Yeast Lipids – A Food of the Future?

An evaluation of the functionality of *Rhodotorula toruloides* lipids in a model food product

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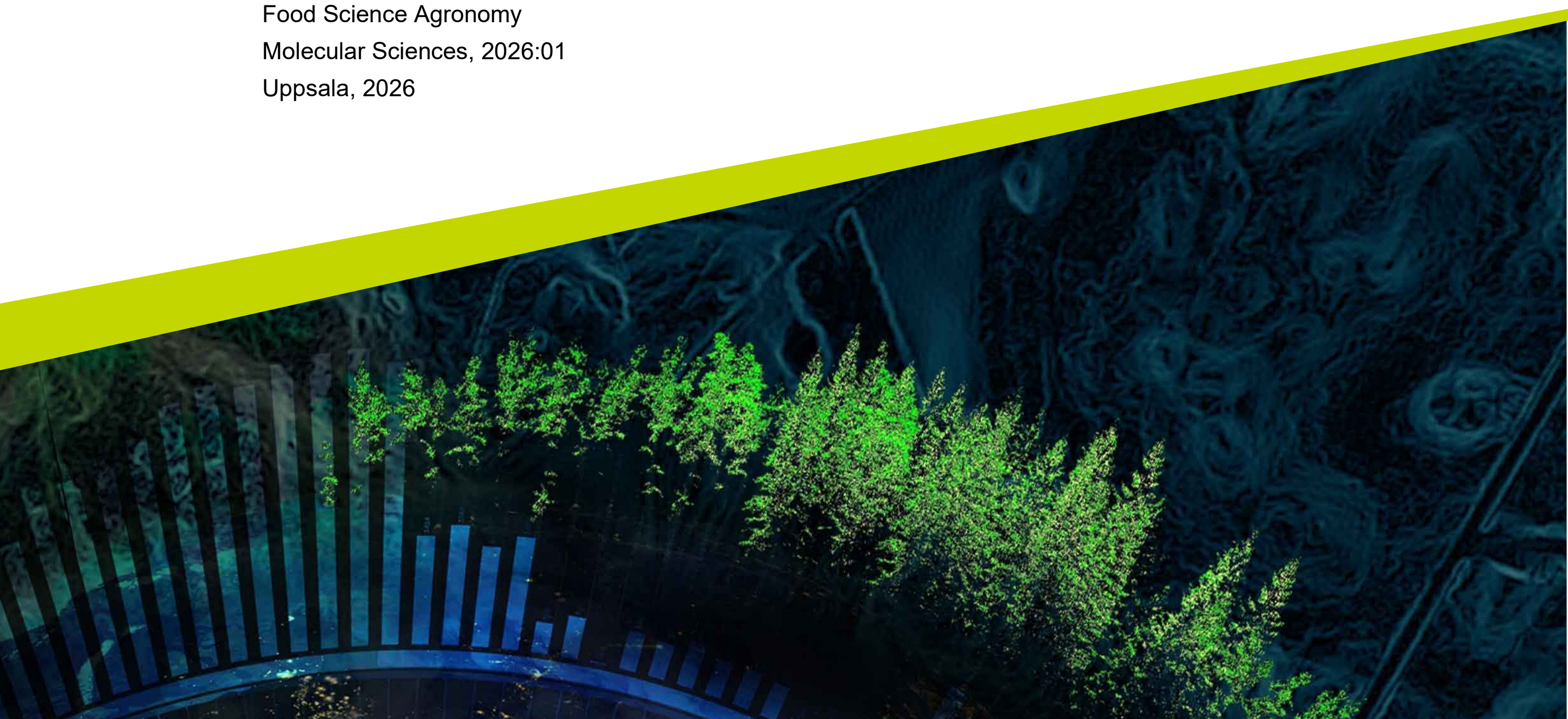

Yeast Lipids – A Food of the Future? An evaluation of the functionality of Rhodotorula toruloides lipids in a model food product

Jästlipider – En framtidsmat? Utvärdering av funktionaliteten av Rhodotorula toruloides lipider i ett modellivsmedel

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Abstract

This study explores the incorporation of yeast lipids into complex food products, using a pea protein emulsion gel as a model system. Some yeast strains can produce lipids up to 80% of their weight, and these lipids could have the potential to replace use of vegetable oils, as a less resource-intensive alternative. *Rhodotorula toruloides* CBS14 is one such yeast strain, that also produces brightly coloured antioxidants, carotenoids. The potential for the use of these in food was explored through texture, stability and colour measurements in this study.

The yeast oil was extracted from the dried biomass using hexane extraction and emulsified into a protein slurry, and then heated to form a gel. Rapeseed oil was used as a control. Texture Profile Analysis was used for textural measurements of the gels where hardness, springiness and cohesiveness were used as the textural parameters. Colour was measured using a Konica Minolta CR-400 , and lipid oxidation was used as a shelf life indicator and measured through TBARS. Gel syneresis was measured after 7 days through weight difference due to driploss.

The yeast oil samples created a thick, spreadable emulsion with a bright orange-pink colour before gelation, compared to the rapeseed oil gel that was thinner and pourable, with a pale beige colour. After gelation the yeast oil gels displayed more air bubbles than the rapeseed oil gels, indicating more incorporated air in emulsification, or less dispelled air during subsequent centrifuging. The yeast oil gels were significantly harder than the rapeseed oil control, and cohesiveness was slightly lower, however the difference was not statistically significant. The colours of the gels are clearly different from each other with a ΔE of 26,9, but no significant change occurred in the samples from day 2 to day 7 ($\Delta E < 2{,}6$). The yeast oil gels were more red, yellow and darker than the rapeseed oil samples as expected. Lipid oxidation did not differ significantly in any of the samples after 7 days. The yeast oil gels displayed significantly less syneresis (1,1-1,8%) than the rapeseed oil gels (11,3-11,5%) after 7 days.

The results in this study indicate that yeast oil from *R. toruloides* has potential as a food ingredient. It seems to have potential in replacing some colourants, stabilisers and emulsifiers in emulsified protein gel products. Repeat measurements over longer time is needed for any conclusions as to antioxidant effect on lipid oxidation in the yeast oil.

Keywords: Rhodotorula toruloides, microbial oil, yeast lipids, novel food, emulsion gel, alternative fats, pea protein gel

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Abbreviations

Abbreviation	Description
BHT	butylated hydroxy toluene
CIE	International Commission on Illumination (Commission internationale de l'éclairage)
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EFSA	European Food Safety Authority
GRAS	Generally Recognised As Safe
MDA	Malondialdehyde
QPS	Qualified Presumption of Safety
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
SCO	Single Cell Oil
SD	Standard Deviation
TBA	thiobarbituric acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	trichloric acid
TEP	tetraetoxypropane
TPA	Texture Profile Analysis
UFA	Unsaturated fatty acid
VO	Vegetable oil

1. Introduction

1.1 Yeast oil in food

Vegetable oils (VO) are among the fastest growing food commodities in the world, as well as being used for production of various chemicals and the main feedstock for biodiesel (Fridrihsone et al. 2020; OECD and Food and Agriculture Organization of the United Nations 2016). However, oil crops require relatively high amounts of arable land for the amount of energy that they produce, making them a sustainability challenge (Beyer and Rademacher 2021).

When produced for food application, crops like rapeseed are pressed to produce 30–50% w/w rapeseed oil. While the remaining rapeseed meal can be used as feed, not all of it is. Finding alternative sources for oil production can therefore decrease global waste (Skoulou et al. 2011).

One promising alternative source of oils is to use microbial fermentations to produce oils and fats. The biotechnological utilisation of the lipid metabolism has recently been gaining attention, and is a field undergoing rapid development. These lipids are often called microbial oils, and are produced by oleaginous fungi. Some oleaginous fungi are able to accumulate lipids of 20 to more than 80% of their total biomass (Passoth & Müller 2025). Oil from oleaginous yeasts especially may represent an alternative to VOs, as most of these yeasts can utilise a variety of substrates to produce oils, like underutilised side streams and second generation substrates. These include crude glycerol from biodiesel production and lignocellulose hydrolysate, as well as volatile fatty acids (Passoth & Müller 2025).

Since these yeast oils are quite similar to vegetable oils in their composition they could both be used as an alternative to oil crops in production of biodiesel, but also in other applications. Oleaginous yeasts were used in Germany during both world wars, where they were allowed to grow on residues from the paper industry to produce fat. This fat was used for baking or as a replacement for butter. The processes were stopped after the wars ended and vegetable oils could be imported again, due to the high production costs (Abeln and Chuck 2021; Lundin 1950). Oils from *L. starkeyi* have been successfully tested in fish feed given to arctic char (*Salvelinus alpinus)*, where no significant difference was found in flesh taste and quality, nor in fish performance (Blomqvist et al. 2018). This shows that yeast oils can be used in food and feed applications. Further confirming that yeast oils have a similar functionality could therefore free up arable land for alternative crop productions, as well as make use of residues from other industries to produce food.

1.2 Rhodotorula toruloides

Rhodotorula toruloides is a non-conventional basidiomycetes oleaginous yeast of the family Sporidiobolaceae. *R. toruloides* can use various low-value substrates, such as lignocellulose hydrolysate and wheat straw hydrolysate to grow. From these it can produce several different products like intracellular single cell oil (SCO) as well as carotenoids and D-arabitol. SCO is the main product with potential for application in food, feed and biofuel production. The carotenoids give the yeasts their characteristic red colour (Zhao et al. 2022). In a study performed by Nagaraj et al. (2025) the four carotenoids found to be produced were mainly torulene and torularhodin which have a high antioxidant effect, as well as some β-carotene and γ-carotene. The yeast obtained total carotenoid levels of 332 ± 27.32 µg/g dry weight, which is comparable to raw carrots, a known carotenoid-rich food (average of 288,3 µg/g dry weight, n=380, Jourdan et al. 2015) .

Often yeasts produce a limited variety of fats, with major fatty acids being stearic acid (18:0) and oleic acid (18:1). Linoleic acid (18:2) and linolenic acid (18:3) can also be produced in smaller amounts (up to 10%) depending on the culture conditions. Consumption of these unsaturated fatty acids are important for human health. However, there is no evidence to support that *R. toruloides* would have the ability to produce longer fatty acids, like EPA (20:5) or DHA (22:6), which are especially vital for human health (Passoth & Müller 2025).

Lipids, and especially unsaturated fatty acids (UFAs), are prone to oxidation. This process can be accelerated by exposure to heat, light, oxygen and microbial activity. This can cause rancidity in food products, leading to strongly unpleasant off-flavours. To avoid this, antioxidants are often added to food products which can prolong shelf-life. As *R. toruloides* naturally produces antioxidants along with the oil, use of additional antioxidants might not be necessary. As stated, the antioxidants also give the fat a bright red colour, also desirable in some plant based meat analogs. In such a product, a yeast oil from *R. toruloides* has the potential to replace vegetable oils, antioxidants and artificial colourants. This can appeal to both a “clean label” and an environmentally conscious market.

It is worth noting that while *Rhodotorula spp.* are generally recognised as safe (GRAS), the genus is not included in the European Food Safety Authority’s (EFSA) list of organisms of organisms with Qualified Presumption of Safety (QPS) (Fallahi et al. 2023; EFSA BIOHAZ Panel 2026). A further examination of food safety, and food safe substrates is therefore needed before *R. toruloides* lipids can be industrially produced for human consumption.

1.3 Pea protein gels as a model food product

The incorporation of such microbial oils into food systems requires evaluation of their functional properties. In this context, plant-based protein gels provide a useful model system. As demand for sustainable protein sources increases, plant proteins such as pea protein are gaining importance, particularly in regions like Northern Europe where soybean cultivation is limited. Pea protein isolate can be used to form gels that mimic the texture of various food products, including meat analogues and dairy alternatives.

1.3.1 Texture

The incorporation of fats into food products is often done to improve flavour, texture and mouthfeel. To quantify how an addition of fat affects textural aspects as well as storage effects, several methods can be used.

Compression test is one objective sensory measurement that can be used to describe textural parameters like stiffness, deformation and stress-strain behaviour. The standard method in the industry is Textural Profile Analysis (TPA) using a two-bite test. This relies on a machine compressing a sample twice by a certain percentage of the sample height to see how the sample deforms and the force that is required to do so (Peleg 2019).

1.3.2 Colour and Stability

Fats can affect the colour of a gel, both through inherent colour and emulsion stability, which can be measured using the CIELAB system. CIELAB is one of the most widely used colour-space models for quantifying colours, and is the standard used in colour industries like paint and textiles, as well as in food production (Shange et al. 2025).

Oils are also subject to oxidation and rancid off-flavours, and increase in such substances can be quantified using the Thiobarbituric Acid Reactive Substances (TBARS) assay. The TBARS assay is currently considered the standard way to detect lipid oxidation and antioxidant activity in foods. This is partly because it is highly accessible and easy to carry out. Malondialdehyde (MDA) is one end product formed by lipid peroxidation decomposition, and is targeted by the TBARS assay (Zeb & Ullah 2016).

Gel stability can be observed by measuring syneresis with aging. Syneresis is the process of a gel losing water over time, also known as driploss. It affects the sensory quality of a food gel and is an important parameter in food production (Mizrahi 2010).

1.4 Aim

The aim of the thesis is to evaluate the functionality of a yeast oil made from *R. toruloides* as compared to rapeseed oil. Differences in texture, colour and gel-aging will be quanitifed. A heat-set protein gel made from pea protein isolate will be used as a model food product to simulate a plant-based protein alternative to meat.

2. Method

2.1 Lipid Extraction

A mixture of hexane and isopropanol (3:2, v/v) was added to an Erlenmeyer flask containing dried *Rhodotorula toruloides* CBS 14 biomass cultured on glucose. The contents were stirred for 5 minutes and then filtered through a 0.45 µm filter under water pressure. The filtrate was added to a separatory funnel, and 6.7% Na_2SO_4 in destilled water (w/v) was added, a volume equal to approximately half of the filtrate volume. These were mixed, and then left to separate for one hour. The lower isopropanol phase was discarded and the upper hexane phase was recovered. The hexane was evaporated under N_2 gas. This was based on a method as described by Hara and Radin (1978).

2.2 Preparation of gels

This method is based on a publication by Auer et al. (2025) with slight modifications. Briefly, dry ingredients (12% w/w pea protein isolate and 1.5% NaCl) were dispersed in destilled water and stirred for 1 hour. The raw materials used was Pisane C9 from Cosucra groupe Warcoing. The pH of the solution was adjusted to 7.00 using 1.0M NaOH (10 -100 µl). The suspensions were treated with ultrasound at a frequency of 20kHz with amplitudes corresponding to 300W (30s), 525 W (30s) and 750 W (60 s). The solutions were kept on ice to limit temperature increase from the sonication process. 15% w/w rapeseed or the hexane yeast oil were added gradually to the system using an Ultra Turrax T25 at 8 500 rpm during 30 s, then further emulsified at 13 500 rpm for 30s. 12 ppm (1% of protein weight) TI Transglutaminase (Moo Gloo, Modernist Pantry) was added to increase gel firmness. Approximately 3 mL of the mixture was added to test tubes (10 mm Ø) and centrifuged to remove entrapped air. The samples were then heated in a water bath at 50 degrees (the temperature optimum for transglutaminase; Auer et al. (2025)) for 60 min and then raised to 95 degrees. When 95 degrees was reached, samples were left in the water bath for a further 15 mins. The samples were then cooled and kept at 4 degrees for 24 hours. Each oil-protein gel was prepared in duplicates.

2.3 Textural & stability measurements

2.3.1 Textural Profile Analysis (TPA)

Gel texture was measured using Texture Profile Analysis (TPA) using two-cycle compression test after 1 and 7 days. TPA was performed using Stable Micro

Systems TA-HDi Texture Analyser. Samples were cut into a cylindrical shape with a thickness of 10 mm, and diameter of 10 mm. Two measurements were carried out for each duplicate. The settings applied were 35 mm diametric cylindrical probe in aluminium, a strain of 50%, 2 mm/s speed and 5 s of waiting time between the first and second measurement. The main quantified parameters of the sample texture were hardness, cohesiveness and springiness. Hardness is equal to the peak force measured in the first compression. The cohesiveness is defined as the area underneath the second compression curve divided by the area under the first compression curve. This measures the brittleness of the sample, with lower values meaning a more brittle sample. The cohesiveness ranges from 0-1. The springiness is a measure of how much a product recovers of its original height and is expressed as a percentage (0-100%) or a ratio. The results for the rapeseed oil gel and the yeast oil gel were compared to each other, as well as to samples after 7 days of storage.

2.3.2 Colorimetric Analysis

Colour of the gels was measured on day 2 and 7 days to quantify colour change as an indication of shelf life and oxidation. The values are presented using the CIELAB system which was created in 1976 by the International Commission on Illumination known as CIE, where LAB stands for L^* (lightness), a^* (red vs green scale) and b^* (yellow vs blue scale) (Westland et al. 2012). Lightness is measured on a scale from 0 (black) to 100 (white). A positive a^* value indicates a more red colour and negative values a more green colour. For b^* negative values indicate blue and positive values indicate yellow (Westland et al. 2012). The colour profile of the samples was measured using a Konica Minolta CR-400 using the CIE xyY system (defined in 1931; Westland et al. 2012), but values were converted to the CIELAB system for analysis as it is the most widely used in the food industry. All conversions were made using the standard CIE illuminant A (D65) and the observer 2°, which are the default settings for the Konica Minolta CR-400. Conversion from xyY values to trismustulus values (XYZ) were done as described by Westland et al. (2012), see Equation 1. Further conversion from trimustulus values to CIELAB values were done as described by Hunt & Pointer (2011), see Equation 2.

Equation 1.

$$X = \left(\frac{x}{y}\right)Y, \qquad Y = \left(\frac{y}{y}\right), \qquad Z = \left(\frac{1-x-y}{y}\right)Y$$

Equation 2.

$$L^{*} = 116f\left(\frac{Y}{Y_n}\right) - 16,$$

$$a^{*} = 500\left[f\left(\frac{X}{X_n}\right) - f\left(\frac{Y}{Y_n}\right)\right],$$

$$b^{*} = 200\left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right]$$

$$\text{where } f\left(\frac{Y}{Y_n}\right) = \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} \; for \; \left(\frac{Y}{Y_n}\right) > \left(\frac{6}{29}\right)^{3} \; and \; f\left(\frac{Y}{Y_n}\right) = \left(\frac{841}{108}\right)\left(\frac{Y}{Y_n}\right) + \frac{4}{29} \; for \; \left(\frac{Y}{Y_n}\right) \leq \left(\frac{6}{29}\right)^{3}$$

$$and \; f\left(\frac{X}{X_n}\right) = \left(\frac{X}{X_n}\right)^{\frac{1}{3}} \; for \; \left(\frac{X}{X_n}\right) > (\frac{6}{29})^{3} \; and \, f\left(\frac{X}{X_n}\right) = \left(\frac{841}{108}\right)\left(\frac{X}{X_n}\right) + \frac{4}{29} \; for \; \left(\frac{X}{X_n}\right) \leq \left(\frac{6}{29}\right)^{3}$$

$$and \; f\left(\frac{Z}{Z_n}\right) = \left(\frac{Z}{Z_n}\right)^{\frac{1}{3}} \; for \; \left(\frac{Z}{Z_n}\right) > (\frac{6}{29})^{3} \; and \, f\left(\frac{Z}{Z_n}\right) = \left(\frac{841}{108}\right)\left(\frac{Z}{Z_n}\right) + \frac{4}{29} \; for \; \left(\frac{Y}{Y_n}\right) \leq \left(\frac{6}{29}\right)^{3}$$

In this equation n represents the trismustulus value of the the standard CIE illuminant A (D65), where $X_n = 95.04$, $Y_n = 100$ $Z_n = 108.8$.

The colour changes (ΔE) between two samples can be compared using Equation 3.

Equation 3.

$$\Delta E = \sqrt{(a_x - a_0)^2 + (b_x - b_0)^2 + (L_x - L_0)^2}$$

In this equation x represents the objective and 0 represents the reference.

The ΔE value is a mathematical expression of the difference in colour between two samples, and a value under 2.6 corresponds to a difference only noticeable if the samples are set side by side (Shange et al. 2025).

The measurements were repeated in technical duplicates for each sample using different measuring points.

2.3.3 Lipid oxidation (TBARS)

Lipid oxidation was measured through the TBARS assay after 1 and 7 days for an indication of difference in shelf life due to the type of oil used. The method aims to detect the lipid oxidation product malondialdehyde (MDA), and other Thiobarbituric Acid Reactive Substances (TBARS), which form a pink complex with thiobarbituric acid (TBA) as outlined by Miller (1998). 1 g of sample was homogenized with 9.1 ml 10% trichloric acid (TCA) in 0.2M H_3PO_4 and 0.2 ml butylated hydroxy toluene (BHT) (0.2 mg/ml in methanol). This mixture was then filtered through a 6 µm filter. 1.5 ml of the filtered solution was mixed with 1.5 ml of 0.02 M thiobarbituric acid solution (TBA). A sample blank was prepared with equal amounts of liquid sample and distilled water. This was repeated in technical duplicates for each sample. To quantify the levels of TBARS, a standard curve was created using 25 µM tetraetoxypropane (TEP) mixed with 10% TCA in 0.2M H_3PO_4 and 0.02 M TBA. All samples and standards were incubated on a heating block for 30 min at 85°C, and then cooled under running water. Absorbance was measured at 530 nm against distilled water. The absorbance value obtained from each sample blank was deducted from all samples and standards. The concentration of TBARS in the sample solution was calculated using the standard curve. The crude sample concentration was then calculated using Equation 4, where 72.06 is the molecular weight of MDA (g/mol).

Equation 4.

$$MDA\ (\mu g/g\ sample) = \frac{(2 \times \Bigl(\text{conc. MDA}\frac{\text{µmol}}{\text{L}}\Bigr) \times \Bigl(9.3 + \Bigl(\frac{\text{sample weight}}{100} \times \text{moisture content \%}\Bigr)\Bigr) \times \frac{72.06}{\text{sample weight (g)}}}{1000}$$

2.3.4 Syneresis

Syneresis was measured by placing a known weight of sample in a test tube and storing for 7 days. The contents were placed on a tissue paper after storage, allowing any exuded liquid to be absorbed. The remaining gel weight was then measured to indicate the amount of driploss, and therefore how much syneresis has occurred in the gel matrix.

3. Results and Discussion

3.1 Hexane extraction

The yeast oil was extracted from dried *R. toruloides* biomass using hexane extraction. 93.74 grams of dried *R. toruloides* biomass was mixed with 1000 mL hexane: isopropanol solution (HIP) (3:2, v/v). After separation and evaporation, the weight of recovered lipids was equal to 46.7 grams. This would determine the fat content in the dried biomass to 49.8%, comparable to an earlier test where the same biomass was determined to have a fat content of 48.3% (Y. N. Nagaraj, personal communication) using the Folch method (Folch et al. 1957).

3.2 Preparation of gels

Protein slurries were prepared and ultrasonicated, and then emulsified with the yeast oil and rapeseed oil respectively. There was an apparent difference in thickness and viscosity after the oils were emulsified into the protein slurries. The yeast oil emulsion became thick and paste-like, holding its shape after deformation. The control rapeseed emulsion was more gelationus and pourable. The differences can be observed in Figure 1. The characteristic red colour of the *R. toruloides* yeast oil gave the emulsion an orange-pink colour, as can also be observed in Figure 1.

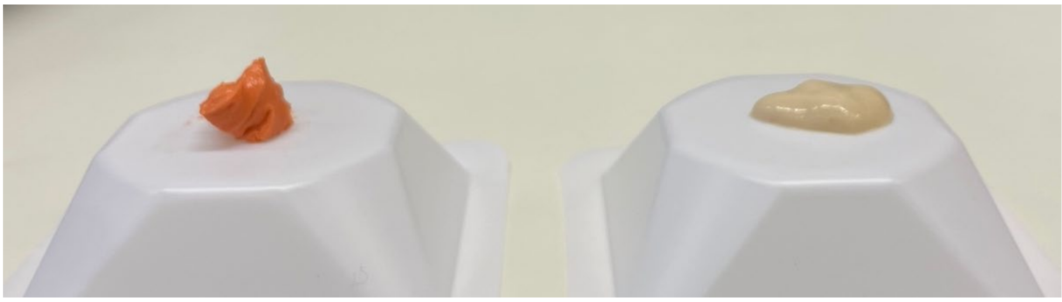

Figure 1. Protein gels after emulsification with yeast oil (left) and rapessed oil (right), before heat induces gelation.

After heat-induced gelation the yeast oil samples appeared to have a more porous structure with larger air bubbles than the rapeseed oil samples. These observations can indicate inherent emulsifying properties in the yeast oil. *R. toruloides* oils are known to contain phospholids (Mishra et al. 2024) which have emulsifying properties due to their amphiphilic nature. These can coat the air-water and oil-water interface, reducing surface tension and allowing for smaller, more numerous, dispersed particles. Compounds in the yeast oil seem to have acted as emulsifiers, coating the surface of dispersed air and possibly oil droplets in the system. This would increase the friction force, which is directly related to emulsion thickness, and explain the thicker emulsion before gelation, as well as the more porous structure after gelation.

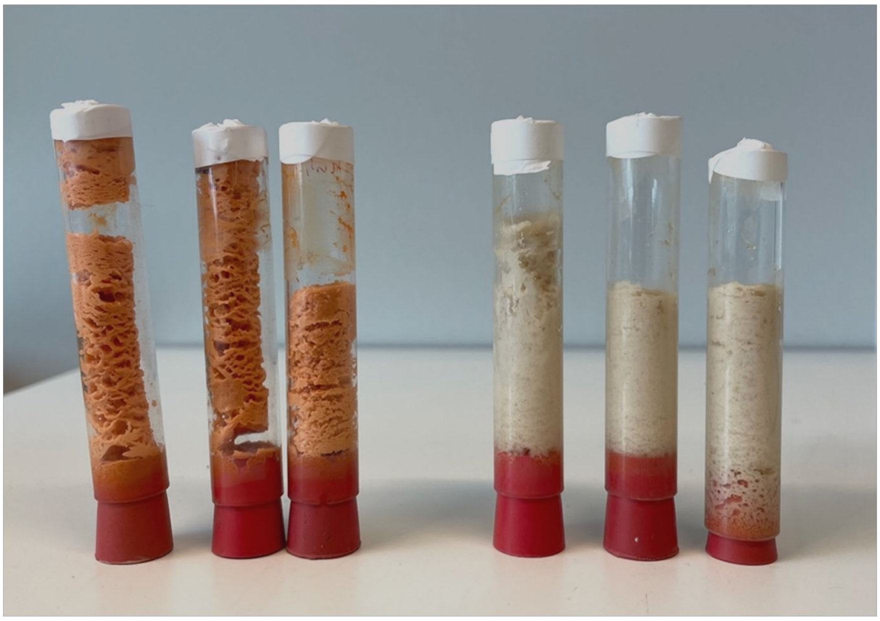

Figure 2. Protein gels after heat induced gelation with yeast oil (left) and rapessed oil (right).

3.3 Texture Profile Analysis (TPA)

The results from TPA using the Stable Micro Systems TA-HDi Texture Analyser are interpreted using a TPA curve, as shown in Figure 3. The maximum force obtained in the test corresponds to the y-value of the first peak and is an expression of the gel hardness. As seen in Figure 4 the yeast oil samples had an average hardness greater than that of the rapeseed oil samples. The average yeast oil hardness on day 2 was 92.4 with a standard deviation (SD) of ± 37.6. The average rapeseed oil hardness was lower, at 37.6 (SD ± 8.5). Little change had occurred on day 7 with yeast oil samples giving average values of 93.9 (SD ± 12.7) and rapeseed oil gels 40.1 (SD ± 7.1). The relative standard deviation (RSD) is between 13-41% for these, meaning the small increase in hardness from day 2 to day 7 could not be statistically confirmed. This high variance was most likely due to issues with the probe and machine in question. However, the difference in hardness between the yeast oil samples and rapeseed oil samples was significant (p = 0.017, *). This was probably due to better emulsification and a thicker emulsion achieved in the gel preparation.

The cohesiveness values were slightly lower for yeast oil samples and slightly higher for rapeseed oil samples, meaning yeast oil samples would be more brittle and rapeseed oil samples more bouncy and elastic. These differences were however not statistically significant (p = 0.50). Repeating measurements for a larger sample size, or using a different, TPA instrument could give clearer indications of the true cohesiveness in the samples.

No conclusions could be drawn regarding the difference in springiness between the samples. The springiness (expressed as a percentage, 0 - 100%) results for all samples were equal to or slightly over 100%, and have been disregarded. This was most likely due to a malfunctiong probe or machine. The Texture Analyser in question is currently due to be replaced due to a previous history of malfunction. Guiné (2022) states that springiness is considered an important parameter for sensory evaluation and mouthfeel, and thus the absence of results for this parameter limits the scope of the conclusions that can be drawn based on the TPA.

Raw data from all measurements can be found in the Appendix 1.

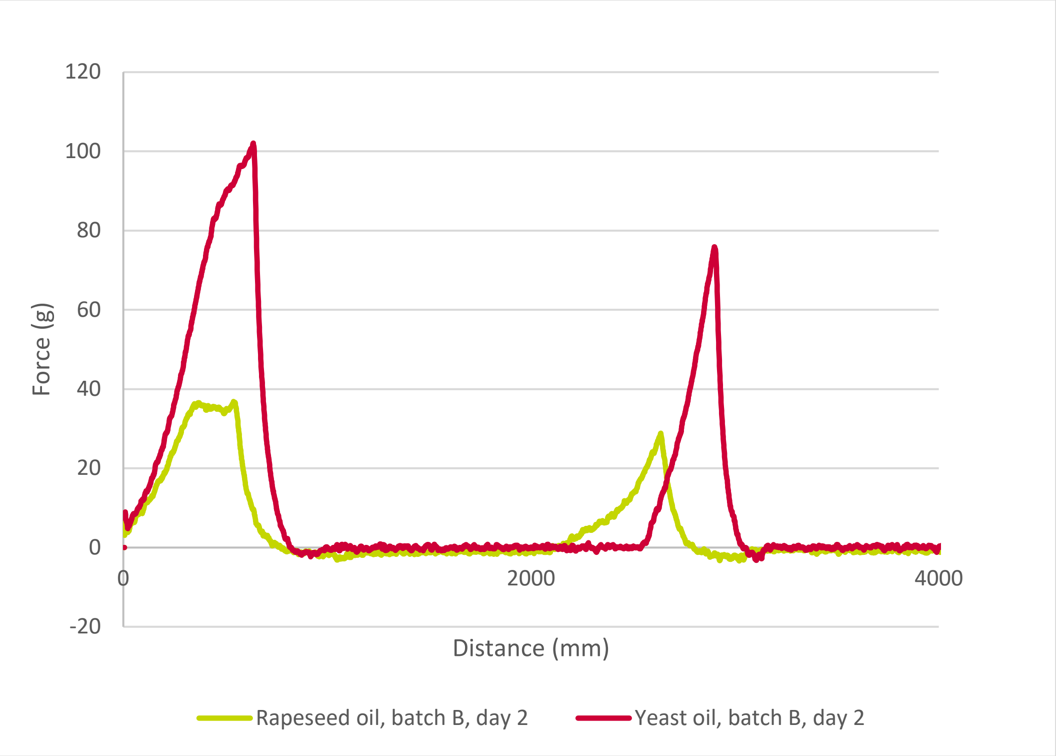

Figure 3. TPA curve with force (g) on the y-axis and distance (mm) on the x-axis. Two curves are included as examples were the light green curve is the resuts from a sample with rapeseed oil, batch B, on day 2 and the red curve is the resuts from a sample with yeast oil, batch B, on day 2.

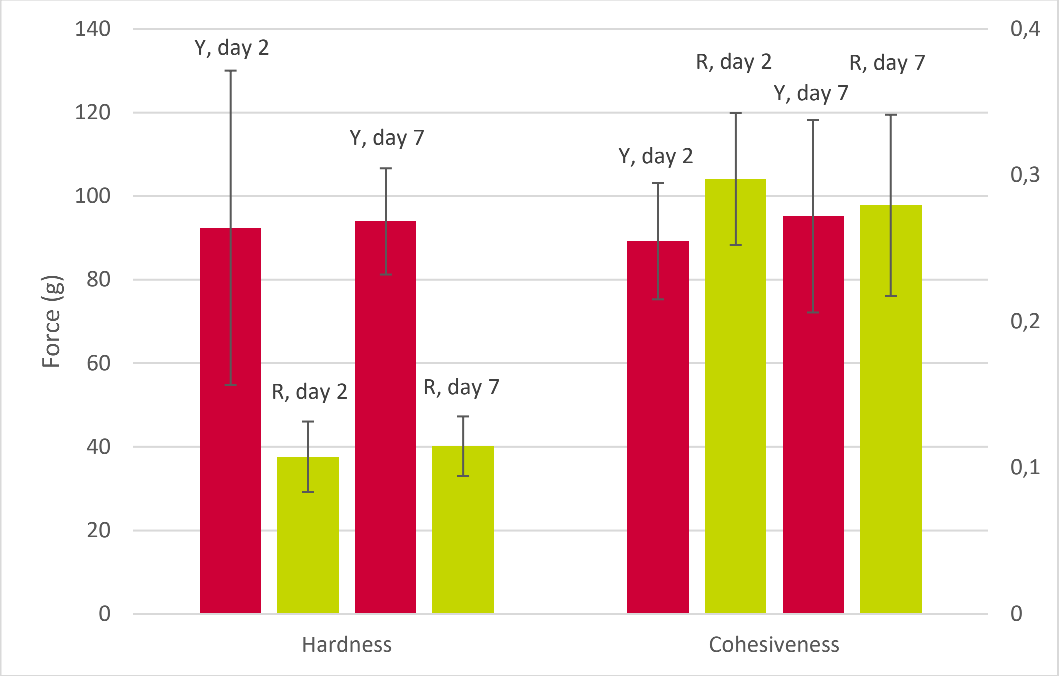

Figure 4. Average hardness and cohesiveness values for yeast oil samples and rapeseed oil samples respectively, as measured on day 2 and 7. The abbreviations Y and R are used to represent the samples; Y= Yeast oil sample, R=Rapeseed oil sample.

Further exploration of the fatty acid composition of the specific biomass used in this study could give insights into differences in melting point and effect on pH in the system. *R. toruloides* lipids generally contain higher levels of saturated FAs than rapeseed oil, and should therefore have a higher melting point (Passoth & Müller 2025; Matthaus et al. 2016). It is possible that the yeast lipids were fully or partially solidified as TPA was conducted after samples had been stored at 4°C, which would contribute to sample hardness. Because fatty acids are weak acids with structure-dependent pKa values, their degree of dissociation varies, and they can therefore influence the pH of the system (Christie & Han, 2010). In this method, the pH of the protein slurry was adjusted, but not post emulsification. pH is an important parameter that affects both emulsification and gelation abilities (Liu et al. 2021; Khalesi et al. 2024). A better understanding of the FA composition and corresponding pKa values would therefore give more insight in to how these may have affected the samples.

3.4 Colorimetric Analysis

The largest colour difference between the samples was in the red/green axis, shown as the *a** value. Where the yeast oil samples had a positive value (indicating a red colour), the rapeseed oil samples had a negative value, meaning a slightly green hue (see Figure 5). The yeast oil samples had a distinct red hue from mere observation, but it was an unsaturated colour. The primary red colour used by Westland et al. (2012) had an $a*$ of +62.5 as a point of comparison. The yeast oil samples displayed average $a*$ values of +17.1 and +17.7 (SD ± 2.2, ± 1.1), indicating a soft, non-saturated red. The rapeseed oil gels had average $a*$ values of -6.1 and -6.2 (SD ± 0.5, ± 03), compared to Westland et al. (2012) primary green that had an $a*$ value of -82.5, and true grey being $a^* = 0$ (CIE 2004). The rapeseed oils therefore had elements of green but were closer to a grey colour.

The yeast oil and rapeseed oil samples were more similar on the yellow/blue axis, where both had positive $b*$ values. The yeast oil had average $b*$ values equal to 53.3 on both day 2 and day 7 (SD ± 0.9, ± 0.2), higher than that of the control rapeseed oil samples. The control samples had average $b*$ values of 41.1 and 41.2 (SD ± 0.7, ± 0.3).

The yeast oil samples were slightly darker than the rapeseed oil samples, likely due to the pigments in the extracted yeast oil. The yeast oil samples had a slightly lower $L*$ (70.2 ± 0.51 and 70.7 ± 0.55) than the rapeseed oil samples (74.2 ± 0.55 and 75.5 ± 0.37), where 0 = pure black and 100 = pure while. Raw data from all measurements can be found in the Appendix 2.

This colorimetric analysis was consistent with the fact that the extracted yeast oil contains high levels of carotenoids, contributing with both red and yellow pigments, as well as giving the yeast oil a darker colour than that of rapeseed oil. The carotenoid content of *R. toruloides* CBS 14 lipids was quantified in a study from 2025 by Nagaraj et al., though growing conditions was different from the sample used in these experiments. The total carotenoids in the dried yeast biomass cultivated on wheat straw hydrolysate was quantified to 332 ± 27.32 µg/g dry weight, where the main carotenoids were torularhodin (60.2%), followed by torulene (31.6%) and β-carotene (7.7%), as well as small amounts of γ-carotene. These where quantified using UHPLC-PDA, and the total fat content of the dry biomass was under 30%. The total fat content therefore differs depending on the cultivation conditions, where the biomass grown on glucose used in these experiments was 49.8%. This indicates it is possible that the fat composition and therefore level of carotenoids also differ. The peak absorbance of β-carotene as described by Ambrico (2024) is 453 nm, ɣ-carotene at 462 nm, torulene at 483 nm and torularhodin at 498 nm. As the β-carotene absorbs light in the blue/cyan spectrum, the colour is perceived as more yellow, and torularhodin and torulene more orange to red as they absorb light in the blue/magenta spectrum. The extracted yeast oil had a deep red colour which indicates that the levels of torularhodin and torulene were high in the glucose-cultivated sample used in these experiments, similar to the study by Nagaraj et al. A full quantification of how carotenoid profiles differ based on cultivation methods would give more insight in how *R. toruloides* lipids can be useful in real-life applications in food products, side-stepping the need for added synthetic colourants.

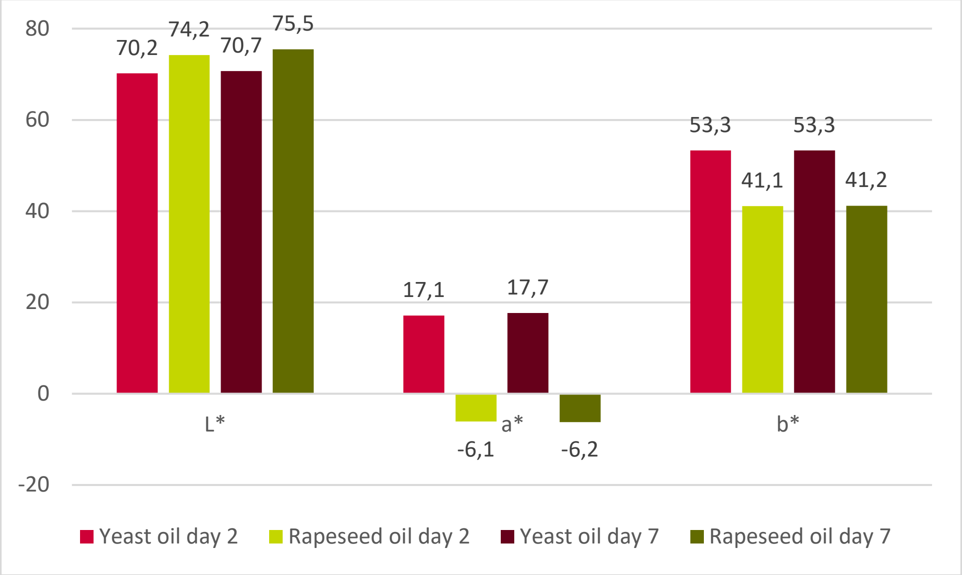

Figure 5. Average CIELAB results for yeast oil samples and rapeseed oil samples on day 2 and day 7 respectively.

Very little colour change occurred in all samples with a *ΔE* under 2.6, corresponding to no discernable difference before and after storage, unless samples are placed directly next to each other. The yeast oil samples displayed a *ΔE* of 1.15 (SD ± 0.46), and the rapeseed oil samples a *ΔE* of 0.95 (SD ± 0.09). However, the colour difference between the yeast oil and control rapeseed oil samples was very obvious to the human eye, with a *ΔE* of 26.9 (see Figures 1, 2 and 6).

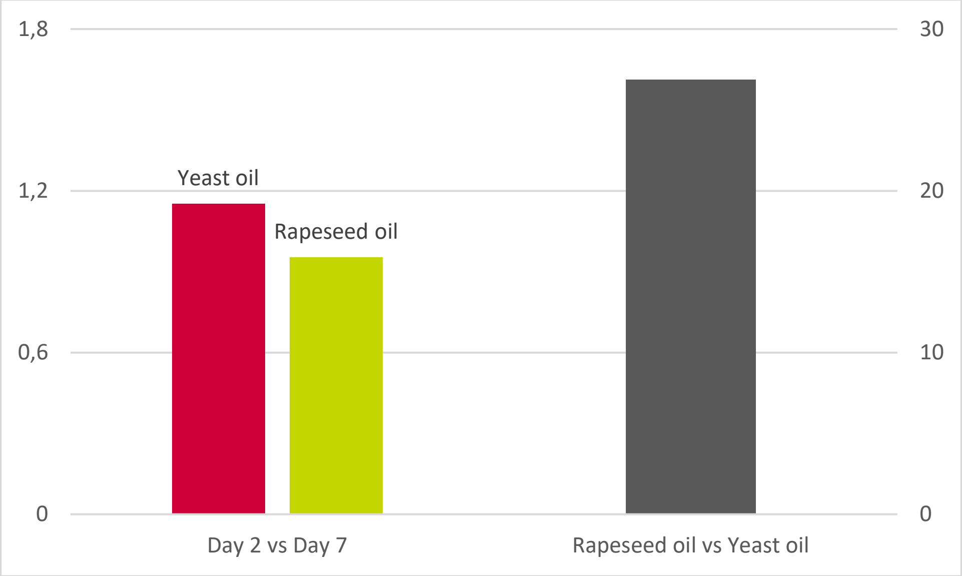

Figure 6. Colour differences between yeast oil samples and rapeseed oil samples respectively on day 2 versus day 7 (left). Colour difference between yeast oil samples and rapeseed seed oil samples overall (right).

3.5 Lipid oxidation

The amount of malondialdehyde (MDA) in the samples was determined using the Thiobarbituric Acid Reactive Substances assay (TBARS) as described in section 2.3.2. MDA is a lipid oxidation product and was used as an indicator for lipid oxidation. Anything below 0.150 µg MDA/g is considered a low level of lipid oxidation in raw and processed meats (Bertolín et al. 2019). The average MDA in all samples was lower than 0.04 µg MDA per gram of sample on day 2, see table 1. However, the RSD for the samples range from 6% up to 36%. This indicates that the levels of MDA in the samples were near the limit of quantification. On day 7, no increase in MDA could be observed, and in some samples there was a marginal decrease in concentration of MDA. As both samples contained unsaturated fats which spontaneously oxidize over time, these results seemed unlikely. This further indicates that the levels of MDA were close the limit of quantification. The results indicate that there was no significant difference in lipid oxidation between the yeast oil and rapeseed oil samples over 7 days of storage at 4°C. Raw data can be found in the Appendix 3.

In short, no conclusions could be drawn as to how rapeseed oil and this yeast oil differ in lipid oxidation over time based on these limited measurements. The tests would need to be replicated over longer time in order to draw any definite conclusions. Further tests were unfortunately not possible in this project due to time constraints.

Table 1. Average absorbance values at 530nm and related concentrations of MDA from TBARS assay on day 2.

Sample	**Blank sample Absorbance**	**TBA sample Absorbance**	**True Absorbance**	**Concentration MDA (µmol/L)**	**Concentration MDA µg/g**
Yeast oil A	0.004	0.039	0.035	0.008	**0.012**
SD					± ***0.004***
Yeast oil B	0.005	0.055	0.051	0.014	**0.021**
SD					± ***0.002***
Rapeseed oil A	0.002	0.072	0.070	0.022	**0.031**
SD					± ***0.002***

Rapeseed oil B	0.003	0.084	0.081	0.026	**0.038**
SD					± ***0.007***

A standard curve was generated using samples with known concentrations of TEP, and their absorbance was measured, as shown in Figure 7. See table A3.3 in Appendix 3 for exact amounts used. This standard curve was used to relate the measured absorbance to the concentration of MDA in all yeast and rapeseed oil samples. The R^2 for the standard curve was equal to 0.9992.

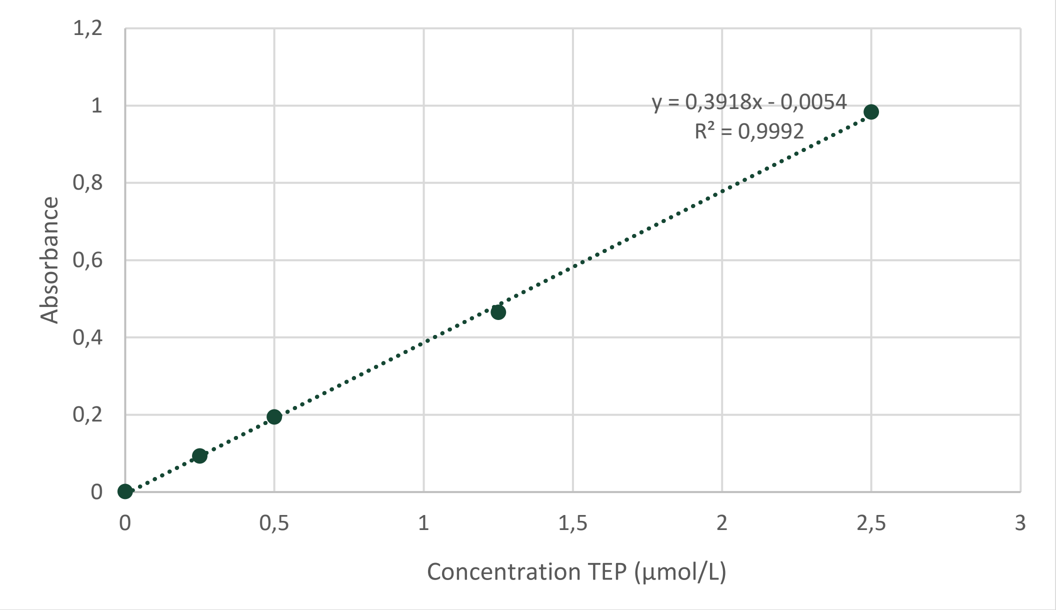

Figure 7. Standard curve using known concentrations of TEP and the resulting absorbance values at 530 nm.

3.6 Syneresis

Samples of 2-3 g were added to tubes the day after the gels were prepared (day 2). They were stored in 4°C until day 7, when they were weighed again to quantify water loss. In 1991, Sun and Breene described how the syneresis of a protein gel during storage is caused by increased cross-linkage between protein molecules which squeezes out entrapped water from within the gel. The water that is forced out can also be referred to as driploss. The rapeseed oil samples had an average driploss of 11.3-11.5% with a relative standard deviation (RSD) of 7.8-11.7% (see Figure 8). The yeast oil samples had a lower average driploss of 1.1-1.8% (RSD 3.30-22.9%). While the RSD shows some variation between samples, the difference between the rapeseed oil samples and the yeast oil samples is highly statistically significant (***), as the p-value is <0.0001 (0.000034). Raw data can be found in table in Appendix 4. When entrapped water is forced out during storage, textural components and mouthfeel can alter slightly. In some products it could also be a highly undesirable trait. For example, a customer might not appreciate an emulsified vegetarian sausage dripping with water when removed from the package. The lower driploss in the yeast oil samples therefore shows potential for usefulness in product formulations where driploss would pose an issue. For comparison, in a study by Khoder et al. from 2020, tofu samples (a common protein gel food stuff) displayed syneresis values between 15.7 and 26.6% after 24 hours at 4°C.

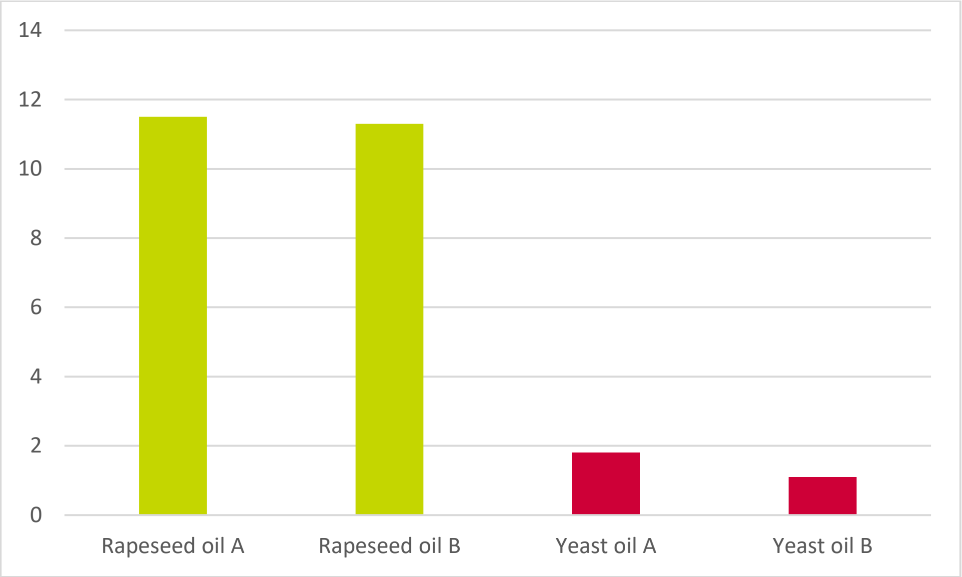

Figure 8. Average driploss (%, w/w) in rapeseed oil samples to the left, showing batch A and B. Average driploss in yeast oil samples to the right, showing batch A and B.

4. Conclusions

From the results in this study it can be concluded that yeast oil from *R. toruloides* has potential as a food ingredient. It seems to have promising emulsifying properties, resulting in a thicker emulsion than when using rapeseed oil. Using the yeast oil in food formulations could therefore act as a substitution for both vegetable oils and added emulsifiers. As there are some emulsifiers that spark health concerns, this can appeal to consumers trying to omit them from their diets (Partridge et al. 2019). The results from this project also indicate that yeast oil substitution could potentially give an emulsion with a lower fat percentage while maintaining emulsion thickness. As more fat is added to an oil-in-water emulsion, the interface between the two increases and the mixture thickens. This means yeast oil could be used in food formulations where a lower fat percentage is necessary for nutrition or otherwise, with no need for added thickeners. The yeast oil gels also displayed significantly lower syneresis than the rapeseed oil samples, further proving the stability of the formed emulsion gels.

The *R. toruloides* oil's orange-red colour seems to transfer well to an emulsified gel product, with an even dispersion and a good colour stay for 7 days. This yeast oil could therefore provide a fat source as well as sidestepping the need for synthetic colourants, whose use have also been criticised due to health concerns. Naturally this also limits the area of application for *R. toruloides* oil to products where this colour would be desirable, like in plant-based meat, poultry and fish alternatives.

Not much can be said on how *R. toruloides* lipids differ from rapeseed oil based on the results from this study as time constraints did not allow further tests. It can atleast be concluded that they hold up similarly after 7 days of storage. Further exploration can give insight to how *R. toruloides* oil holds up to lipid oxidation and what role the inherent carotenoids play here.

One challenge to address is large-scale food-safe cultivation of *R. toruloides*, and subsequent extraction. Second generation substrates, like waste products from the food industry show potential, with molasses being a promising alternative. The production of microbial oils as an alternative to VOs in biodiesel production has been explored on several fronts (Passoth & Müller 2025). However, yield and productivity are currently too low to be commercially competitive. As oleaginous fungi produce lipids intracellularly, the cells need to be lysed and for biodiesel production they then need to be extracted. The extraction methods, like hexane extraction used in this study, requires large amounts of time, energy and solvents, which contribute greatly to the increase in production costs. The extraction of the yeast oil also means the protein, fiber and minerals of the yeast biomass is lost, but in food applications these could add value. Yeast proteins are regarded as highly digestible, and many western diets generally contain far to little fiber, with mineral deficiencies also being a concern. If *R. toruloides* could instead be used in food formulations without lipid extraction, utilising the full homogenized yeast cells, the production costs could be decreased significantly while improving nutritional aspects. This could also make microbial oil sources more commercially competitive. *R. toruloides* products may become a future meat alternative, with fats, proteins, emulsifiers, stabilisers, colourants, minerals, all in one food ingredient, with some fiber as an added bonus! Further studies on how a homogenised *R. toruloides* biomass would work in food formulations can give more insight to the viability of this idea. The next step in exploring the food-value of *R. toruloides* oil would be to map out the flavour, and see what reservations and possibilities this might present.

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Appendix 1, Texture Profile Analysis

Table A1.1. Hardness, area under peaks 1 and 2 and cohesiveness for rapeseed oil samples, day 2 and 7 respectively.

Rapeseed oil samples	**Hardness**	**Area Peak 1**	**Area Peak 2**	**Cohesiveness**
Day 2.				
RA1	47.3	128.0	38.4	0.299
RA2	39.4	117.1	27.4	0.234
RB1	36.9	98.3	31.0	0.316
RB2	26.8	56.5	19.2	0.339
Day 7.				
RA1	50.8	142.5	30.1	0.212
RA2	37.2	107.4	26.2	0.244
RB1	36.6	80.1	25.6	0.320
RB2	35.9	70.1	24.0	0.343

Table A1.2. Hardness, area under peaks 1 and 2 and cohesiveness for yeast oil samples, day 2 and 7 respectively.

Yeast oil samples	**Hardness**	**Area Peak 1**	**Area Peak 2**	**Cohesiveness**
Day 2.				
YA1	53.5	159.8	32.6	0.204
YA2	73.9	223.9	54.3	0.243
YB1	140.2	331.1	95.0	0.287
YB2	102.1	255.7	73.2	0.286
Day 7.				
YA1	76.8	267.3	54.3	0.203
YA2	91.8	245.9	67.7	0.275
YB1	103.9	361.5	90.2	0.250
YB2	103.2	188.6	67.8	0.360

Appendix 2, Colorimetric Analysis

Table A2.1. Results of Minolta measurements for yeast oil samples, day 2 and day 7.

Yeast oil samples	***Y***	***x***	***y***
Day 2			
Yeast oil A	34.8	0.468	0.422
	34.9	0.465	0.420
Mean	**34.8**	**0.466**	**0.421**
SD	*0.064*	*0.002*	*<0.001*
Yeast oil B	36.31	0.458	0.429
	36.3	0.466	0.424
Mean	**36.3**	**0.462**	**0.426**
SD	*0.007*	*0.006*	*0.004*
Day 7			
Yeast oil A	34.9	0.469	0.422
	35.2	0.467	0.423
Mean	**35.0**	**0.468**	**0.422**
SD	*0.240*	*0.001*	*<0.001*
Yeast oil B	37.1	0.463	0.423
	37.2	0.460	0.423
Mean	**37.1**	**0.461**	**0.423**
SD	*0.106*	*0.001*	*<0.001*

*Table A2.2. Results of Minolta measurements for rapeseed oil samples, day 2 and day 7. *The measurement marked in yellow is treated as an outlier and is not included in charts showing averages.*

Rapeseed oil samples	***Y***	***x***	***y***
Day 2			
Rapeseed oil A	50.3	0.385	0.425
	49.8	0.385	0.425
Mean	**50.0**	**0.385**	**0.425**
SD	*0.325*	*<0.001*	*<0.001*
Rapeseed oil B	48.2	0.385	0.426
	48.9	0.389	0.427
Mean	**48.6**	**0.387**	**0.427**
SD	*0.523*	*0.003*	*0.001*
Day 7			
Rapeseed oil A	49.4	0.386	0.426
	51.6*	0.424*	0.402*
Mean	**50.5**	**0.405***	**0.414**
SD	*1.54*	*0.027*	*0.016*
Rapeseed oil B	48.2	0.386	0.426
	49.1	0.384	0.425
Mean	**48.6**	**0.385**	**0.425**
SD	*0.601*	*0.001*	*<0.001*

Table A2.3. L, a*, b* results for yeast oil samples, day 2 and day 7.*

Yeast oil samples	***L****	***a****	***B****
Day 2			
Yeast oil A	69.9	18.6	53.2
	69.8	18.1	52.1
Mean	**69.9**	**18.3**	**52.6**
SD	*0.030*	*0.343*	*0.760*
Yeast oil B	70.0	13.9	53.8
	70.9	17.9	54.2
Mean	**70.4**	**15.9**	**54.0**
SD	*0.660*	*2.87*	*0.232*
Day 7			
Yeast oil A	70.0	18.9	53.4
	70.1	18.2	53.4
Mean	**70.1**	**18.5**	**53.4**
SD	*0.076*	*0.480*	*0.002*
Yeast oil B	71.3	17.1	53.4
	71.3	16.5	53.0
Mean	**71.3**	**16.8**	**53.2**
SD	*0.025*	*0.450*	*0.268*

Table A2.4. L, a*, b* results for rapeseed oil samples, day 2 and day 7. *The measurement marked in yellow is treated as an outlier and is not included in charts showing averages as standard deviationof a* is greater than the average.*

Rapeseed oil samples	***L****	***a****	***B****
Day 2			
Rapeseed oil A	73.5	-6.4	40.5
	74.1	-5.5	42.1
Mean	**73.8**	**-5.9**	**41.3**
SD	*0.473*	*0.626*	*1.10*
Rapeseed oil B	74.7	-6.5	40.7
	74.5	-6.2	40.9
Mean	**74.6**	**-6.4**	**40.8**
SD	*0.155*	*0.190*	*0.157*
Day 7			
Rapeseed oil A	74.30	-6.0	41.0
	80.3*	14.0*	42.5*
Mean	**77.3**	**4.00**	**41.8**
SD	*4.23*	*14.2**	*1.09*
Rapeseed oil B	73.6	-6.1	40.8
	74.0	-6.6	40.3
Mean	**73.8**	**-6.4**	**40.5**
SD	*0.304*	*0.307*	*0.313*

Table A2.5. Quantified colour changes for all samples, comparing day 2 and day 7. Outlier not included in calculations

Difference	**Yeast oil A**	**Yeast oil B**	**Rapeseed oil A**	**Rapeseed oil B**
ΔE	0.825	1.48	1.02	0.888

Appendix 3, Lipid Oxidation

Table A3.1. Absorbances and concentration of MDA in all samples on day 2. The blank sample absorbance is subtracted from the TBA sample absorbance to obtain true absorbance. The concentration of MDA was then obtained from the standard curve using the true absorbance. Equation 1 was then used to convert the unit from µmol/L to µg/g.

Sample	**Blank sample Absorbance**	**TBA sample Absorbance**	**True Absorbance**	**Concentration MDA (µmol/L)**	**Concentration MDA (µg/g)**
Rapeseed oil A	0.002	0.06	0.058	0.017	0.025
	0.002	0.084	0.082	0.027	0.038
Mean	**0.002**	**0.072**	**0.070**	**0.022**	**0.032**
SD	*0*	*0.017*	*0.017*	*0.001*	*0.002*
Rapeseed oil B	0.003	0.064	0.061	0.018	0.027
	0.004	0.104	0.100	0.034	0.049
Mean	**0.004**	**0.084**	**0.081**	**0.026**	**0.038**
SD	*0.001*	*0.028*	*0.028*	*0.005*	*0.008*
Yeast oil A	0.004	0.043	0.039	0.010	0.014
	0.004	0.034	0.030	0.006	0.009
Mean	**0.004**	**0.039**	**0.035**	**0.008**	**0.012**
SD	*0*	*0.006*	*0.006*	*-0.003*	*-0.004*
Yeast oil B	0.003	0.047	0.044	0.012	0.017
	0.006	0.063	0.057	0.017	0.024
Mean	**0.005**	**0.055**	**0.051**	**0.014**	**0.021**
SD	*0.002*	*0.011*	*0.009*	*-0.002*	*-0.003*

Table A3.2. Absorbances and concentration of MDA in all samples on day 7. The blank sample absorbance is subtracted from the TBA sample absorbance to obtain true absorbance. The concentration of MDA was then obtained from the standard curve using the true absorbance. Equation 1 was then used to convert the unit from µmol/L to µg/g.

Sample	**Blank sample Absorbance**	**TBA sample Absorbance**	**True Absorbance**	**Concentration MDA (µmol/L)**	**Concentration MDA µg/g**
Rapeseed oil A	0.005	0.040	0.035	0.008	0.012
	0.003	0.046	0.043	0.011	0.017
Mean	**0.004**	**0.043**	**0.039**	**0.010**	**0.014**
SD	*0.001*	*0.004*	*0.003*	*-0.004*	*-0.006*
Rapeseed oil B	0.007	0.061	0.054	0.016	0.023
	0.006	0.038	0.032	0.007	0.010
Mean	**0.007**	**0.050**	**0.043**	**0.011**	**0.017**
SD	*0.001*	*0.0163*	*0.016*	*0.001*	*0.001*
Yeast oil A	0.005	0.069	0.064	0.020	0.028
	0.019	0.038	0.019	0.002	0.003
Mean	**0.012**	**0.054**	**0.042**	**0.011**	**0.016**
SD	*0.010*	*0.022*	*0.012*	*-0.001*	*-0.001*
Yeast oil B	0.02	0.036	0.016	0.001	0.001
	0.001	0.043	0.042	0.011	0.016
Mean	**0.011**	**0.040**	**0.029**	**0.006**	**0.009**
SD	*0.013*	*0.005*	*-0.008*	*-0.009*	*-0.013*

Table A3.3. Volumes and absorbances used to compute standard curve.

Standard curve (ml TEP added)	**Blank sample Absorbance**	**TEP sample Absorbance**	**True Absorbance**	**Concentration TEP (µmol/L)**
0	0.005	0.006	0.001	0
0,1	0	0.093	0.093	0.25
0,2	0.015	0.209	0.194	0.5
0,5	0	0.465	0.465	1.25
1	0	0.983	0.983	2.5

Appendix 4, Syneresis

Appendix A4.1. Weights of samples on day 2 compared to day 7, and the driploss defined as the decrease in weight by weight and percantage, w/w.

Sample	**Tube weight (g)**	**Tube+sample weight before storage (g)**	**Sample weight day 2 (g)**	**Sample weight day 7 (g)**	**Driploss (g)**	**Driploss (%, w/w)**
Rapeseed oil A1	6.93	9.30	2.37	2.12	0.25	**10.6**
Rapeseed oil A2	6.92	9.64	2.72	2.38	0.34	**12.5**
Rapeseed oil B1	6.95	9.51	2.56	2.25	0.31	**11.9**
Rapeseed oil B2	6.96	10.08	3.12	2.79	0.33	**10.7**
Yeast oil A1	6.93	9.84	2.91	2.86	0.05	**1.7**
Yeast oil A2	6.99	9.32	2.33	2.29	0.04	**1.8**
Yeast oil B1	6.92	9.26	2.34	2.31	0.03	**1.3**
Yeast oil B2	6.94	9.98	3.04	3.01	0.03	**0.9**

Table A4.2. Average driploss in all samples.

Sample	**Average sample weight (g), day 2**	**Average sample weight (g), day 7**	**Average driploss (g)**	**Average driploss (%, w/w)**
Yeast oil A	2.62	2.58	0.05	**1.8**
RSD (%)				***3.3***
Yeast oil B	2.69	2.66	0.03	**1.1**
RSD (%)				***22.9***
Rapeseed oil A	2.55	2.25	0.30	**11.5**
RSD (%)				***11.7***
Rapeseed oil B	2.84	2.52	0.32	**11.3**
RSD (%)				***7.8***

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