and 2) lignocellulosic biomass (wheat straw).

Finally, this work aimed to compare two different downstream processes described for MEL recovery from glucose or soybean oil (SBO) broth. The first one was based on one-stage liquid-liquid extraction with ethyl acetate, as described by Morita, et. al [18]. The second procedure is based on two-stage liquid-liquid extraction, as described by Rau, et. al [13].

2. Experimental

2.1. Diafiltration modelling and detoxification

Diavolumes are defined as:

\[ D = \frac{V_W}{V_F} = \frac{V_P}{V_F} \]  

(1)

Where \( V_W \) is the water fed into the system. Assuming a system operating at constant volume (45 ml) and without accumulation \( (V_0 = V_t) \), thus \( V_W = V_P \).

The concentrations on the permeate, the carbon substrates losses, and inhibitory compounds removal were calculated according to:

\[ C_{P,x} = C_{R,x}(1 - R_x) \]  

(2)

\[ Substrate \ loss = 1 - \frac{C_{R,x}}{C_{F,x}} \quad V_R = V_F \]  

(3)

\[ Toxic \ removal = \frac{C_{R,y}}{C_{F,y}} \quad V_R = V_F \]  

(4)

For the organic Brønsted acids (AH) used in the model solutions, the dissociation degree \( (\alpha) \) of their conjugate base \( (A^-) \) was taken into account according to the acid-base equilibria that is reached when these weak acids are dissolved in water at a given pH. This effect was considered since different rejections can be observed at the molecular level for the organic acid and the respective conjugate base. \( C_{F,x,HA} \) represents a given organic solute considering both forms, the
acidic and the respective conjugate base. Furthermore, it was assumed that the pH (pH = 2) and temperature (25 °C) will not change through time.

2.2. Yeast strain and growth medium

*Pseudozyma antarctica* PYCC 5048\(^T\) was obtained from the Portuguese Yeast Culture Collection (PYCC), CREM, FCT/UNL, Portugal. Inoculum was prepared by incubation of stock cultures at 28 °C, 250 rpm, for 48 h, in liquid medium containing D-glucose (40 g/l), NaNO\(_3\) (3 g/l), MgSO\(_4\)-7H\(_2\)O (0.3 g/l), KH\(_2\)PO\(_4\) (0.3 g/l) and yeast extract (1 g/l) [5,7].

All shaking flasks were inoculated with 10% v/v of inoculum culture and incubated at 28 °C, 250 rpm during all bioconversion time. All media were autoclaved at 121 °C and 1 bar for 20 min. Furthermore, the ratio between medium volume and flask/vessel volume was 1/5.

2.2.1. Glucose based medium (control)

The cultivation medium containing D-glucose as carbon source (40 or 72 g/l glucose) was supplemented with NaNO\(_3\) (3 g/l), MgSO\(_4\)-7H\(_2\)O (0.3 g/l), KH\(_2\)PO\(_4\) (0.3 g/l) and yeast extract (1 g/l) (control 1) [5,7].

When assessing the effect of sulphate/sulphuric acid, media was supplemented with 2.4 – 2.5 g/l of sulphates with the purpose to assess the effects of these ions on yeast’s growth (control 2).

2.2.2. Biological oils based media

One formula was prepared with an oil concentration of 80 mL/L\(_{MEDIUM}\) plus supplements: NaNO\(_3\) (2 g/l), MgSO\(_4\) (0.2 g/l), KH\(_2\)PO\(_4\) (0.2 g/l), and yeast extract (1 g/l) [13,19].

The oils assessed were crude rapeseed oil, crude SBO, waste frying oils (WFO) and poultry oil. Crude oils were donated by Sociedade Ibérica de Biocombustíveis e Oleaginosas, S.A. (IBEROL, Portugal); WFO were obtained from a chain of fast food restaurants in Lisbon, Portugal; poultry oil was obtained from AVIBOM AVÍCOLA, S.A. (Portugal). The characteristics of these raw materials were obtained from certificates of analysis (CoA) issued by LNEG (Portugal).

2.2.3. Growth media containing organic acids and furan compounds

Shaking flasks containing acetic acid (5 g/l), formic acid (5.4 g/l) and 2-furfuraldehyde (0.6 g/l) supplemented with glucose (40 g/l), MgSO\(_4\)-7H\(_2\)O (0.3 g/l), KH\(_2\)PO\(_4\) (0.3 g/l), and yeast extract (1 g/l) were assessed.

2.2.4. Growth media containing wheat straw hydrolysates

Both detoxified hemicellulosic hydrolysates from NF membranes (80 % v/v) and non-detoxified hemicellulosic hydrolysate were supplemented with D-glucose (40 g/l), KH\(_2\)PO\(_4\) (0.3 g/l), and yeast extract (1 g/l).

2.3. Environmental assessment

The experimental data obtained from the production of MEL from non-mineral oils were submitted to a consulting company to perform a short environmental assessment. This analysis uses a single metric that converges different factors: Human health, use of resources, and the effect on ecosystems. The databases used were ECOINVENT (Switzerland) and Agri-footprint gouda © (The Netherlands).

2.4. Nanofiltration membranes

A commercial polyamide NF membrane was used for diafiltration and for setting up the rejection profile under the conditions set up above.

2.5. MEL recovery

Two different MEL recovery procedures were carried out: one based on one-step liquid-liquid extraction with ethyl acetate, as described by Morita, et. al [18]; other based on two-step liquid-liquid extraction, as described by Rau, et. al [13]. Extractions were performed in separator funnels under manual agitation.
2.6. Analytical methods

2.6.1. Yeast growth and viability

Cell growth was followed by quantification of cell dry weight (CDW). CDW was determined with 1 ml of culture broth.

2.6.2. Sugar and oligosaccharides concentration

Collected supernatants or samples from retentates and permeates were analysed for glucose, xylose, formic acid, acetic acid, and furfural quantification in a high performance liquid chromatography (HPLC) system (Merck Hitachi, Darmstadt, Germany) equipped with a refractive index detector (L-2490, Merck Hitachi, Darmstadt, Germany) and an Rezex ROA-organic acid H+ column (300 mm ~ 7.8 mm, Phenomenex), at 40°C. Sulphuric acid (0.005 M) was used as mobile phase at 0.5 ml/min. For Xylooligosaccharides (XOS) quantification the same HPLC system was used coupled to a Rezex RSO-Oligosaccharide Ag⁺ 4% column (200 mm ~ 10.0 mm, Phenomenex), at 80°C. Water was used as mobile phase at 0.15 ml/min.

2.6.3. MEL and fatty acid profile

The total fatty-acid pattern of biological samples was determined by methanolysis of freeze-dried culture broth as reported before [5,7,20]. The resulting reaction was extracted with hexane (1 ml) and deionized water (1 ml). 1 μl of the organic phase was analysed by gas chromatography (GC) to determined fatty acid concentrations against C7:0 internal control/standard [20]. MELs were quantified through the amount of C8, C10 and C12 fatty acids [7].

3. Results and discussion

3.1. MEL production from biological oils

Figure 1 revealed that biomass yield from glucose is lower than that obtained from oils. The highest biomass values were achieved with crude SBO (29.5 ± 7.5 g/l) and poultry oil (27.0 ± 2.0 g/l). Table 1 show that the MEL titres achieved from glucose (3.79 ± 0.42) are much lower than those obtained from biological oils. This value is similar to others obtained previously by other authors using glucose as carbon source. Morita et al. achieved 3.5 g MEL/l with *P. antarctica* T-34 and under absence of nitrate [18].

![Figure 1](image)

Plot presenting data coming from cell dry weight on different growth media. Carbon substrates: Glucose (dark dashed blue line), orange dashed line), crude rapeseed oil (pale blue dash line), poultry oil (purple dashed line), and crude SBO (green dashed line) (Mean ± SD, n = 2).

The differences observed on biomass production from different oils can be due to autoxidation of polyunsaturated lipids of oils, as are not refined oils. Autoxidation involves free radical chain reactions that are promoted when oils are exposed to light, heat, ionizing radiation, metal ions, or metalloprotein catalysis [21]. In consequence, it may result in oxidative stress due to the production of reactive oxygen species (ROS). This may explain why the MEL titres and biomass are lower for WFO compared to crude oils.

In the case of poultry oil, probably it achieved similar values of MEL titres as crude SBO because it comes from category 3 animal source [22], it may contain other nutrients that weren’t detected in the characterization analysis of oils. It might have minerals or proteins that could be used by the cells. Moreover, it has the higher percentage of saturated fats (27.3 %) and lower iodine value (92...
glucose/100g) compared to SBO (15.7%; 92 glucose/100g). Hence, its oxidation is less probable comparing to the other oils.

3.2. Environmental assessment

Figure 2 illustrates that the oils with lower total impact are the WFO (303.9 mPt). This is because this type of oil is a waste from food industry and domestic activities. The other three oils, are seven or eight times greater on environmental impact than WFO. Further in Figure 2, the effect on the ecosystems account between 62 and 78% of total environmental impact. This impact on ecosystems is mainly due to the extensive land use for the crops.

3.3. Diafiltration and detoxification

One operational problem to face was the high content of solids on hemicellulosic hydrolysates. Suspended or dissolved solids represent an operational problem because they may cause membrane fouling [23]. It might increase the osmotic pressure and lead to the reduction of effective trans membrane pressure and permeate flux [23].

Figure 3 illustrates the performance of the diafiltration. Also it shows that the model that was proposed did not fit with the experimental values. This permeation differences between the model and experimental results could be due to interactions between membrane-solutes that may interfere with solutes rejections [24]. In addition to that, hydrolysate’s composition is more complex than model solutions. Nonetheless, fouling is another factor that may affect solutes’ rejection and operation times [24].

NF-PA2 membrane removed 80.14 ± 4.84 % of 2-furfuraldehyde, 56.36 ± 6.75 % of formic acid, and 42.88 ± 4.06 % of acetic acid with up to 17.23 ± 2.29 % of D-xylose losses.

3.4. Bioconversion of wheat straw hemicellulose hydrolysate for MEL production

The retentate obtained from NF-PA2 could promote the growth of P. antarctica and MEL production in 14 days (Table 2). During this experiment, P. antarctica could achieve higher values of biomass at day 14th in the detoxified hydrolysate (9.5 ± 0.5 g/l) than the control 1 (8.0 ± 0.0 g/l). Moreover, the medium containing 80 % v/v of non-detoxified hemicellulosic hydrolysate was not able to support P. antarctica growth.
Table 1
Data obtained of at day 10 of bioconversion of the different biological oils that were assessed. It includes nitrate consumption, substrate consumption, MEL titres, volumetric productivity, and yield (27 °C, 250 rpm) (Mean ± SD, n = 2).

<table>
<thead>
<tr>
<th></th>
<th>Nitrate consumption (NO₃⁻/NO₂⁻)</th>
<th>Substrate consumption (Cᵢ/C₁)</th>
<th>MEL titre (g/l)</th>
<th>Productivity (g/l·day⁻¹)</th>
<th>Y (P/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (72 g/l)</td>
<td>47.8%</td>
<td>100%</td>
<td>3.79 ± 0.42</td>
<td>0.38 ± 0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Crude SBO (72 g/l)</td>
<td>100%</td>
<td>92.7%</td>
<td>13.66 ± 1.58</td>
<td>1.37 ± 0.16</td>
<td>0.13</td>
</tr>
<tr>
<td>Poultry oil (72 g/l)</td>
<td>100%</td>
<td>91.9%</td>
<td>12.89 ± 1.13</td>
<td>1.29 ± 0.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Crude Rapeseed oil (72 g/l)</td>
<td>100%</td>
<td>94.7%</td>
<td>11.46 ± 2.18</td>
<td>1.15 ± 0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>WFO (72 g/l)</td>
<td>100%</td>
<td>99.3%</td>
<td>8.26 ± 0.85</td>
<td>0.83 ± 0.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 2
Substrate consumption, MEL titre, volumetric productivity, and yield obtained from the bioconversion using wheat straw hemicellulosic hydrolysate from NF-PA2 membrane at 14th day (27 °C, 250 rpm) (Mean ± SD, n = 2).

<table>
<thead>
<tr>
<th>Substrate consumption (Cᵢ/C₁)</th>
<th>MELtitre (g/l)</th>
<th>Productivity (g/l·day⁻¹)</th>
<th>Y (P/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 g/l Glucose (Control 1)</td>
<td>100 %</td>
<td>2.84 ± 0.05</td>
<td>0.20 ± 0.0</td>
</tr>
<tr>
<td>40 g/l Glucose + Sulphuric acid (Control 2)</td>
<td>100 %</td>
<td>1.24 ± 0.21</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>80 % non-detoxified hydrolysate</td>
<td>0 %</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>80 % Detoxified hydrolysate</td>
<td>97 %</td>
<td>1.47 ± 0.09</td>
<td>0.10 ± 0.01</td>
</tr>
</tbody>
</table>

Figure 3
Plot representing the theoretical and experimental permeation of sugars and toxic compounds on NF-PA2 membrane. Continuous lines represent theoretical data. Dashed lines represents experimental data including error bars. Horizontal red dashed lines denotes either 20 % of permeation of substrates or 90 % of permeation of toxic compounds (Mean ± SD, n = 2).
This demonstrates that this detoxification procedure has been successful towards MEL production.

Figure 4 and Figure 5 illustrates the whole process from upstream to bioconversion. There can be appreciated the changes in the parameters measured for this trial. Something that clearly stands out is that there is an increase of xylose concentration as glucose is depleted by the yeast at day 7.

This hydrolytic capacity of \textit{P. antarctica} (not measured in this study) can be followed by the appearance/disappearance of xylo-oligosaccharides (XOS) – penta-, tetra-, tri- and di– and monosaccharides during the bioconversion step [25]. In Figure 4 the concentration of total XOS decreases along the timeframe of the bioconversion step. In fact, when xylose concentration rises, the concentration of total XOS decreases. This observation confirms that this yeast has xylanolytic capabilities, as previously described by Faria, et al. [6]. However, further enzymatic analyses are needed to quantify the hydrolytic activity from \textit{P. antarctica} PYCC 5048. Moreover in Figure 4 can be noticed that xylose concentration drops by 10\textsuperscript{th} day. This indicates the ability of the yeast to metabolize xylose and probably because of that the yeast was able to increase biomass. The capability of \textit{P. antarctica} PYCC 5048\textsuperscript{T} to use pentoses as carbon source has been reported before by Faria, et al in batch mode [5]. These authors were able to achieve 4.9 \(\pm\) 1.9 gMEL/l (20 g/l glucose + 20 g/l xylose) and 4.8 \(\pm\) 1.0 gMEL/l (40 g/l xylose) with biomass concentrations from 9.1 – 10.6 g/l. These titres reached by Faria, et. al in batch mode are nearly twice as higher as those gotten in this study [5].

Nonetheless, it is important to point out that in this study a more complex mixture was added and these authors worked with defined medium.

The results might suggest that the yeast use glucose to grow and to produce the hydrolytic enzymes needed to breakdown other substrates.

3.5. Downstream processing

Data in Table 3, shows that the method that requires more solvent is the two-stage liquid-liquid extraction (3.10 \(\pm\) 0.52 l/gMEL).

Although it requires more than three times of solvent comparing to liquid-liquid – extractions with ethyl acetate, the former is able to isolate MEL with higher purity (75.95 \(\pm\) 7.97\%) than the latter (5.79 \(\pm\) 0.13 \%). Nonetheless, Rau, et. al, reached 100 \% w/w of purity with the two-stage process, but this study was able to improve the yield of these authors (Y = 0.08) [13]

In fact, the latter procedure pulls a high amount of oils in the purified product (43.27 \(\pm\) 2.66 \%).

Furthermore, the lower yield in the latter (0.39 \(\pm\) 0.11) indicates that losses of product are even greater than in the former (0.51 \(\pm\) 0.13).

Liquid-liquid extraction with ethyl acetate is more effective for MEL recovery from glucose broth than from SBO broth, reaching nearly 13 times more purity. Moreover, the amount of fatty acids extracted from glucose broth was considerably lower than from SBO medium. Regarding solvent intensity, there are no significant differences since the same procedure was done for both media.

Although MEL recovery from lignocellulosic hydrolysate broth was not performed, the process is expected to be similar to that from glucose broth.
Figure 4
Detoxification and bioconversion of wheat straw hemicellulose hydrolysate (80% v/v) supplemented with 40 g/l of glucose. Plot summarizes the changes of glucose (purple dashed line), xylose (red dashed line), formic acid (green dashed line), acetic acid (pale blue dashed line), furfural (dark blue line), XOS (indigo continuous line), and biomass (orange line) in the detoxified wheat straw hydrolysate. It begins with the upstream processing. This includes its reception (crude) and detoxification through NF membrane. Day 0 of bioconversion shows the values after supplementation with glucose (40 g/l) and autoclaving (Mean ± SD, n = 2).

Figure 5
Bioconversion of wheat straw hemicellulose hydrolysate (80% v/v) supplemented with 40 g/l of glucose. Plot summarizes the changes of glucose (purple dashed line), xylose (red dashed line), formic acid (green dashed line), acetic acid (pale blue dashed line), XOS (indigo continuous line), and 2-furfuraldehyde (dark blue line) in the non-detoxified wheat straw hydrolysate. It begins with the reception (crude). Day 0 of bioconversion shows the values after supplementation with glucose (40 g/l) and autoclaving (Mean ± SD, n = 2).
Therefore, L/L – extractions with ethyl acetate seems to be the most suitable extraction procedure to isolate MEL from hydrophilic broths. **4. Conclusions**

This work compares for the first time two main carbon sources to produce MEL production: biological oils and lignocellulosic substrates. The advantages of using oils is that they are renewable sources and achieve high MEL titres comparing to lignocellulose hydrolysates. Nonetheless, their downstream processing becomes compelling since the target product has similar miscible properties of oils and yields tend to be low, which in turn increases operational costs. Furthermore, their environmental impact seems to be high because of the extensive use of land that is needed for the crops. In addition to that, it is a product targeted to human consumption and it is a highly volatile commodity in the international markets.

Also, this is the first time that MEL is produced from hemicellulose hydrolysates. In the literature, only the cellulose-rich (solid) fraction of pre-treated wheat straw was used for MEL production [7]. The supplementation with glucose can now be replaced by glucose obtained from the cellulose through enzymatic hydrolysis, by combining the solid fraction with the detoxified liquid fraction.

In the other side, lignocellulose is an hydrophilic substrate where the isolation of MEL using L/L extraction becomes easier and less solvent intensive. In addition to that, a material that is considered an agricultural waste can be reused to obtain a product with applications in many areas. Also wheat straw is a renewable material that doesn’t need to use more land area to obtain this raw material. Nevertheless, the operational costs of NF membranes and lower MEL titres represents the major drawback for the use of this carbon source.

Finally, weighing the pros and cons of each carbon substrate, it seems that the best carbon source are the lignocellulosic substrates.

The positive aspects worth the efforts done to improve the technologies to exploit them and to reduce their operation costs. In the long term, it would turn sustainable the production of biosurfactants. Furthermore, they certainly will contribute to reduce the impact of the human activities and consumption of non-renewable resources.

**5. References**


[2] D. Kitamoto, H. Isoda, T. Nakahara, Functions and potential applications of glycolipid biosurfactants – from energy-saving materials to...

### Table 3

MEL recovery using two different procedures on two different carbon sources (oils and sugars) used in the medium. The reported parameters are purity/concentration, yield of operation (Y) and solvent intensity (litres of solvent per gram of produced MEL). (Mean ± SD, n = 2 (SBO); n = 3 (glucose)).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Purity (%)</th>
<th>Y (MELg/MELf)</th>
<th>Solvent intensity (l solvent/gMEL)</th>
<th>Purity (%)</th>
<th>Y (MELg/MELf)</th>
<th>Solvent intensity (l solvent/gMEL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72 g/l</td>
<td>MEL</td>
<td>5.79 ± 0.13</td>
<td>0.39 ± 0.11</td>
<td>75.95 ± 7.97</td>
<td>0.51 ± 0.13</td>
<td>1.16 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Fatty acids</td>
<td>43.27 ± 2.66</td>
<td>---</td>
<td>0.0 ± 0.0</td>
<td>---</td>
<td>3.10 ± 0.52</td>
</tr>
<tr>
<td>40 g/l</td>
<td>MEL</td>
<td>79.66 ± 11.31</td>
<td>0.82 ± 0.12</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Fatty acids</td>
<td>1.32 ± 0.60</td>
<td>---</td>
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</table>


