Change in stability and characteristics of different sunflower press cake mixtures during fermentation with *Lactiplantibacillus plantarum*

Master's Thesis KLGM10

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Preface

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Abstract

Food's impact on the climate is getting more awareness, leading to an interest in finding possible solutions. One such is to use waste products to create new products. Sunflower press cake (SFPC) is a waste product from sunflower oil production with great potential because of its high protein content. The nutritional content, as well as its physicochemical properties, are affected by the source, for example, if the seeds are hulled or dehulled, as well as the pressing method during oil extraction. Together with whey, a waste product from cheese production, SFPC has the possibility of being a balanced nutritional food product. To increase the nutritional bioavailability and to create tasty food, the aim of this project was to ferment sunflower press cake dispersions.

The addition of whey and homogenisation was investigated by analysing the pH change during fermentation, the viscosity before and after fermentation and the physical stability before and after fermentation. The results show differences between the SFPC where the dehulled and cold-pressed ones had the highest protein content and lowest insoluble fibre content. The solvent-extracted press-cake had the lowest dry matter and fat content. The fermentation with the cold-pressed and hulled cake led to the lowest pH and it had the shortest lag phase. It was also the most appetising looking, being beige while the others were brown. The methods showed whey affects both the stability and the fermentation, mainly by decreasing the pH further. The homogenisation influenced the stability, but it was not conclusive. In the future, it is recommended to try another lactic acid bacteria to see if it is possible to find a more suitable one. It is also recommended to analyse if the cold-pressing or dehullisation are affecting the results the most.

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1 Introduction

In the past decade, plant-based protein has become an important part of many people's diets. One of the reasons is that the consumption of a diet rich in plant-based proteins lowers the risk of for example getting cancer, diabetes, and heart-related disorders. (Kumar et al. 2022) At the same time, plant-based proteins often are a more climate-friendly choice. Compared to meat and dairy, plant-based proteins have a lower climate impact and use less land, water, and energy to produce. (Karlsson Potter and Röös 2021) Another way to create climate-friendly and sustainable food products is to use waste products. An example of a plant-based by-product is the sunflower press cake (SFPC). It is left after the production of sunflower oil and is currently often sold as animal feed or as a raw material to produce biofuel. (Gültekin Subaşı et al. 2021) The high protein content, low traces of antinutritive contents as well as the absence of toxic substances are valuable characteristics, making the press cake a possible substitute for soy within the alternative-protein industry. (Pickardt et al. 2014)

The SFPC can have different compositions depending on the source, for example, if the sunflower seeds are hulled or dehulled but also on the different processes used during oil extraction. It will determine both the nutritional composition and physicochemical properties. (Rataj 2021) The dehulled press cakes have lately gotten more attention. It is higher in protein and lower in fat, causing farmers to be able to sell the dehulled SFPC for a higher price. Also, the hulls can be sold and used as an energy source instead of fossil fuels. However, the driving economic factor is the oil, leading the profit from the hulls only to be marginal. Together with the need for an extra processing step to dehull the seeds are the demand among companies not strong. (Dauguet et al. 2016)

A process for oil extraction that is becoming more popular is cold pressing. It includes pressing and sometimes filtering the oil but the use of organic solvents as well as heating is prohibited. This leads to the conservation of naturally present nutritional components in the seeds, such as polyphenols, chlorophylls, and carotenoids. (Dordevic et al. 2020) Warm-pressed oil extraction is like cold-pressed but heat above 50°C is also used to increase the yield; often also the seeds can be pre-roasted. If the press-cake is used after pressing, for example as animal feed, the heat should be optimised between a high temperature for increased oil yield and a low temperature to not affect the protein quality or oxidise the phenolics. (Frandsen et al. 2019) The last commonly used process for oil extraction is solvent extraction, which is the final stage after the mechanical pressing from the previous steps. The most common solvent to use is hexane and the extraction itself is considered a mass transfer where the oil is extracted from the pores of the plant particles to the liquid bulk. (Baümler, E., Carrín, M., Carelli, A. 2016)

Current research in the food sector addresses protein properties and how they can be modified by mechanical, thermal, enzymatic, or biological treatment to increase the nutrient content as well as the bioavailability. (Scharff et al. 2022) By applying homogenisation, a processing step which uses pressure to break particles into smaller pieces, to dispersions it is possible to modify the physicochemical properties and inactivate microbes. (Escobar Gianni et al. 2020) Another way to modify a product is to use lactic acid bacteria (LAB) to ferment it. This could be used both to enhance the flavour and for nutritional aspects. LAB can produce secondary metabolites with natural preservative properties, peptides with health beneficial attributes, and exopolysaccharides

that allow modifying the texture and sensory features of the final products. (Madsen Kjærulf et al. 2021)

One of the most common fermented products eaten today is yoghurt, making it suitable to compare it to a fermented SFPC dispersion. Conventional yoghurt is defined as a fermented milk product which has a pH lower than 4.5 and a final LAB density higher than 10^8 cfu/g. The strains used are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. (Montemurro et al. 2021) For plant-based yoghurt products, the strain *Lactiplantibacillus plantarum* has recently been used with positive results. The fermentation has led to increased bacteria concentration and increased antioxidant activities. (Jin Hur et al. 2014, Canaviri Paz et al. 2020, Hang et al. 2020, Nawal et al. 2022) *L.plantarum* is a versatile bacterium which can be found in many different environments and for different food applications. It is classified as "generally recognized as safe," and has a "qualified presumption of safety". (Hang et al. 2020, Solval et al. 2019) It is a bacterium which only grows in a rich medium containing a carbon source, amino acids, peptides, fatty acids, vitamins, and nucleic acids, and because of this has become auxotrophic for several vitamins and amino acids. (Teusink, 2006) Nonetheless, fermentation with *L.plantarum* has been shown to increase the concentration of free amino acids, soluble fibres and total phenols. This leads to higher protein digestibility as well as an increased nutritional value of the final product. The increased viscosity of conventional yoghurt is because the pH is lowered beyond the isoelectric point of the caseins, causing them to gelatinize into a cohesive protein network. This is known to be one of the main challenges when producing a plant-based yoghurt. The proteins present in plant sources are not affected by acidification, causing them not to gel. (Montemurro et al. 2021)

Whey, another waste product in abundance, is a carbohydrate-rich yellowish liquid that is left after cheese production. (Mabrouki et al. 2022) There are two different types of whey; sweet whey, which is obtained after enzymatic coagulation of dairy products and sour whey, which is from cottage cheese production. (Szudera-Kończal et al. 2020) It has been shown that a combination of whey and sunflower press-cake can become a balanced nutrient-rich raw material, being high in protein, fibre, and several essential nutrients. (Rataj 2021) Except for increasing the nutritional content, the addition of whey is also a possible way to increase the viscosity. Whey is known to be accessible and efficient during microbial fermentation, mainly because it is rich in lactose, nitrogenous compounds, and other nutrients. (Nawal et al. 2022)

1.1 Aim

The aim of the study is to ferment four different sunflower press cakes (SFPC) and compare the results regarding the change in pH, viscosity, and stability as well as connect it to the difference in nutritional content between the SFPC. How the addition of whey and homogenisation affects the pH, viscosity and stability will also be analysed and compared to the pure SFPC samples. The goal is to find the press cake and methods that give the products after fermentation both an increase in stability (being less separated with time) and in viscosity, for sensory reasons.

2 Materials and methods

2.1 Sunflower press cakes

During this project, four different types of sunflower press cakes were used. They were from three different companies, but all underwent different processing during oil manufacturing and therefore varied in their basic compositions. The first type was from VivaOrganic GmbH, Cloppenburg, Germany (VO) and contained 10% hulled and 90% dehulled sunflower seeds. It was heated before pressing with steam (80°C). The second one was from Schalk Mühle GmbH & Co KG, Ilz, Austria (SM) and was already a powder since the company sold it as a protein powder after cold oil pressing (55-65°C). This one was made from dehulled sunflower seeds. The third and fourth ones were both from Cargill GmbH, Riesa, Germany and are named C1 and C2 in the report. Both are made from hulled sunflower seeds but C1 is warm pressed (75-80°C) and C2 is warm pressed but also solvent-extracted with hexane.

Before the press cakes could be used, they had to be ground with the ultra-centrifugal mill ZM 2000 (Retsch GmbH, Haan, Germany). VO, C1, and C2 were first ground to a size smaller than 1.0 mm and then 0.2 mm, but since SM already was a powder, it was ground directly to a size smaller than 0.2 mm. All powders were then stored at room temperature until use.

2.2 Sweet whey

The sweet whey used for the project is called *Bayolan sweet whey powder halal and kosher* and was provided by Bayerische Milchindustrie eG. The nutritional content can be seen in *Table 1* below.

Table 1. Shows the nutritional content of the sweet whey powder.

Protein content, %	*12.9*
Lactose, %	*≥ 72*
Ash, %	*7.1*
Fat, %	*0.9*
Dry matter, %	*98.9*

2.3 Dry matter

The dry matter was determined by first heating the crucibles for 1h at 103°C to remove all potential contaminants. The crucibles were then cooled down in a desiccator for 30 minutes. The next step was to weigh the crucibles and then add approximately 1g of sample. All samples were performed in triplicates and dried in the oven (Memmert, Fisher Scientific GmbH, Swords, Germany) at 103°C. After 3h the samples were put into a desiccator to cool down and then they were weighed again before being put into the oven, to see whether a constant dry mass was achieved. After a

minimum of 1h, the samples were put into the desiccator to cool down for the last time and then weighed. All weights were used to calculate the dry matter with *Equation 1*.

Dry matter, % = (1 - (Weight after drying - Crucible) / (Weight before drying - Crucible)) • 100 (1)

2.4 Protein

The protein contents were determined with the Kjeldahl method. First, approximately 5 g of catalysator (1 kg K_2SO_4 + 25 g $CuSO_4 x 5H_2O$), approximately 0.3 g of sample, and 10 ml of sulphuric acid 96% were added to the Kjeldahl flasks. All samples were performed in triplicate. The flasks were then slowly heated in a block (K-436 Büchi, Flawil, Switzerland) for 2h until all samples had turned bright green. Then the samples were put into a distillation plant (Distillation Unit B-234, Büchi, Flawil, Switzerland) to be distilled. The distilled samples were then mixed with 3 drops of indicator (methyl red + methyl blue). The last step was then to titrate the distilled samples with 0.1 mol/L HCl until the colour changed from blue/ green to colourless, just before reaching purple. The sample weight and titrated amount were used to calculate the protein concentration with *Equation 2*.

Protein, % = (Titrated amount sample - Titrated amount blank) • 14.008 • 0.1 • 6.25 / (Sample weight • 10) (2)

The protein solubility was determined by first weighing approximately 1 g of sample and mixing it with 50 ml of deionised water in a cup. The pH was measured and adjusted to approximately pH 7 or 9, depending on the trial, with NaOH or HCl. The water-sample mix was then stirred for 2h by stirring at 250 rpm using (Comet CIMAREC Multipoint, ThermoFisher Scientific, Waltham, USA). After being stirred the samples were centrifuged for 30 min at 500 g and 4°C. Then the supernatant was separated and used for the Kjeldahl method, as explained above, but with 5 mL of supernatant instead of 0.3 g of sample and 0.01 mol/L HCl instead of 0.1. All samples were performed in duplicate. The water volume, sample weight and titrated amount were used with *Equation 3* to determine the protein solubility. *Amount soluble protein* and *amount total protein* are calculated using *Equation 2*.

Protein solubility, % = Amount soluble protein • Volume water • 100 / (Amount total protein • Sample weight) (3)

To compare the soluble protein content is also the adjusted soluble protein calculated according to *Equation 4*. It is defined as the soluble protein per 100 g of the powdered product instead of soluble protein per 100 g of protein in the product.

Adjusted protein solubility, % = Protein solubility • Amount total protein / 100 (4)

2.5 Fat

The fat content was determined by first adding 4-5 stones to the glass cups, heating the set up for 2h at 103°C in order to remove all potential contaminants. After the glass cups and stones had been

cooled down in a desiccator for 30 minutes the set-up was weighed. Then approximately 5g of sample was filled into a cellulose filter tube. All samples were measured in duplicate. Inside each cellulose filter, a ball of glass fibre was added for the sample not to spill out later on. 150 ml of petroleum ether was added to each glass cup before being put into the Soxtherm Apparatus (C. Gerhardt GmbH & Co. KG, Königswinter, Germany).

The software (Soxtherm Manager, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) was used to start the extraction method. First, there was a 30-minute cooking phase. Second, a part of the petroleum ether was distilled off with a reduction interval of 3 min and 30 s until approximately one cm was below the sleeve. This was followed by the extraction phase at 150°C with 1 h 20 min of subsequent distillation of the excess petroleum ether. When the program was done the original set-up, now also containing oil, was put into the oven (Memmert, Fisher Scientific GmbH, Swords, Germany) for 1h at 103°C before being weighed again. The weights were used with *Equation 5*.

Fat content, % = (Set up weight with oil - Set up weight) / Sample weight • 100 (5)

2.6 Ash

The ash was determined by first heating the cups for 1h at 550°C in the oven (Nabertherm GmbH, Lilienthal, Germany) to remove all potential contaminants. After the cups had been cooled down in a desiccator for 30 minutes the cups were weighed and approximately 1g of sample was added. All samples were performed in triplicates and then put into the heater at 550°C. After 3-4h the samples were taken out and put into a desiccator to cool down before being weighed again. All weights were used to calculate the ash content with *Equation 6*.

Ash content, % = (Weight after burning - Cup) / (Weight before burning - Cup) • 100 (6)

2.7 Fibre

The fibre content was determined by enzyme kit (Total Dietary Fiber Assay Kit K-TDFR-100A, Megazyme Ltd., Wicklow, Ireland) according to AOAC Method 991.43 and the use of the previously mentioned analyses Ash and Protein. First, the samples in quintuplicate were weighted to 1.000g and mixed with approximately 40 ml of deionised water and 50 µl of enzyme α amylase. Also, two blanks were prepared. The samples were then put into a 95°C hot shaking water bath (GFL 1983, Lauda Society for Laboratory technique mbH, Burgwedel, Germany) for 35 min before being cooled down to 60°C. The pH was measured and corrected to approximately 7.5 before 100 ml of protease was added. In the meantime, the water bath was cooled down to 60°C and then the samples were put inside for 30 minutes. Then, amyloglucosidase was added and the samples were put back into the water bath for 30 minutes.

After the enzyme process was finished the filtration was started. The first step was to preheat the filters for 1h at 103°C, let them cool down and then measure the weights. Then, the samples were filtered and cleaned with 10 ml of deionised water. The next step depends on whether soluble or insoluble fibre was analysed. For insoluble fibre, the filter and the remaining sample were cleaned with 10 ml 95% ethanol and acetone twice. For soluble fibre, the filtrate from insoluble fibre (before the cleaning steps) was used and 95% ethanol was added. The samples were then stored

for 1h before being filtered and cleaned with 15 ml of 78% ethanol, 95% ethanol and acetone twice. When all samples were done, they were heated to 103°C for 1h before being weighed.

The last steps were then to use the filtrate with samples for either protein determination or ash, as explained above. All results were used with *Equation 7*.

Fibre content, % = ((Residue weight 1 + Residue weight 2) / 2 - Ash content - Protein content - Blank) / ((Sample weight 1 + Sample weight 2) / 2) • 100 (7)

The blank in *Equation 7* is calculated according to *Equation 8* below.
Blank = (Residue weight 1 + Residue weight 2) / 2 - Ash content - Protein content (8)

2.8 Homogenisation/ pre mixing

Before the homogenisation, the press cake mixtures were prepared. A 10% or 15% dry matter of press cake was used. When whey was used it was pre-mixed with water to a 6% dry matter, meaning the total mixture had an approximate dry matter of 15-20%. The mixture was mixed with the Ultra-Turrax (Ika-Labortechnik, Janke and Kunkel GmbH, Staufen, Germany) for 5 minutes at 10 000 rpm to make the particle sizes even smaller. To further improve the stability half of the dispersions were homogenised with a two-stage homogenizer (APV 2000, SPX Corporation, Charlotte, North Carolina, United States) at 80 MPa (first stage 65 MPa and second stage 15 MPa). Time and throughput were not measured. It is assumed that homogenisation was performed at room temperature.

2.9 Sterilisation

After the homogenisation, the prepared mixtures were sterilised by heating them to 85°C for 10 minutes to prevent other microbes to grow during the fermentation. The reason the samples were not autoclaved was that previous tests had shown the press cakes to become very gel-like upon too high heating. After sterilisation, the samples were stored at 4°C until used.

2.10 Physical stability

Throughout the project, several stability tests were performed. Both to check if the 85°C heating had an effect on the samples as well as to see if there was a change after fermentation. The samples were centrifuged for 60 min at 3000 rpm and 24°C according to Jeske et.al (2019). Then the supernatant was removed. The weight of the sample and the weight of the supernatant were used according to *Equation 9* to calculate a percentage of stability where a high percentage meant more separation, thus a more unstable sample. The values before and after fermentation were then compared.

Stability, % = Supernatant/ Total sample (9)

2.11 Viscosity

The viscosity was measured before and after fermentation to see if there was a viscosity increase, as often seen in yoghurt. The viscosity was measured with Rotary Rheometer Physica Anton Paar (Thermo Fisher Scientific, Waltham, USA). The concentric cylinder geometry CC25 DIN / Ti 02180077 (Thermo Fisher Scientific, Waltham, USA, do = 34 mm, di = 31.4 mm, h = 47.1 mm) was used for all measurements. Also, 22 ml of sample was used every time and the settings were set at 0.01 for 1 min and logarithmic increase from 0.01 to 1000 with every measurement taking 15 s. All tests were performed at 25°C.

The viscosity data is presented in two ways. One is the exponentially decreasing power law as seen in *Equation 10* where a is a constant and b is the law's exponent. The other presentation way is shown by first plotting the data double logarithmic, thus making it linear. From this is it possible to calculate the slope of the data from *Equation 11* where c is the slope and d is the value where the graph crosses the y axis.

$$Y = ax^{-b} \tag{10}$$
$$Y = cx + d \tag{11}$$

2.12 Preparation of *L.plantarum*

At the beginning of the project, the lactic acid bacteria were prepared. The MRS Vegetone was adjusted to pH 6.2 before pouring 50 ml into a small flask. Two cryo pearls were added to the flask and the starter culture was inoculated in an anaerobic chamber for two days at 30°C. 50 ml of the starter culture was then poured into a larger flask and mixed with MRS Vegetone to a concentration of 1%. The culture was inoculated in an anaerobic chamber overnight at 30°C. The optical density was checked before pouring 1.5 ml of the culture into tubes. The tubes were stored at -78°C until usage.

2.13 Fermentation

The fermentation started after the pre-measurements of the mixtures by adding 1.5 ml of prepared *L.plantarum* to 85 ml of the respective mixture in an autoclaved cup. The fermentation was done in duplicates in two different batches. Before every fermentation started the pH loggers were calibrated at pH 4 and 7. Afterwards, the samples were put into a 30°C-water bath for 24 h with constant stirring at 150 rpm using (Comet CIMAREC Multipoint, ThermoFisher Scientific, Waltham, USA). During the time the pH was logged every 3 minutes. From the data was the rate of acidification calculated with the slope function in excel.

3 Results

3.1 Data for raw material

The four different press cakes are compared throughout the project, and it starts with the nutritional content. As seen in *Table 2* there is a great difference between the press cakes, the major ones

being protein and fat content. Also, for soluble protein and both fibre types, large differences can be seen.

VO is in the middle for most data. SM has the highest protein and ash content as well as the highest protein solubility at both pHs. It is also the press cake that differs in colour, being beige while the others are brown/grey. C1 has the highest dry matter, fat and insoluble fibre content while having the lowest soluble fibre content. C2 has the lowest dry matter and fat content. It is also very near C1 in fibre content.

Table 2. The nutritional content of the four sunflower press cakes. The percentages are in w/w. All analyses were performed in duplicate or triplicate and then a mean value was calculated as well as the standard deviation of the mean. Soluble protein is per 100 g of protein and adjusted soluble protein is per 100 g of powder.

Type	*VO*	*SM*	*C1*	*C2*
Dry matter, %	*96.1 ± 0.17*	*93.7 ± 0.24*	*97.8 ± 0.16*	*92.2 ± 0.040*
Protein, %	*35.5 ± 0.43*	*48.9 ± 0.31*	*24.8 ± 0.24*	*31.2 ± 0.70*
Fat, %	*6.47 ± 0.064*	*9.16 ± 0.051*	*19.6 ± 0.052*	*0.663 ± 0.013*
Ash, %	*5.41 ± 0.082*	*7.18 ± 0.055*	*5.21 ± 0.063*	*7.03 ± 0.090*
Soluble protein, % (pH 7)	*3.93 ± 0.37*	*10.4 ± 0.060*	*7.42 ± 0.32*	*9.68 ± 0.60*
Adjusted soluble protein, % (pH 7)	*1.40 ± 0.13*	*5.08 ± 0.030*	*1.84 ± 0.080*	*3.02 ± 0.19*
Soluble protein, % (pH 9)	*18.2 ± 0.61*	*34.1 ± 0.19*	*21.1 ± 0.80*	*13.3 ± 0.35*
Adjusted soluble protein, % (pH 9)	*6.44 ± 0.22*	*16.7 ± 0.20*	*5.24 ± 0.20*	*4.16 ± 0.11*
Insoluble fibre, %	*37.7 ± 1.24*	*26.7 ± 0.59*	*42.5 ± 2.69*	*42.5 ± 1.3*
Soluble fibre, %	*4.17 ± 0.50*	*5.52 ± 0.097*	*1.13 ± 0.50*	*1.64 ± 0.51*

3.2 pH change

3.2.1 No whey no homogenisation

The pH change for the four press cakes without whey and without homogenisation during fermentation can be seen in *Table 3*. The lowest start pH is seen for VO, which also has the lowest end pH. When looking at the graphs for the fermentation (*Figure 1 below* and *Figure A1* in the appendix) it can be seen that the slope plans out later for VO compared to the other press cakes. This also means that the pH might have been even lower if more time was allowed. SM has the highest start pH but the second-lowest end pH. C2 has the highest end-pH.

When looking at "time of the lag phase" and "time to lower the pH with 1" the results are correlating. SM has the lowest time for both, then C1, VO and last C2. In general, the lag phase is 4.5h and it takes an additional 5-6h to lower the pH with one. The differences in the additional time to lower the pH can be explained by the rate of acidification, which is the maximum slope during the fermentation. The slope is the highest for SM and the lowest for C2.

Table 3. Calculated values from both batches of fermentation of SFPC no whey no homogenisation. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The time of the lag phase is determined by the time when the start pH has lowered by 0.1.

No w no h	*VO*	*SM*	*C1*	*C2*
Start pH	*6.0 ± 0.1*	*6.3 ± 0.05*	*6.1 ± 0.03*	*6.2 ± 0.07*
End pH	*4.3 ± 0.04*	*4.4 ± 0.2*	*4.6 ± 0.07*	*5.1 ± 0.06*
Time of lag phase, h	*4.92 ± 0.70*	*4.36 ± 0.64*	*4.41 ± 0.27*	*5.04 ± 0.30*
Rate of acidification, pH/h	*-0.240 ± 0.0076*	*-0.321 ± 0.027*	*-0.295 ± 0.017*	*-0.224 ± 0.038*
Time to lower pH with 1, h	*10.7 ± 0.76*	*9.26 ± 0.21*	*9.34 ± 0.18*	*11.8 ± 0.59*

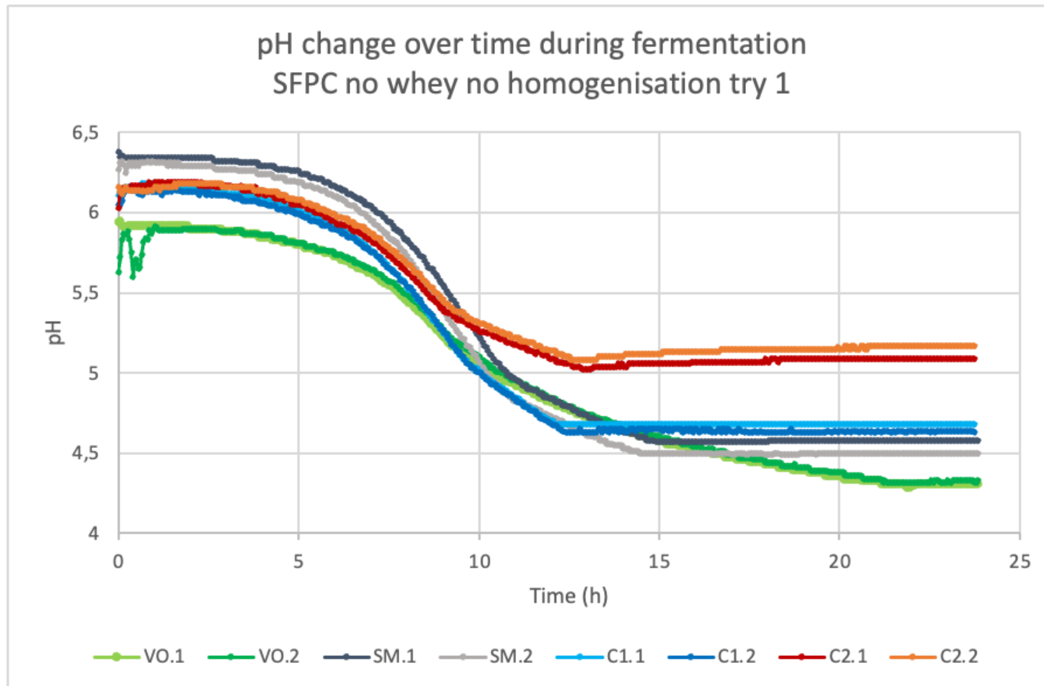


Figure 1. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were not homogenised before fermentation, also no whey was added. This was the first try. The green samples are VO, black/ grey are SM, blue are C1 and red/orange are C2.

3.2.2 With whey no homogenisation

In *Table 4* the pH change for the four press cakes with whey and without homogenisation during fermentation is shown. A difference can be seen between the samples where the biggest difference is between C2 and SM where C2 did not have as much decrease in pH as the rest of the samples and SM had more of a decrease than the rest. The absolute change in pH for SM and C2 was 2.0 and 1.3 respectively.

When comparing the data to *"SFPC without whey without homogenisation"* it is clear that the whey has an effect. The end-pH went from 4.3, 4.4, 4.6, and 5.1 to 4.3, 4.1, 4.3, and 4.7. Two aspects to note are the small difference between the VO samples with and without the whey as well as the big difference between the C2 samples. In addition, it is also interesting that SM reached a lower pH than VO and is thus the sample with the lowest end-pH of all four press cakes.

The time of the lag phase and time to lower the pH with 1 is still correlating even if it has an exception, as seen in *Table 4*. The order of time of lag phase (lowest to highest) is C1, VO, C2 and SM while the order of time to lower pH with 1 is C1, SM, VO and C2. This means that all samples stayed the same except for SM which had a long lag phase but then lowered the pH quickly. This is also seen in the rate of acidification, where SM has the highest rate and VO has the lowest.

Table 4. Calculated values from both batches of fermentation of SFPC with whey no homogenisation. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The time of the lag phase is determined by the time when the start pH has lowered by 0.1.

With w no h	*VO*	*SM*	*C1*	*C2*
Start pH	*5.9 ± 0.03*	*6.1 ± 0.03*	*6.0 ± 0.02*	*6.0 ± 0.01*
End pH	*4.3 ± 0.06*	*4.1 ± 0.04*	*4.3 ± 0.09*	*4.7 ± 0.09*
Time of lag phase, h	*4.74 ± 0.17*	*5.19 ± 0.17*	*4.40 ± 0.20*	*4.79 ± 0.095*
Rate of acidification, h^-1	*-0.141 ± 0.17*	*-0.308 ± 0.0068*	*-0.236 ± 0.021*	*-0.204 ± 0.0088*
Time to lower pH with 1, h	*11.8 ± 0.29*	*9.90 ± 0.23*	*9.81 ± 0.44*	*11.9 ± 0.37*

3.2.3 No whey with homogenisation

The pH change for VO and SM during fermentation without whey and with homogenisation can be seen in *Table 5*. Due to blockage of the homogeniser, thus leading to no sample coming out, there is no data for C1 and C2. It is assumed the press cakes would have the same correlations as the non-homogenised samples.

The main differences between the samples are the start pH, rate of acidification and the time to lower pH with 1. The start pH is higher for SM, the rate of acidification is faster for SM and the time to lower with 1 is shorter for SM. When compared with the previous trials is it interesting to note that SM reaches the lowest pH when combined with whey, but without whey, the lowest pH

is reached by VO. Homogenisation has no effect on the outcome. The absolute change is however always greater for SM.

Table 5. Calculated values from both batches of fermentation of SFPC no whey with homogenisation. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The time of the lag phase is determined by the time when the start pH has lowered by 0.1. The data for C1 and C2 are missing due to a problem with the homogeniser.

No w with h	*VO*	*SM*
Start pH	*5.9 ± 0.02*	*6.3 ± 0.06*
End pH	*4.3 ± 0.02*	*4.4 ± 0.07*
Time of lag phase, h	*4.52 ± 0.13*	*4.73 ± 0.66*
Rate of acidification, h^-1	*-0.224 ± 0.011*	*-0.341 ± 0.019*
Time to lower pH with 1, h	*10.7 ± 0.10*	*9.89 ± 0.25*

3.2.4 With whey with homogenisation

In *Table 6* the pH change for fermentation *"with whey with homogenisation"* is seen. The data for C2 is missing and there are only two fermentations for C1 due to a problem with the homogeniser, as mentioned earlier. SM has the highest start pH, and rate of acidification as well as the lowest end pH and time to lower the pH to 1. VO has the lowest start pH, rate of acidification, time of lag phase and time to lower pH with 1 as well as the highest end-pH. C1 has the lowest time of lag phase.

When looking at *Figure 2* below and *A6* in the appendix is it clear that the pH could have been even lower for the samples if the time for the fermentation would have been prolonged. When comparing all samples from all fermentations by how much the curves have flattened by the end of the fermentation it can be seen that the whey causes a longer fermentation. Homogenisation has no effect on the outcome.

Table 6. Calculated values from both batches of fermentation of SFPC with whey with homogenisation. All values are the average from all fermented samples (duplicates on both batches except for one batch of VO when there was only one sample) with the standard deviation of the mean. The time of the lag phase is determined by the time when the start pH has lowered by 0.1. The data for C2 is missing due to a problem with the homogeniser.

With w with h	*VO*	*SM*	*C1*
Start pH	*5.9 ± 0.02*	*6.2 ± 0.05*	*6.0 ± 0.007*
End pH	*4.3 ± 0.03*	*4.1 ± 0.04*	*4.2 ± 0.01*
Time of lag phase, h	*5.49 ± 0.29*	*5.01 ± 0.54*	*4.83 ± 0.25*

Rate of acidification, h^-1	*-0.190 ± 0.011*	*-0.312 ± 0.015*	*-0.244 ± 0.0076*
Time to lower pH with 1, h	*12.6 ± 0.31*	*9.59 ± 0.48*	*10.3 ± 0.21*

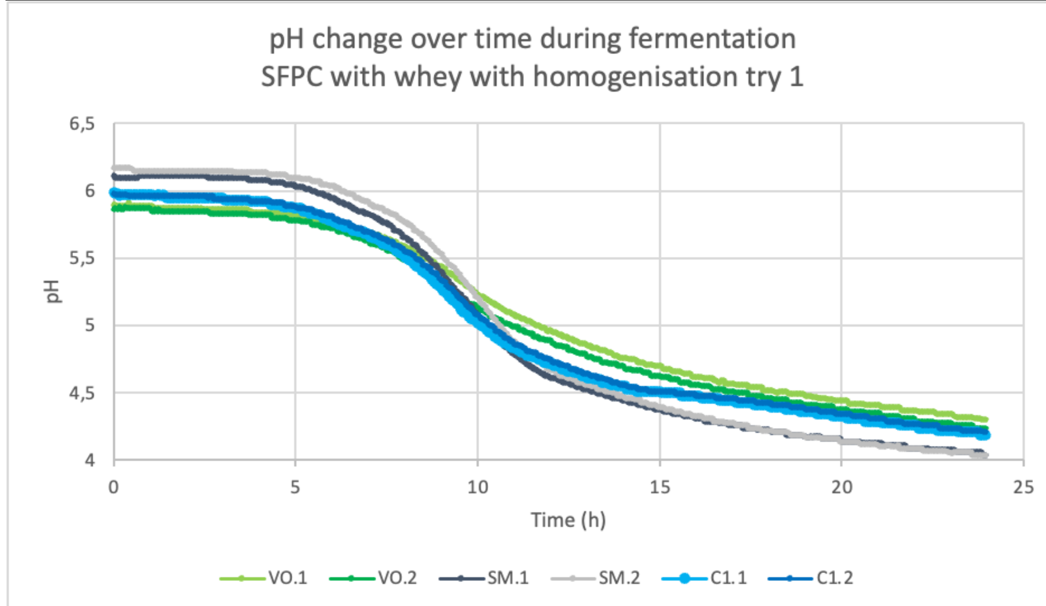
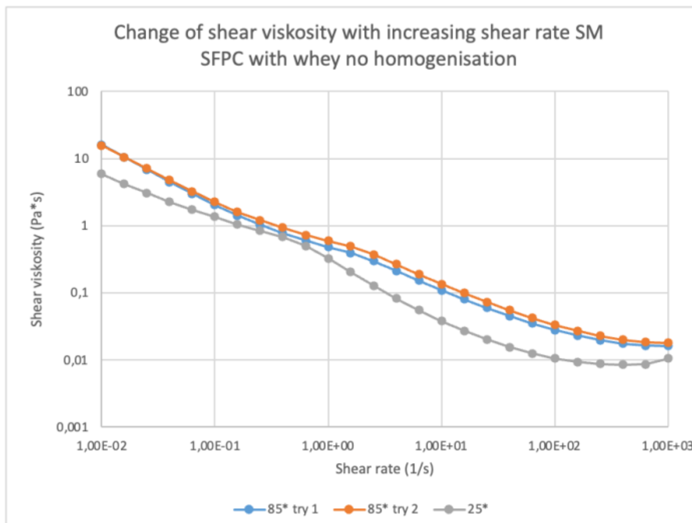


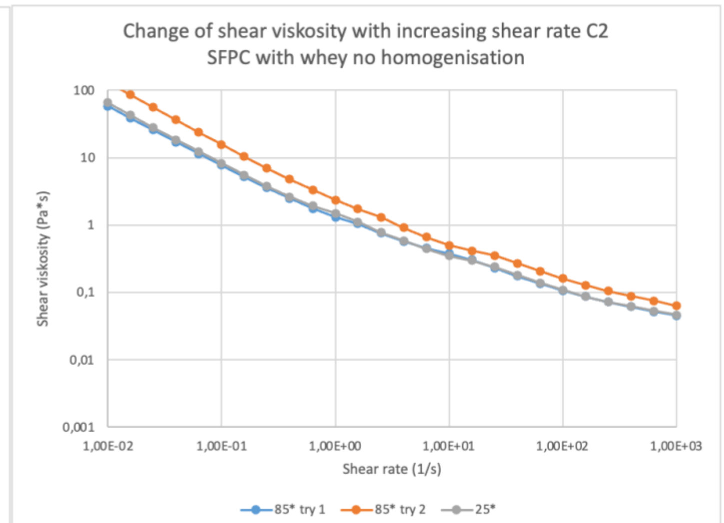
Figure 2. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were homogenised before fermentation and whey was added. This was the first try. The green samples are VO, black/ grey are SM and blue are C1.

3.3 Viscosity

3.3.1 Viscosity change with heating

The first viscosity experiment can be seen in *Figures 3a* and *b* below as well as in *Figures A9a* and *b* in the appendix. These were performed to check how much the sterilisation (85°C for 10 min) affected the press cakes. All the tests were performed before fermentation. It can be noted that, in general, there is not much of a difference between the heated and non-heated samples. There is however a clear difference for SM, which is shown as the grey line (25°C) being further away from the 85°C samples compared to the other press cakes. For C2 it can also be noted that there is a difference between the first and second try with 85°C. The reason for this is unknown.





Figures 3a and b. Shows the shear viscosity plotted against the shear rate for SFPC SM and C2 (10 % w/w) at temperatures 85 ℃ and 25 ℃. These samples were not fermented or homogenised. Also, no whey was added.

For almost all viscosity trials, no matter the SFPC or method, a bump can be seen in the middle of the graph before being flattened again. In *Figures 12a* and *16a* in the appendix, however, two spikes can be seen.

3.3.2 No whey no homogenisation

In *Table 7* the viscosity before and after fermentation can be seen for all four press cakes without whey and without homogenisation. For all measurements, all values are lower after fermentation, except for the slope of C2. The slope after fermentation for C2 does however have a quite large error bar. The negative slope seen for all four samples means that the press cakes have a shear thinning or pseudoplastic behaviour.

C2 has the highest start value, followed by VO, C1 and last SM, meaning C2 is the thickest and SM the thinnest when not using any force. The press cakes' start values have the same order both before and after fermentation. However, the slope does not follow the same trends. SM has the greatest slope before fermentation but the lowest after fermentation and C2 has the second to lowest slope before fermentation but the greatest one after. As mentioned before, the slope after fermentation has a large error bar, meaning that the conclusions are unsure. The changes in the order of the slopes mean that the fermentation led SM to go from the most shear thinning behaviour to the least while C2 went from almost the least to the most.

Table 7. Calculated values for the viscosity before and after fermentation for No whey no homogenisation for all four press cakes. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The slope is from the double logarithmic plots and the start value is the value when crossing the y-axis.

No w no h		*VO*	*SM*	*C1*	*C2*
Slope, kg/m	*Before ferm*	*-1.42 ± 0.022*	*-1.95 ± 0.027*	*-1.61 ± 0.026*	*-1.47 ± 0.013*
	After ferm	*-0.623 ± 0.57*	*-0.121 ± 0.023*	*-0.286 ± 0.10*	*-2.40 ± 2.6*
*Start value, Pa*s*	*Before ferm*	*56.5 ± 21*	*5.88 ± 1.6*	*15.6 ± 6.1*	*107 ± 25*
	After ferm	*13.4 ± 13*	*2.45 ± 0.64*	*5.99 ± 2.2*	*53.0 ± 57*

3.3.3 With whey no homogenisation

The viscosity before and after fermentation for all four press cakes with whey and without homogenisation can be seen in *Table 8*. As with the previous results, most data decreased after fermentation. The exception is the power law for SM where the exponent went from -0.524 to

-0.548. However, it is a very small increase and the constant number in the power-law decreased significantly.

As with *"no whey no homogenisation",* C2 has the highest start value, followed by VO, C1 and last SM. The press cakes' start values have the same order both before and after fermentation. Also, here the slope does not follow the same trends. SM has the greatest slope before fermentation but the lowest after fermentation and C2 has the second to lowest slope before fermentation but the greatest one after.

Table 8. Calculated values for the viscosity before and after fermentation for With whey no homogenisation for all four press cakes. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The slope is from the double logarithmic plots and the start value is the value when crossing the y-axis.

With w no h		*VO*	*SM*	*C1*	*C2*
Slope, kg/m	*Before ferm*	*-1.47 ± 0.028*	*-1.90 ± 0.062*	*-1.67 ± 0.084*	*-1.48 ± 0.029*
	After ferm	*-0.550 ± 0.018*	*-0.517 ± 0.038*	*-0.535 ± 0.022*	*-0.622 ± 0.018*
*Start value, Pa*s*	*Before ferm*	*50.2 ± 9.1*	*6.14 ± 2.0*	*13.5 ± 12*	*85.3 ± 19*
	After ferm	*7.24 ± 2.0*	*3.57 ± 1.5*	*4.43 ± 1.9*	*67.2 ± 35*

3.3.4 No whey with homogenisation

In *Table 9* the viscosity before and after fermentation without whey and with homogenisation can be seen for VO and SM. As mentioned earlier, there was a problem with the homogeniser and thus there is no data for C1 and C2.

For VO it can be seen that the power-law and slope slightly increased during fermentation, while they decreased for SM. The start values decreased for both. This means that the viscosity when no force is applied became thinner for both. The increase in slope after fermentation for VO means that it gets more of a thinning behaviour as force is applied.

Table 9. Calculated values for the viscosity before and after fermentation for No whey with homogenisation. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The data for C1 and C2 is missing due to a problem with the homogeniser. The slope is from the double logarithmic plots and the start value is the value when crossing the y-axis.

No w with h		*VO*	*SM*
Slope, kg/m	*Before ferm*	*-1.47 ± 0.0083*	*-1.75 ± 0.12*
	After ferm	*-1.59 ± 0.017*	*-0.40 ± 0.040*

Start value, Pa*s	Before ferm	51.2 ± 9.4	12.9 ± 6.8
	After ferm	35.6 ± 6.8	8.68 ± 0.88

3.3.5 With whey with homogenisation

The viscosity before and after fermentation with whey and with homogenisation can be seen in *Table 10*. The data for C2 is missing due to a problem with the homogeniser. The data is like the previous test, with also this time most values decrease with fermentation. For VO there is however one exception with the slope.

When comparing all methods, it is difficult to draw conclusions. The power law is mostly the greatest for *with whey with homogenisation* and the lowest for *with whey no homogenisation*. Both have several exceptions. For the slope there is no clear difference, but the start-values are in general lower for the two trials without homogenisation. The whey increases the start values, but it is not conclusive.

Table 10. Calculated values for the viscosity before and after fermentation for With whey with homogenisation. All values are the average from all fermented samples (duplicates on both batches except for one batch of VO when there was only one sample) with the standard deviation of the mean. The data for C2 is missing due to a problem with the homogeniser. The slope is from the double logarithmic plots and the start value is the value when crossing the y-axis.

With w with h		*VO*	*SM*	*C1*
Slope, kg/m	*Before ferm*	*-1.42 ± 0.018*	*-1.59 ± 0.087*	*-1.44 ± 0.044*
	After ferm	*-2.39 ± 0.75*	*-0.98 ± 0.94*	*-0.783 ± 0.14*
*Start value, Pa*s*	*Before ferm*	*97.6 ± 17*	*29.7 ± 14*	*45.2 ± 4.3*
	After ferm	*53.1 ± 16.5*	*11.4 ± 8.9*	*17.4 ± 3.0*

3.4 Physical stability

Table 11 shows the results from the stability tests for the four press cakes and the four methods. As mentioned earlier, some data is missing due to a problem with the homogeniser. The percentage shows how much supernatant got separated after centrifugation and a low percentage or decrease after fermentation (positive change) is thus wanted. The standard errors of the mean for the change are often greater than the change itself thus leading to a big uncertainty when analysing the results.

When looking at the results for VO it can be seen that the stability decreased the most for *no whey no homogenisation* and the only increase was for *with whey no homogenisation*. When comparing adding whey to homogenisation, the whey increased the stability during fermentation more. However, the absolute percentage of separation after fermentation is lowest for the samples with homogenisation.

The results for SM show three out of the four samples increasing stability with fermentation. The samples having the greatest stability increase are *with whey no homogenisation* and *no whey with homogenisation*. As for VO, the homogenised SM samples are having the lowest percentage of separation after fermentation. The same can be seen for C1. However, when looking at the change is *with whey no homogenisation* the only sample which increased stability during fermentation.

For C2 *with whey no homogenisation* is the only sample which increases stability. Because of the missing data, it is difficult to draw further conclusions. It is, however, assumed all press cakes behave the same after the application of the different methods. It can therefore be concluded that the homogenised samples were always more stable after fermentation and most often more stable before fermentation, compared to the non-homogenised samples.

Regarding the change, *no whey no homogenisation* is always having a negative effect while *with whey no homogenisation* is having a positive effect. For the other two methods, no conclusion can be drawn.

Table 11. Calculated values for the stability tests before and after fermentation for all processes for all four press cakes. All values are the average from all four samples (duplicates on both batches except for one batch of VO when there was only one sample) with the standard deviation of the mean. The data for No whey with homogenisation for C1 and C2 as well as With whey with homogenisation for C2 is missing due to a problem with the homogeniser.

		No whey no homogenisation	*With whey no homogenisation*	*No whey with homogenisation*	*With whey with homogenisation*
VO	*% before*	*40.7 ± 6.33*	*54.9 ± 2.18*	*45.4 ± 3.7*	*40.3 ± 2.02*
	% after	*57.3 ± 10.7*	*51.1 ±1.31*	*50.2 ± 1.2*	*43.1 ± 2.57*
	Change	*-16.7 ± 16.9*	*3.85 ± 2.84*	*-4.84 ± 4.2*	*-2.79 ± 2.38*
SM	*% before*	*53.6 ± 3.66*	*61.4 ± 2.01*	*59.9 ± 1.8*	*53.8 ± 0.857*
	% after	*57.6 ± 1.79*	*54.3 ± 1.28*	*53.6 ± 2.4*	*51.9 ± 2.64*
	Change	*-3.97 ± 2.69*	*7.08 ± 2.75*	*6.30 ± 1.6*	*1.96 ± 2.76*
C1	*% before*	*53.58 ± 6.02*	*59.1 ± 2.68*	-	*48.6 ± 0.103*
	% after	*59.7 ± 2.04*	*55.9 ± 2.04*	-	*50.9 ± 5.42*
	Change	*-6.09 ± 8.00*	*3.19 ± 2.73*	-	*-2.35 ± 5.53*
C2	*% before*	*37.9 ± 2.56*	*44.9 ± 4.41*	-	-
	% after	*43.4 ± 9.23*	*44.5 ± 3.77*	-	-
	Change	*-5.58 ± 8.33*	*0.40 ± 6.59*	-	-

3.5 15% dry matter

After the four press cakes had been fermented with the four different processing combinations (except for when problems with the homogeniser arose) the data was analysed. It was concluded that the SM was the most optimal press cake for fermentation. The trials continued by increasing the dry matter of SM to 15% while doing the same four processing steps (with and without whey, with and without homogenisation). This was done to see if the increase would give the LAB more nutrients, thus lowering the pH more, or if the viscosity would increase, and maybe affect the physical stability.

3.5.1 pH change

In *Table 12* the pH change during fermentation with 15% dry matter for SM can be seen. Both the start-pH and end-pH are similar within the non-whey groups and the with-whey groups. The difference is approximately 0.1 for the start-pH and 0.25 for the end-pH. The rate of acidification shows similar differences between the non-whey and with-whey groups. The rate is greater when no whey is added. Homogenisation has no effect on the outcome of change in pH or rate of acidification.

The *time of lag phase* and *time to lower pH to 1* are difficult to interpret since the results for *with whey no homogenisation* and *no whey with homogenisation* have large error bars. This becomes clear when looking at *Figures 4* below and *Figure A8* in the appendix where one of the four fermented samples for both cases is different from the others. The times are therefore also calculated with only three samples, shown in parenthesis in *Table 12*. When comparing the times these results are similar to the previous ones, having differences depending on if whey is added or not. Both times are shorter if no whey is added. There is a small difference in the *time of lag phase* when adding homogenisation, making the time shorter. The same is not seen for *time to lower pH with 1*.

Table 12. Calculated values from trials of fermentation of SFPC SM 15% dry matter. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean. The time of the lag phase is determined by the time when the start pH was lowered by 0.1.

SM 15%	*No w no h*	*With w no h*	*No w with h*	*With w with h*
Start pH	*6.3 ± 0.03*	*6.2 ± 0.03*	*6.3 ± 0.03*	*6.1 ± 0.02*
End pH	*4.4 ± 0.02*	*4.2 ± 0.08*	*4.4 ± 0.01*	*4.1 ± 0.06*
Time of lag phase, h	*5.73 ± 0.39*	*6.85 ± 1.25 (6.23 ± 0.25)*	*6.03 ± 0.96 (5.57 ± 0.35)*	*6.04 ± 0.46*
Rate of acidification, h^-1	*-0.328 ± 0.013*	*-0.306 ± 0.0020*	*-0.324 ± 0.013*	*-0.310 ± 0.013*
Time to lower pH with 1, h	*10.4 ± 0.17*	*11.3 ± 1.2 (10.75 ± 0.15)*	*11.0 ± 1.1 (10.5 ± 0.41)*	*10.8 ± 0.42*

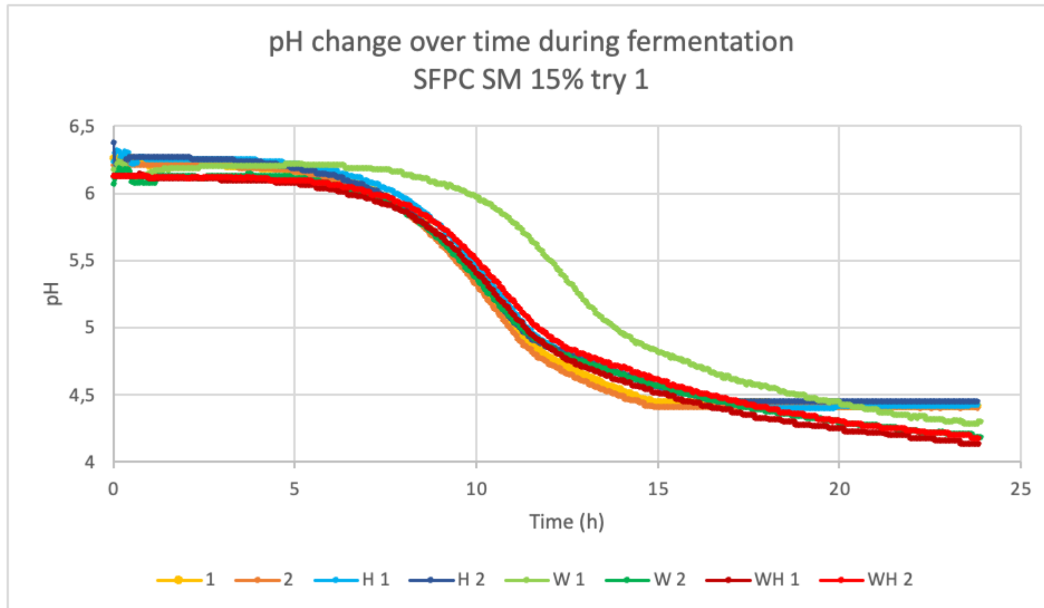


Figure 4. pH change over time during fermentation of different sunflower press cake and whey mixtures. These samples were with a 15% dry matter. H stands for homogenised and W for added whey. The samples with similar colours are the same but from different tries. This was the first try.

3.5.2 Viscosity

The viscosity change during fermentation for 15% dry matter for SM can be seen in *Table 13*. When looking at the slope before fermentation *no whey no homogenisation* has the greatest one while *with whey no homogenisation* has the smallest one. The other two methods correlate somewhat to whey giving a smaller slope but homogenisation does not seem to have an effect. The slopes after fermentation show, that the homogenised samples have the greatest slopes, even though the margins of error also are large. The effect of the whey is unclear, having both the smallest and largest slope. The change between before and after fermentation shows the homogenised samples had an increased slope while the non-homogenised samples decreased.

With whey no homogenisation has the highest start value before fermentation, followed by *with whey with homogenisation*. *No whey no homogenisation* has the smallest start value. It can therefore be concluded whey has a greater effect than homogenisation before fermentation. The start values after fermentation are the greatest for *with whey no homogenisation* and *no whey with homogenisation*. This does not give any conclusions on how whey and homogenisation affect the viscosity. However, the start values were cut more than half during fermentation for the samples with added whey, going from 177 to 81.2 and 120 and 43.6 respectively. The samples without whey did on average decrease by approximately 17%. All start values are however having great margins of error making the conclusions uncertain.

Table 13. Calculated values for the viscosity before and after fermentation for SFPC SM 15% dry matter. All values are the average from all four samples (duplicates on both batches) with the

standard deviation of the mean. The slope is from the double logarithmic plots and the start value is the value when crossing the y-axis.

15% dw		*No w no h*	*With w no h*	*No w with h*	*With w with h*
Slope, kg/m	*Before ferm*	*-1.66* ± 0.043	*-1.46* ± 0.050	*-1.61 ± 0.0065*	*-1.55 ± 0.054*
	After ferm	*-1.28* ± 0.82	*-0.616* ± 0.036	*-3.61 ± 1.9*	*-5.13 ± 3.0*
Start value, Pa/s	*Before ferm*	*59.2* ± 26	*177* ± 48	*84.1 ± 12*	*120. ± 29*
	After ferm	*42.2* ± 5.4	*81.2* ± 34	*79.9 ± 41*	*43.6 ± 21*

3.5.3 Physical stability

In *Table 14* the change in physical stability before and after fermentation for SM 15% dry weight can be seen. The most stable sample before fermentation was *with whey with homogenisation*, and had the least percentage of separation. The least stable sample was *no whey with homogenisation*. After fermentation was the most stable sample also *with whey with homogenisation* while the least stable sample was *with whey no homogenisation*.

The greatest increase in physical stability was seen for *no whey with homogenisation*. After fermentation, the homogenised samples are the most stable while the samples with added whey are the most stable before fermentation. The change shows samples with added whey have less of an increase. Homogenisation does not have an effect on the outcome of the change.

Table 14. Shows calculated values for the stability tests before and after fermentation for SFPC SM 15% dry matter. All values are the average from all four samples (duplicates on both batches) with the standard deviation of the mean.

15% dry weight	*No whey no homogenisation*	*With whey no homogenisation*	*No whey with homogenisation*	*With whey with homogenisation*
% before	*45.3 ± 3.7*	*43.3 ± 1.2*	*45.5 ± 1.5*	*42.1 ± 3.9*
% after	*42.3 ± 5.5*	*46.2 ± 0.79*	*41.2 ± 2.2*	*40.3 ± 2.7*
Change	*2.95 ± 7.5*	*-2.91 ± 0.59*	*3.95 ± 3.6*	*1.80 ± 1.3*

4 Discussion

Waste products are full of potential to create nutritional and sustainable food products. (Li et al. 2022) Therefore, this project aimed to ferment four different kinds of SFPC to see if it was possible to create a stable product which didn't separate with time. During the project, the addition of whey and homogenisation was done to see how the changes would affect the result in regards to pH, viscosity and physical stability. Towards the end of the project, the results were analysed before choosing the settings and which press cake to use for the second part. Thus, the results for the first

(major) part will be discussed before the results for the increased dry matter with SM. First, the difference between the SFPC will be discussed.

4.1 Nutritional determination

As previously mentioned, the nutritional content for VO is in between the other press cakes. This is most likely because it has both hulled and dehulled seeds, meaning it gets some of the characteristics from both types. SM is made from only dehulled seeds and is the only SFPC being cold-pressed. This is probably the reason why it is higher in protein and lower in fat but also why it differs in colour from the other press-cakes. The protein content is in the lower range compared to literature, where the dehulled press cakes have a protein content between 34-44%. The hulled press cakes are most often in the range of 27-29% protein which is in between the other three press cakes. (Dauguet et al. 2016) The reason for VO being higher than the hulled but lower than the dehulled is most likely because of the mixture of both types. C1 and C2 probably differ because of the solvent extraction which left C1 with more oil and C2 with less oil than standard. The reason C1 and C2 are near each other in fibre content but not protein and fat are most likely since they are from the same company but from different steps of the process.

The fat content is also different from the press cakes. The greatest difference is between C1 and C2 which is before and after solvent extraction. The high content in C1 is likely because there only is a shorter pre-pressing before extraction, causing much of the oil to be left in the press cake. The low fat content in C2 compared to VO and SM shows the final effect of the solvent extraction compared to only using mechanical pressing. It correlates to literature, where it has been shown that solvent extraction can lower the fat content to less than 0.5%. (Evangelista et al. 2022). The difference in fat between SM and VO could be because of them being cold-pressed and warm pressed respectively, but also because of SM being dehulled while VO is containing some hulls.

4.2 pH change

SM is the press cake which has the greatest decrease in pH from start to finish and most often it is also the press cake which reaches the lowest pH, similar to those of yoghurt (pH 4.2-4.5). This could be because of the high amount of nitrogen, giving the LAB more or just the right nutrients compared to the other press cakes. Lactobacillus species namely have different specific growth requirements, meaning the nutritional content of the media is very important for optimal growth. (Solval et al. 2019) The lowest pH decrease is always reached by C2. This, however, does not support the hypothesis of nitrogen being the factor which allows the LAB to lower the pH since C1 has a lower percentage of protein, and thus nitrogen. It could be a difference in the number of free amino acids between the press cakes but to draw any conclusions, this would need to be analysed. A possible explanation is the protein solubility which differs between all press cakes. At pH 9, C2 has the lowest solubility, which could mean that the protein is less available. The protein solubility does however not seem to follow the trend and the protein solubility for C2 decreases less compared to the other press-cakes when reaching pH 7. The solubility at pH 4-5 was not analysed but it would be interesting for the future since the fermentation reached those levels.

Another possible argument is the effect of the processing type. SM decreases the most and is the cold-pressed press-cake. According to Ji, Liu and Wang (2020) cold pressing preserves the

proteins and other bioactive ingredients, in peanuts, to a greater extent. It is likely the same is happening in sunflower seeds, thus in this case SM would have the most preserved nutrients. VO and C1 are both warm-pressed but with different nutritional compositions but are decreasing approximately the same. C2 is the only solvent-extracted press-cake and the one reaching the lowest pH. In a study targeting the pressing of *Euphorbia lagascae* seeds was it shown that increased temperature and addition of solvent extraction caused more coagulation in the proteins. (Evangelista et al. 2022) It is possible that the proteins in C2 had coagulated, leading to the LAB not being able to use it as a nutrient to the same degree.

When looking at the slope (rate of acidification), SM is often the greatest one, followed by C1. Either C2 or VO are having the smallest slopes, depending on the method. Interesting is, even though VO is having small slopes of acidification it is reaching low pH, no matter the method. The opposite is seen for C1, which most often has greater slopes (correlating to the time to lower pH to 1) than VO but plans out earlier. This might be because the nutrients are more available for C1, for example, a higher percentage of soluble proteins. In total, however, VO contains more nutrients (higher protein content), causing the LAB to be able to decrease the pH further.

The pH changes clearly show the effect of the addition of whey and/or homogenisation. The samples with added whey reach the lowest pH but *Figures 2, A2, A3* and *A6* also show that the graphs do not plan out during the 24h of fermentation. The pH decreases even further with homogenisation. The reason for lower pH with added whey is probably because of the nutrients available, such as lactose and nitrogenous compounds. This correlates to literature, which shows that *L.plantarum* can lower the pH of a whey media from 5.7 to 4.1. (Nawal et al. 2022) The lower pH with homogenisation is most likely because the smaller particles make it easier for the LAB to reach the nutrients from the increased surface area.

The *time of lag phase* and *time to lower pH with 1* give inconclusive results but most often whey is increasing the times. One reason for this could be that the LAB needs more time to adjust to the nutrients in the whey, but more research would be needed to be done to draw any conclusions. Another reason could be that the pH is lower when whey is added and that the optimal pH for LAB is closer to 6.2-6.4 rather than 5.8-6.1. This is however contradicted by SM having the lowest lag phase during fermentation of *no whey no homogenisation*.

4.3 Viscosity

The most important result regarding the viscosity change during fermentation is that the viscosity decreased in all cases. There are several possible reasons for this. The most likely reason why there is no increase in viscosity, as seen with conventional yoghurt, is the reason mentioned in the introduction, namely that plant-based proteins are not gelling the same way as in dairy products. The viscosity increase in conventional yoghurt starts with casein aggregation and is followed by crosslinking between whey and casein molecules, none of which are present in SFPC and there is no casein in the whey powder. (Sah et al. 2016) This also connects to the fibre in the press cake. Kieserling et al. (2019) showed that the addition of fibre during conventional yoghurt fermentation sometimes increases and sometimes decreases the gelling capacity in the yoghurts. They argue for fine fibres being integrated as fillers in the casein network while the larger fibres act as a steric hindrance. García-Pérez et al. (2005) also showed that the addition of fibre decreases the viscosity

due to steric hindrance, shown as breaking in the gel structure, but that this phenomenon was reversed when more than 1% of fibre was added thanks to the water holding capacity. This leaves two problems regarding the SFPC-whey dispersions. First, there are no caseins present, meaning the fibre would not be able to integrate into the network, nor break it. Second, the water-holding effect was seen when the addition in fibre content was increased, meaning that even though the SFPC contains 26-42% of insoluble fibre per 100 g of press cake it might not be enough when adding 10% of SFPC to the dispersions. Two other reasons that affect the viscosity might be the heating during fermentation as well as the stirring. The fermentation was performed at 30°C, which could create a slight decrease in viscosity, compared to the non-fermented samples where the viscosity was measured one hour after being taken from the fridge.

There is a clear difference in viscosity between the samples. C2 was always the sample with the highest start value while SM had the lowest. When looking at the nutritional content this does not correlate with the dry content, fat content or fibre content. It does however correlate to the protein content but that is not likely to be the explanation. It could be a combination of the nutrients, for example, a low fat content, as well as low fibre content, leads to low viscosity. This would mean the low fat content together with a high fibre content in C2 is the reason for the particles to hold a lot of water, leading to high viscosity. This is followed by C1 and VO, having different amounts of fat and fibre which can bind approximately the same amount of water. SM is the sample with the lowest combination of fat and fibre, and the sample with the lowest viscosity.

The bumps seen in almost all viscosity graphs are because of the particles' delayed movement in the dispersions. The larger particles will need more time and be forced to arrange themselves to follow the flow. The bump shows just when they get arranged, causing an easier flow afterwards. The spikes in *Figures A12a* and *A16a* in the appendix are most likely explained by a clump getting stuck causing increased pressure. This could also explain why the spike goes down quickly since the clump would be easily destroyed.

The start values for the viscosity as well as most data for the physical stability are often having large error bars. One reason is that the fermentation is affecting the sample differently every time, causing different batches having different results. This does, however, not explain why the results before fermentation also have large margins of error. It is often seen that the two tries from the same batch are quite similar, but it differs from the other batch. This eliminates the possibility that it could be a human error when for example pouring in the 20 ml for the viscosity measurements and all factors only applied to the fermentation. One sample type, the 15% *with whey no homogenisation*, both batches were prepared the same day. The results from this time show that it is not the different days that cause the difference, but rather having different bottles to store the samples overnight. It is however interesting to see that the margin of error was decreased for the physical stability of these samples, making it a possibility.

4.4 Physical stability

The conclusions from the stability tests are unconcise. This is probably because of the handling of the centrifuge. The centrifuge took several hours to increase in temperature and because of high demand, it was not always possible to wait until the centrifuge reached 20°C. This led to some samples being centrifuged at 15°C, while others at 20°C and a few above 22°C. Nonetheless, when

looking at the data it is seen that the homogenised samples are more stable than the non-homogenised samples of the same press-cakes. This is likely because of the smaller particle sizes achieved during homogenisation, leading to less sedimentation, flocculation and coalescence. (McClements 2007) The different temperatures were eliminated for the four samples of 15% *with whey no homogenisation* since all samples were prepared the same day and it is the most probable reason for the results being more even. In the future it is recommended to set a lower temperature, thus making it easier to stabilise the centrifuge before starting the measurements.

Another factor of physical stability is viscosity. With increased viscosity, several instability mechanisms are slowed down. This is due to the increased difficulty of particles to move within a thicker continuous phase as well as the prevention of movement from a more packed dispersion. (McClements 2007) In the results, it is seen that C2 has less separation compared to the other samples, except for *with whey with homogenisation* for VO. This correlates to the viscosity results where C2 has shown to have the greatest viscosity and the start-value for *with whey with homogenisation* for VO is almost double of the other three methods. The reason for the high value is unknown.

4.5 15% dry matter

There were several reasons for choosing to continue to work with SM. The main ones were that it reached the lowest pH, lag-time and *time to lower pH with 1*. It was also the press cake with the highest protein content and protein solubility, it was the easiest to handle (only needed one mixing step) and had the least number of problems during the homogenisation.

In general, similar results can be seen for the increased dry matter as for the previous analyses. In *Table 12* is it clear how whey affects the results from fermentation to a greater extent than homogenisation, mainly by reaching lower pH. One reason for this is because of the lower start-value but the main reason is believed to be the higher amounts of nutrients present in the dispersions. This is shown in *Figures A7* and *A8* where it is seen that the graph doesn't plan out, meaning the LAB still has enough nutrients to produce lactic acid and thus, are able to lower the pH.

It is also seen that the addition of whey leads to a longer lag phase, *time to lower pH with 1* and slower acidification. This could be because the viscosity increases from the increase of dry matter in the dispersion. The same results are namely seen for the 10% dry matter samples where the 10% no whey samples are having the shortest lag phase, followed by the 10% with whey, 15% no whey and the longest lag phase is seen for the 15% with whey samples. The viscosity increase is having different effects. One is that particles are more likely to create clumps, making it difficult for LAB to reach everywhere, another is some problems that were seen for the stirrers. When having higher viscosity, the magnetic stirrers were less efficient and thus, the LAB was not spread out as evenly as with the less viscous samples.

The effect whey has on viscosity is clear when looking at *Table 13*. The whey samples are having the greatest start values before fermentation, but it is decreased by more than half during fermentation. As mentioned above, the value before fermentation is most likely due to the increase in dry matter and the great decrease during fermentation is probably due to whey being used as a

nutrient. The decreased acidification seen when whey is added is also not conclusive with previous research, stating *L.plantarum* works best in rich media. The auxotrophic behaviour would correlate to greater acidification as more nutrients are available with the addition of whey. The reason for this not being seen is unknown. (Montemurro et al. 2021)

The physical stability tests show samples with added whey being most stable before fermentation, correlating to the previous explanation about the increase in viscosity. The reason for the homogenised samples being the most stable samples after fermentation could be the stabilising effect homogenisation has over time, especially since the viscosity decreased during fermentation. The homogenisation would therefore be the greater stabilising factor with smaller particles leading to less sedimentation and aggregation. With this background, it would be believed that *with whey with homogenisation* is the most stable sample and *no whey no homogenisation* is the least stable sample. However, this is not true for the *no whey no homogenisation* samples. The reason for this is unknown.

5 Conclusion

The first part of the project showed differences in the result depending on the method used for receiving the press cakes. It was concluded that the cold-pressed and dehulled press cake was the most suitable for fermentation. For the second part of the project, it became clear that the addition of whey has a great effect on the result, for example, it is lowering the pH further but causes a longer lag phase. The homogenisation seems to have an effect on the stability after fermentation but the results are not concise.

In the future, it is recommended to analyse the fibre content before and after fermentation to identify an eventual breakdown. This could explain why there is a viscosity decrease during fermentation. It is also recommended to be more thorough with the temperature of the centrifuge for the stability test, and lower the temperature, to see if the results get more conclusive. Thus, the effect of the addition of whey and homogenisation on physical stability could be clearer and the recommendation for using the methods could be more valid. It would also be interesting to try other bacteria strains to see if they are more suitable for fermentation of SFPC, thus having a lower lag phase or faster rate of acidification. This would therefore be able to increase the efficiency and the yield during a potential scale-up. Lastly, it is recommended to try SFPC which is dehulled and warm-pressed or hulled and cold-pressed to see which has the greatest effect on the outcome since the press cakes during this experiment only had one cold-pressed.

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7 Appendix

7.1 Fermentation graphs

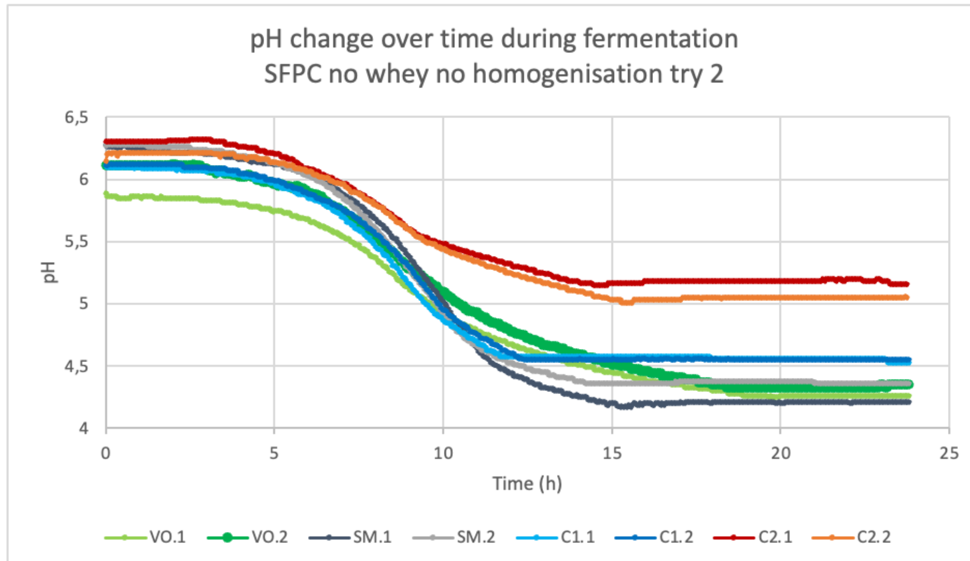


Figure A1. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were not homogenised before fermentation, also no whey was added. This was the second try. The green samples are VO, black/ grey are SM, blue are C1 and red/orange are C2.

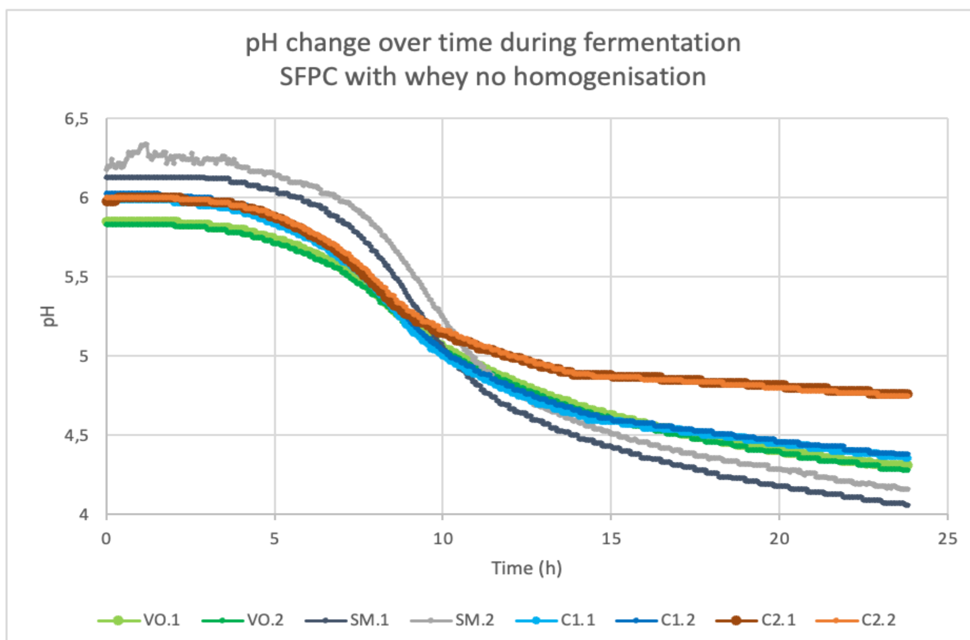


Figure A2. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were not homogenised before fermentation, but whey was added. This was the first try. The green samples are VO, black/ grey are SM, blue are C1 and red/orange are C2.

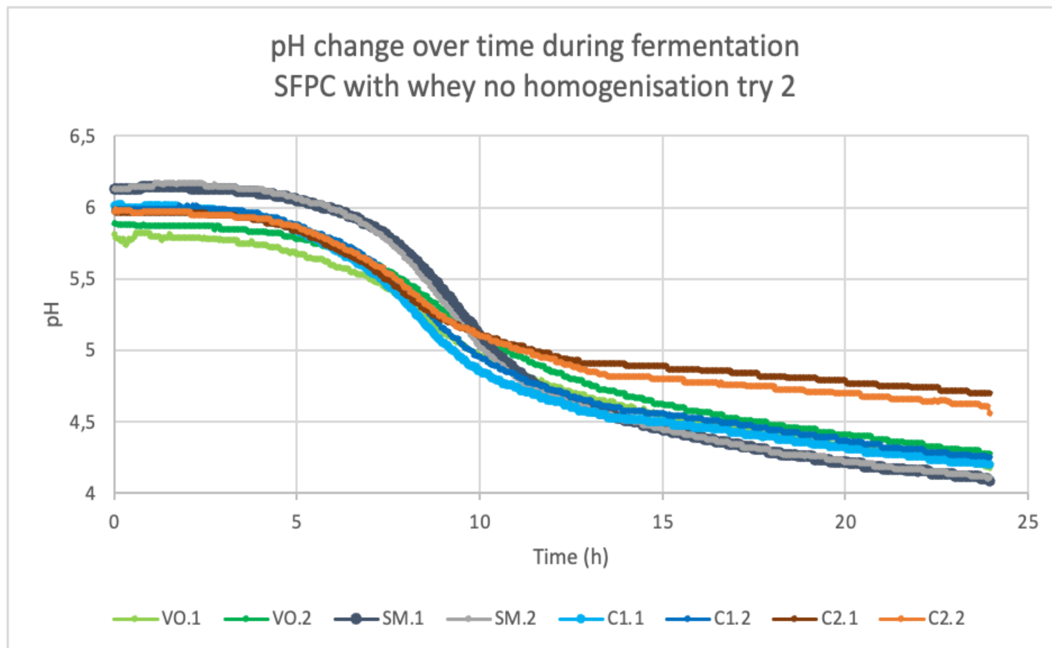


Figure A3. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were not homogenised before fermentation, but whey was added. This was the second try. The green samples are VO, black/ grey are SM, blue are C1 and red/orange are C2.

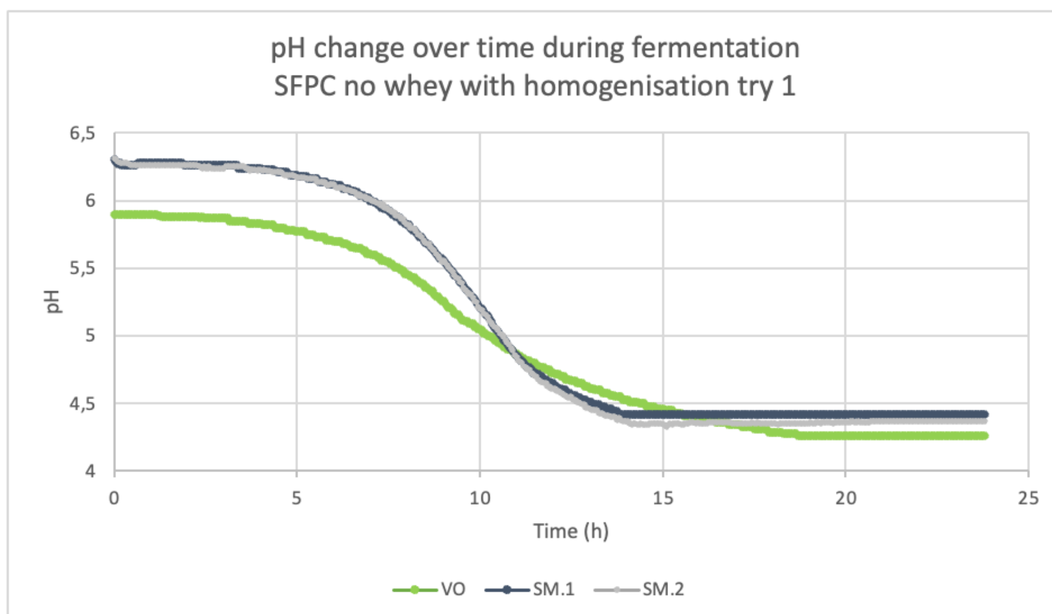


Figure A4. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were homogenised before fermentation, but no whey was added. This was the first try. The green sample is VO and black/ grey are SM.

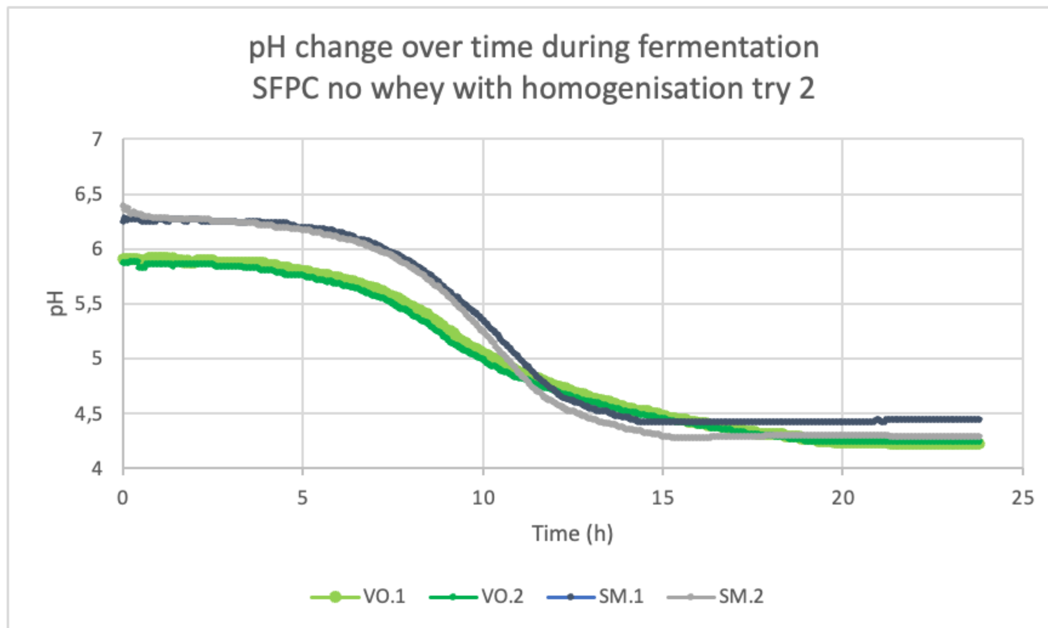


Figure A5. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were homogenised before fermentation, but no whey was added. This was the second try. The green samples are VO and black/ grey are SM.

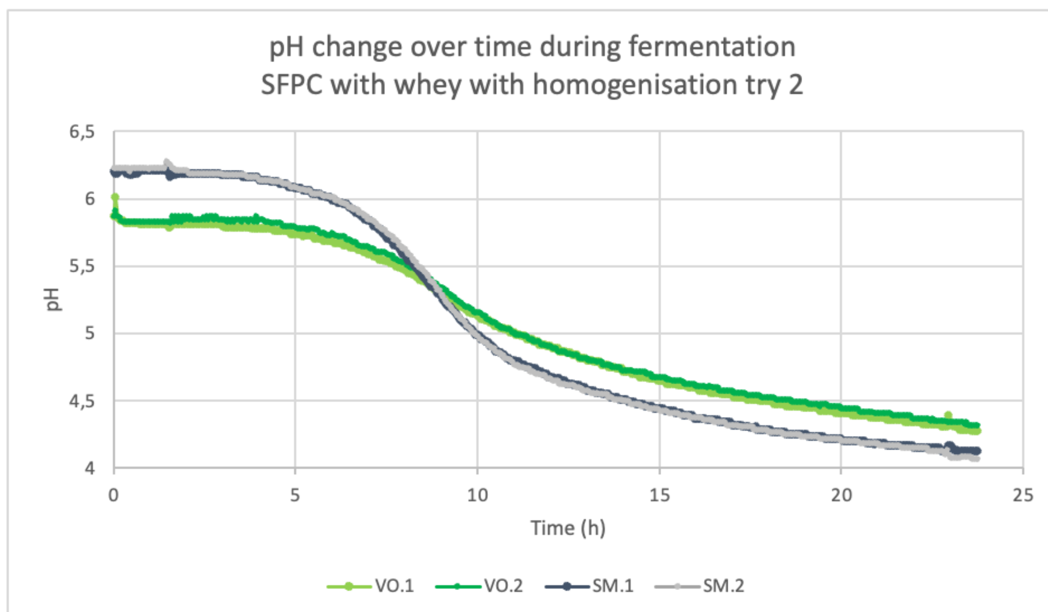


Figure A6. pH change over time during fermentation of different sunflower press cake mixtures (10 % w/w). These samples were homogenised before fermentation, and whey was added. This was the second try. The green samples are VO and black/ grey are SM.

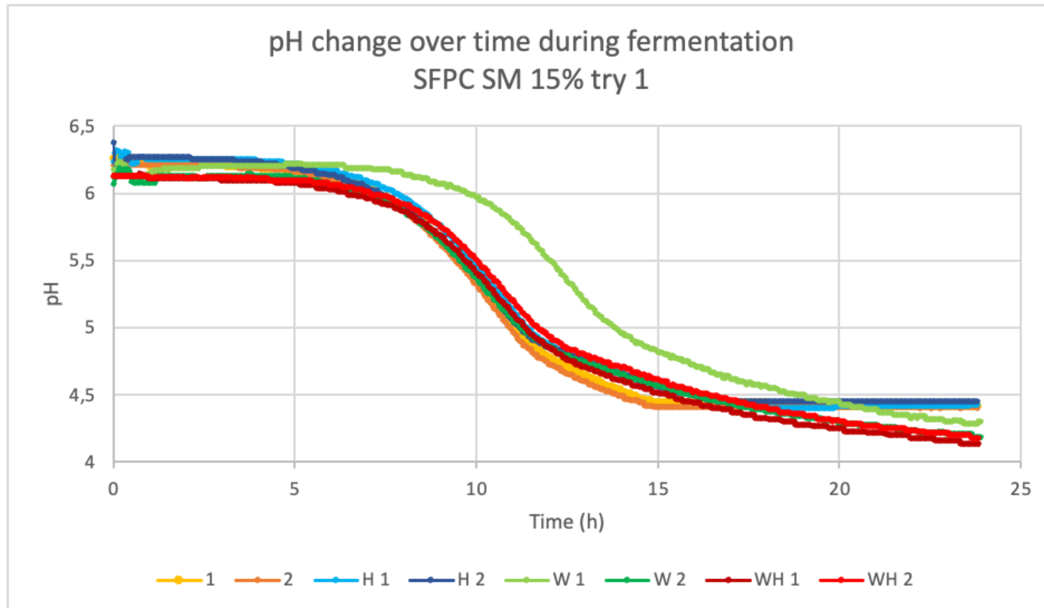


Figure A7. pH change over time during fermentation of different sunflower press cake and whey mixtures. These samples were with a 15% dry matter. H stands for homogenised and W for added whey. The samples with similar colours are the same but from different tries. This was the first try.

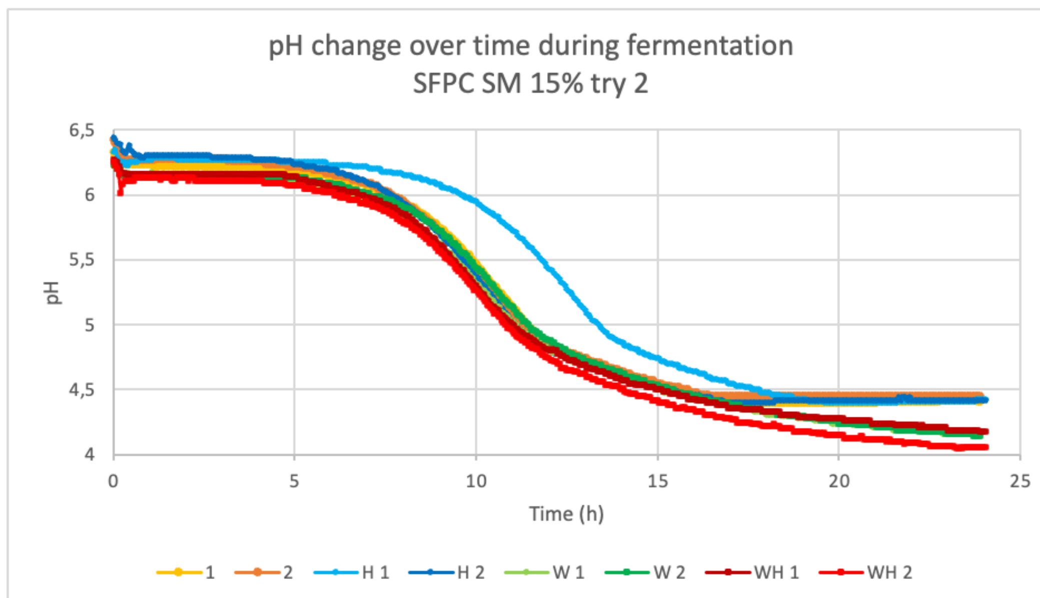


Figure A8. pH change over time during fermentation of different sunflower press cake and whey mixtures. These samples were with a 15% dry matter. H stands for homogenised and W for added whey. The samples with similar colours are the same but from different tries. This was the second try.

7.2 Viscosity data

Table *A1. The equations are obtained from the raw data. The correlation coefficient was above 0.88 for all. The first rows are a and second are b in the equation Y=ax^b. The standard deviation of the mean is also given.*

Exponential data		*VO*	*SM*	*C1*	*C2*
No w no h	*Before ferm*	*1.30 ± 0.48* *-0.707 ± 0.018*	*0.445 ± 0.12* *-0,590 ± 0.057*	*0.559 ± 0.21* *-0.633 ± 0.012*	*3.33 ± 0.73* *-0.704 ± 0.0043*
	After ferm	*0.438 ± 0.34* *-0.590 ± 0.057*	*0.170 ± 0.030* *-0.521 ± 0.015*	*0.243 ± 0.084* *-0.584 ± 0.030*	*1.70 ± 1.7* *-0.626 ± 0.074*
With w no h	*Before ferm*	*1.51 ± 0.21* *-0.703 ± 0.011*	*0.409 ± 0.084* *-0.524 ± 0.035*	*0.510 ± 0.39* *-0.598 ± 0.046*	*2.68 ± 0.62* *-0.698 ± 0.014*
	After ferm	*0.345 ± 0.093* *-0.560 ± 0.026*	*0.197 ± 0.034* *-0.548 ± 0.046*	*0.237 ± 0.62* *-0.548 ± 0.031*	*1.66 ± 1.3* *-0.656 ± 0.045*
No w with h	*Before ferm*	*1.26 ± 0.26* *-0.688 ± 0.019*	*0.686 ± 0.24* *-0.577 ± 0.049*	-	-
	After ferm	*1.32 ± 0.25* *-0.690 ± 0.017*	*0.414 ± 0.050* *-0.604 ± 0.015*	-	-
With w with h	*Before ferm*	*2.78 ± 0.44* *-0.722 ± 0.0072*	*1.11 ± 0.27* *-0.625 ± 0.034*	*1.18 ± 0.015* *-0.711 ± 0.019*	-
	After ferm	*1.54 ± 0.30* *-0.678 ± 0.0052*	*0.528 ± 0.29* *-0.589 ± 0.036*	*0.760 ± 0.31* *-0.646 ± 0.0077*	-

Table A2. The equations are obtained from the raw data. The correlation coefficient was above 0.90 for all. The first rows are a and second are b in the equation Y=ax^b

Exponential data		*No w no h*	*With w no h*	*No w with h*	*With w with h*
15% dw	*Before ferm*	*2.92 ± 1.1* *-0.610 ± 0.023*	*5.22 ± 0.64* *-0.684 ± 0.0088*	*3.77 ± 0.49* *-0.627 ± 0.0065*	*4.62 ± 0.52* *-0.642 ± 0.022*
	After ferm	*1.78 ± 0.24* *-0,615 ± 0.0092*	*2.98 ± 0.83* *-0.644 ± 0.035*	*2.64 ± 0.89* *-0.656 ± 0.042*	*1.84 ± 0.57* *-0.626 ± 0.029*

7.3 Viscosity graphs

7.3.1 Viscosity change with heating

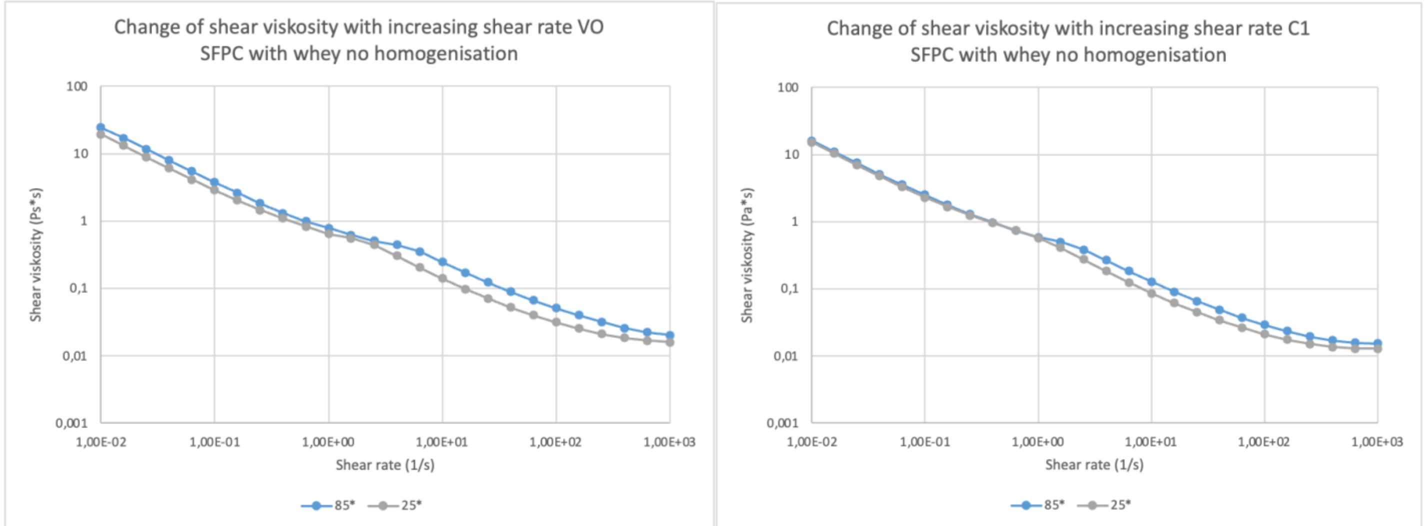


Figure A9a and b. The shear viscosity plotted against the shear rate for SFPC VO and C1 (10 % w/w) at temperatures 85 ℃ and 25 ℃. These samples were not fermented or homogenised. Also, no whey was added.

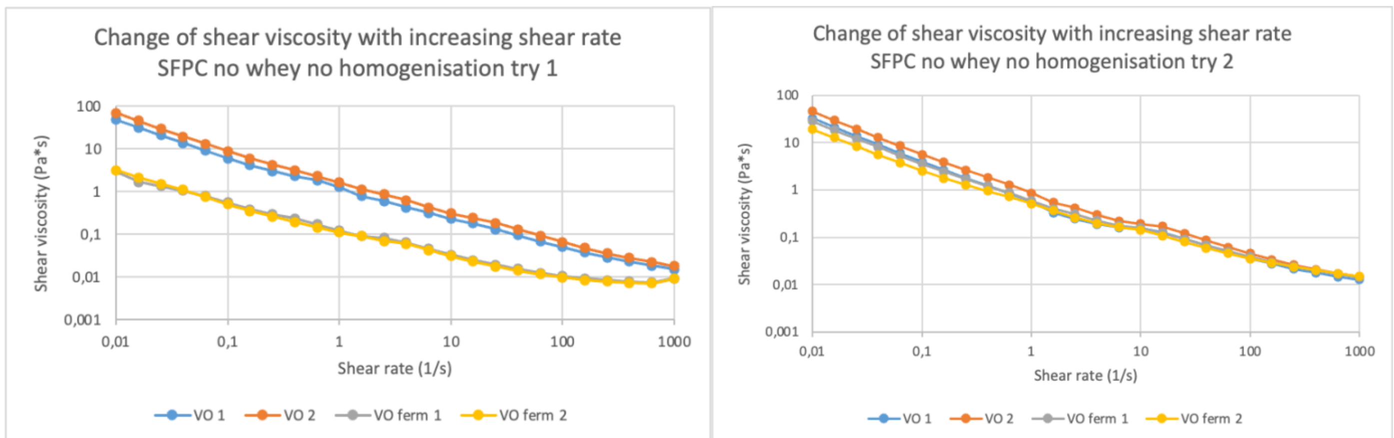


Figure A10a and b. The shear viscosity plotted against the shear rate for VO before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, and no whey was added.

7.3.2 No whey no homogenisation

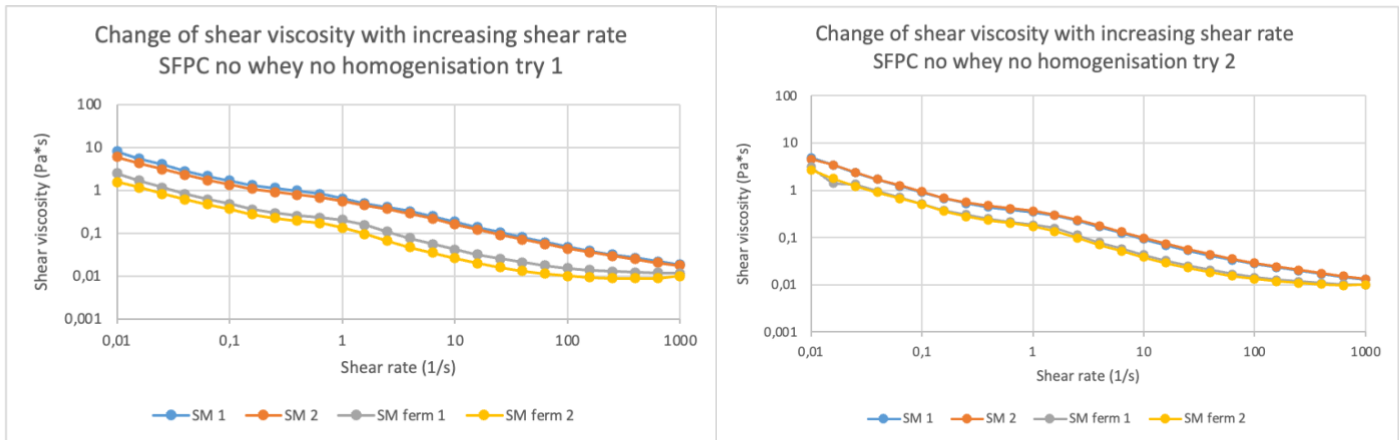


Figure A11a and b. The shear viscosity plotted against the shear rate for SM before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, and no whey was added.

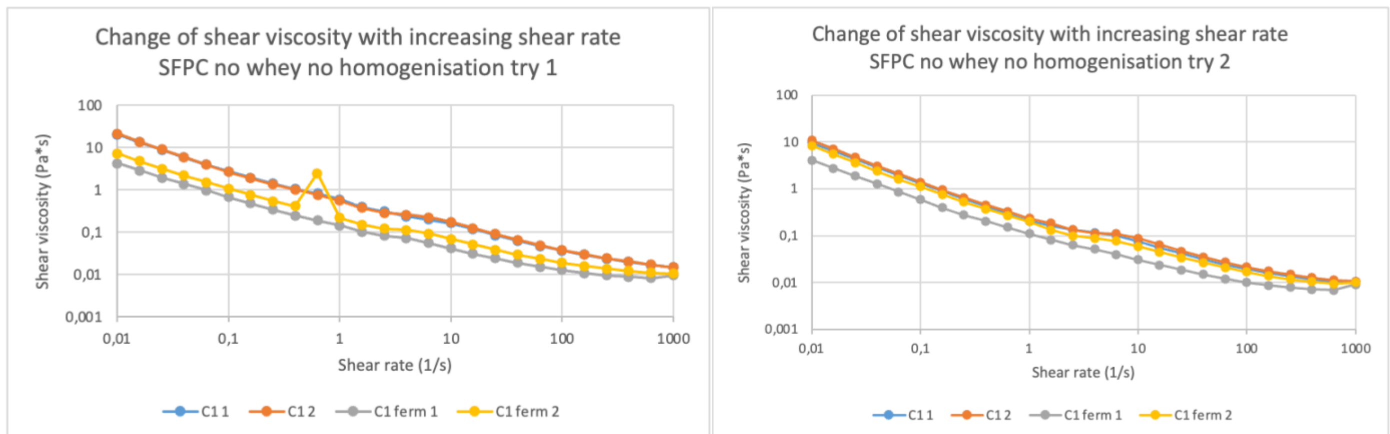


Figure A12a and b. The shear viscosity plotted against the shear rate for C1 before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, and no whey was added.

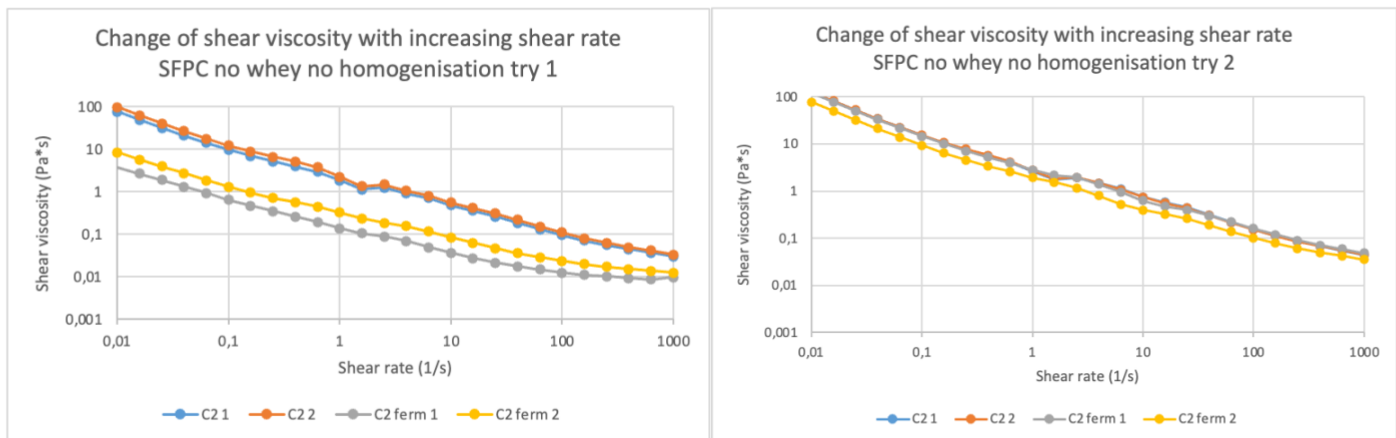


Figure A13a and b. The shear viscosity plotted against the shear rate for C2 before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, and no whey was added.

7.3.3 With whey no homogenisation

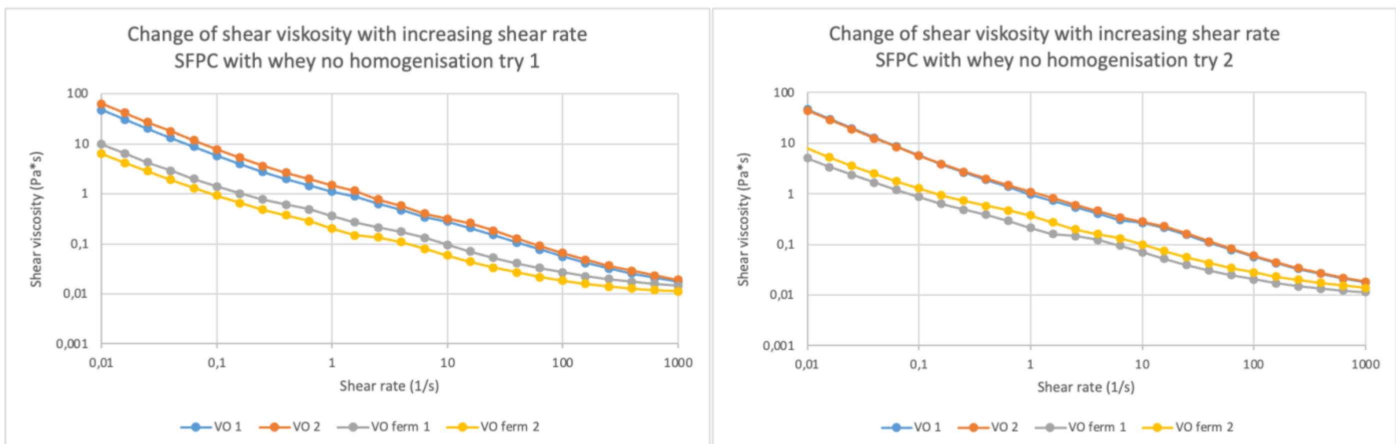


Figure A14a and b. The shear viscosity plotted against the shear rate for VO before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, but whey was added.

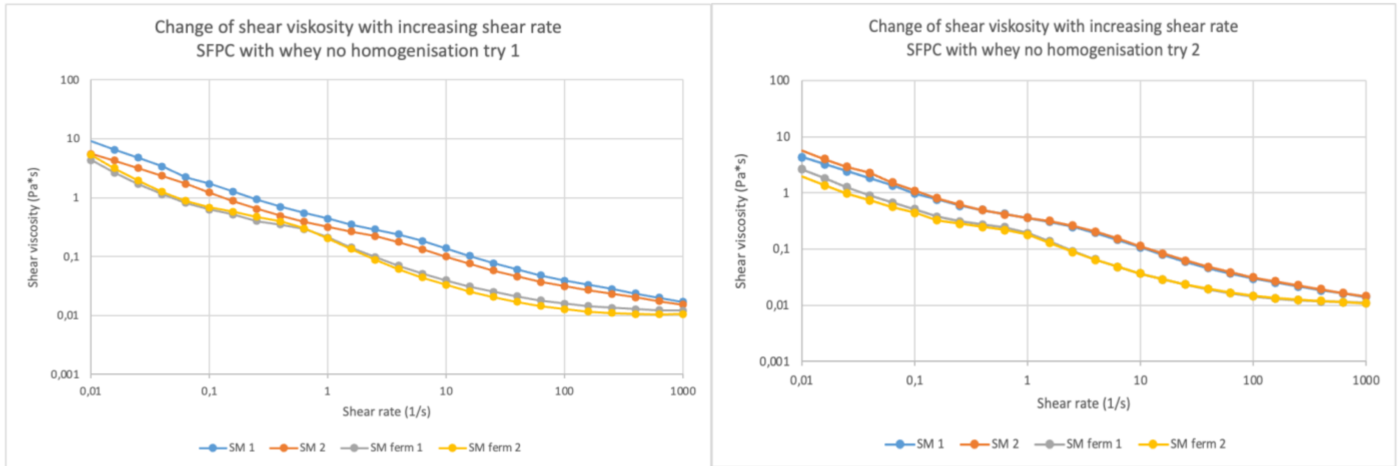


Figure A15a and b. The shear viscosity plotted against the shear rate for SM before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, but whey was added.

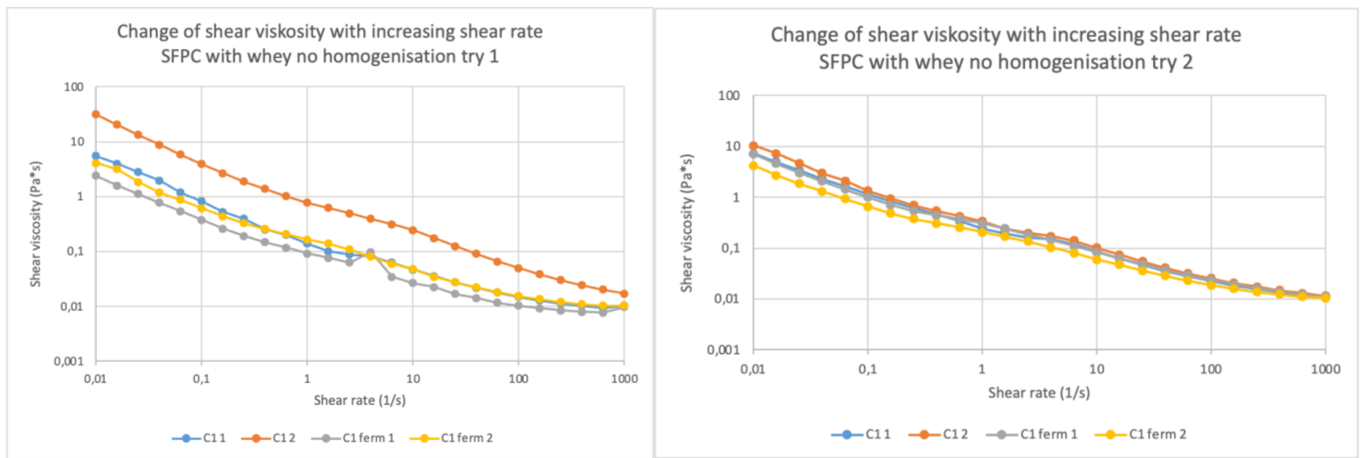


Figure A16a and b. The shear viscosity plotted against the shear rate for C1 before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, but whey was added.

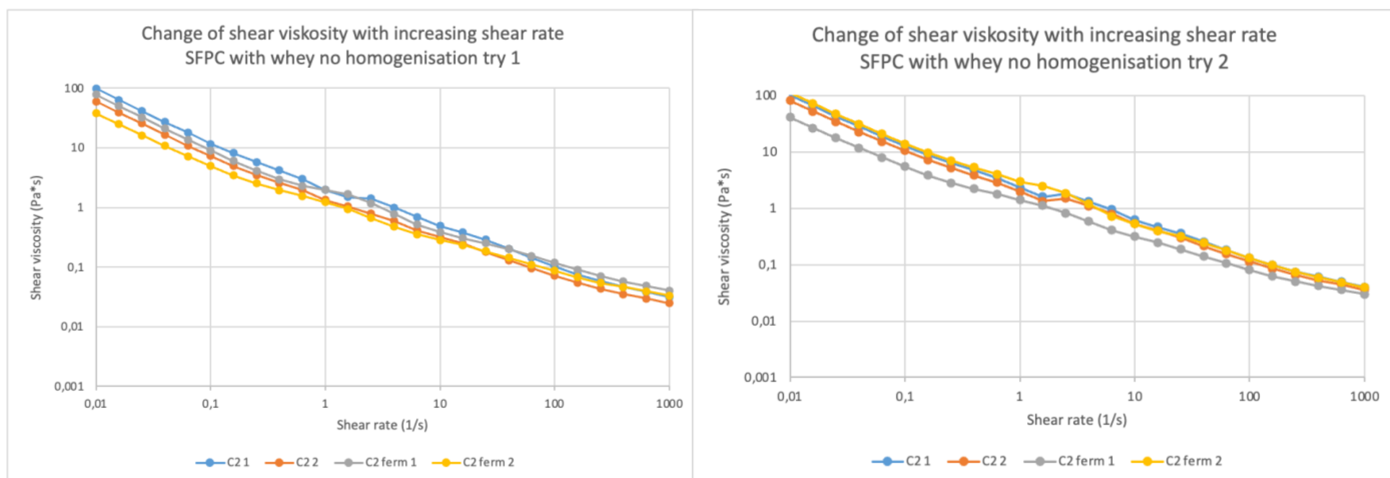


Figure A17a and b. The shear viscosity plotted against the shear rate for C2 before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, but whey was added.

7.3.4 No whey with homogenisation

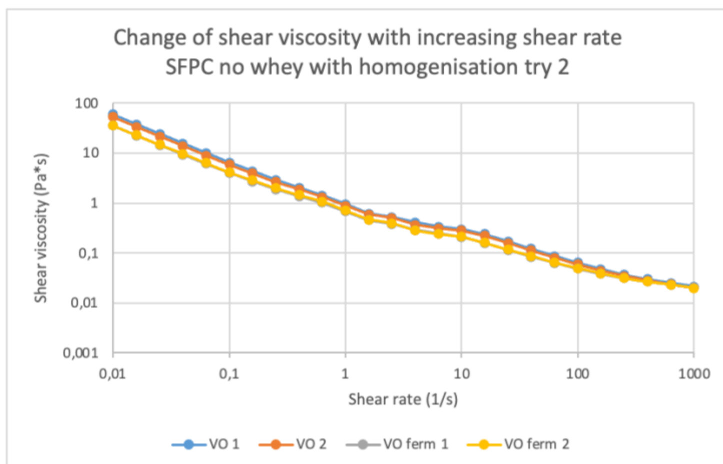
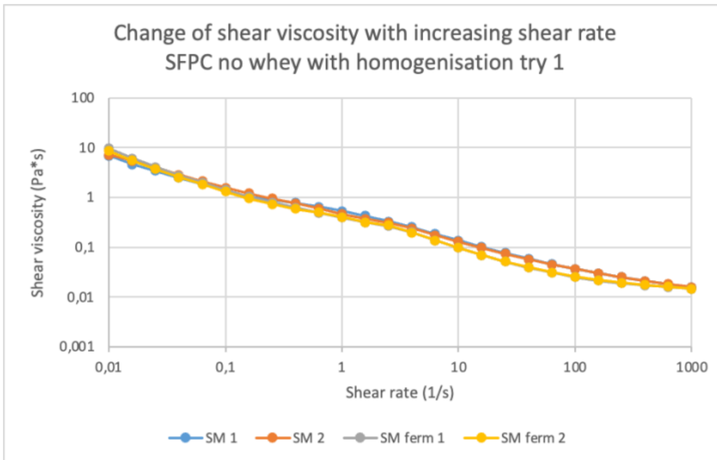


Figure A18. The shear viscosity plotted against the shear rate for VO before and after fermentation. These samples were homogenised, but no whey was added.



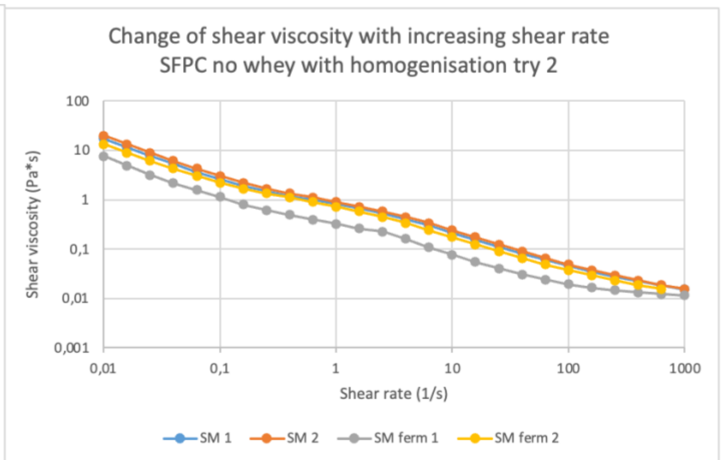
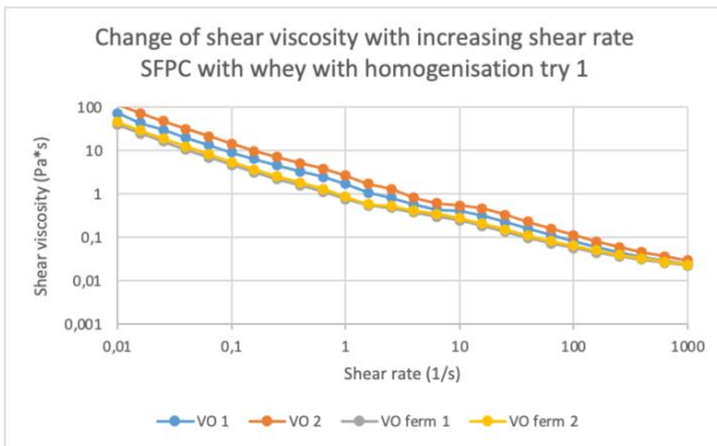


Figure A19a and b. The shear viscosity plotted against the shear rate for SM before and after fermentation. Figura a is try one and b is try two. These samples were homogenised, but no whey was added.

7.3.5 With whey with homogenisation



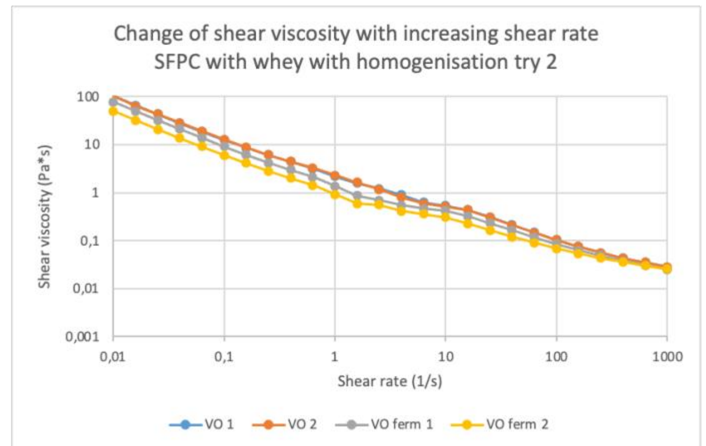
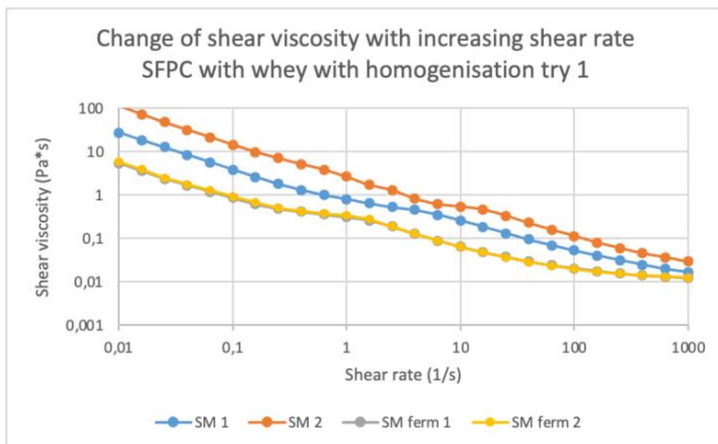


Figure A20a and b. The shear viscosity plotted against the shear rate for VO before and after fermentation. Figure a is the first try and b is the second. These samples were homogenised, and whey was added.



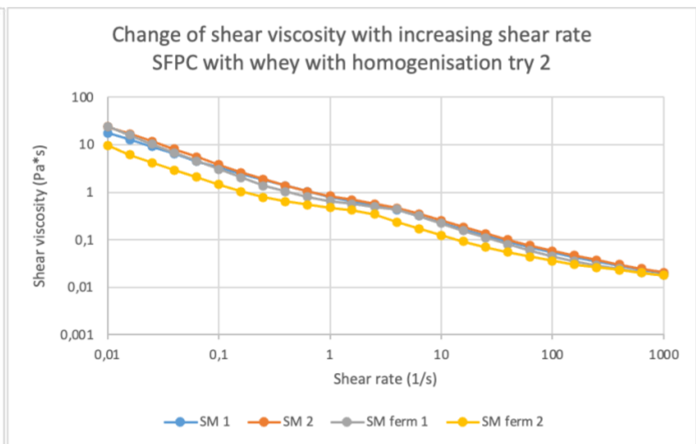


Figure A21a and b. The shear viscosity plotted against the shear rate for SM before and after fermentation. Figure a is the first try and b is the second. These samples were homogenised, and whey was added.

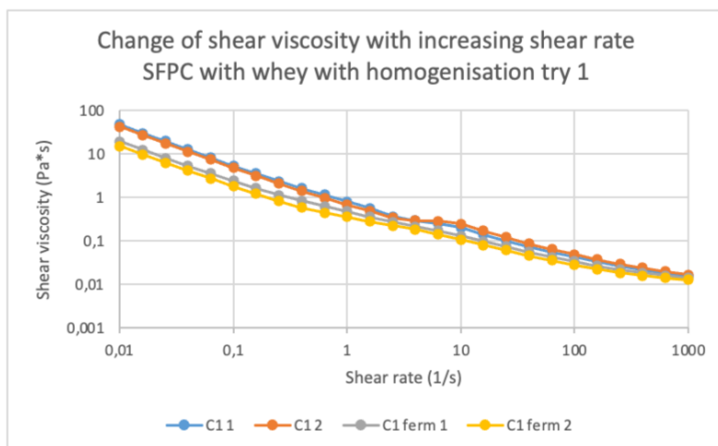


Figure A22. The shear viscosity plotted against the shear rate for C1 before and after fermentation. These samples were homogenised, and whey was added.

7.3.6 SM 15 % dry weight

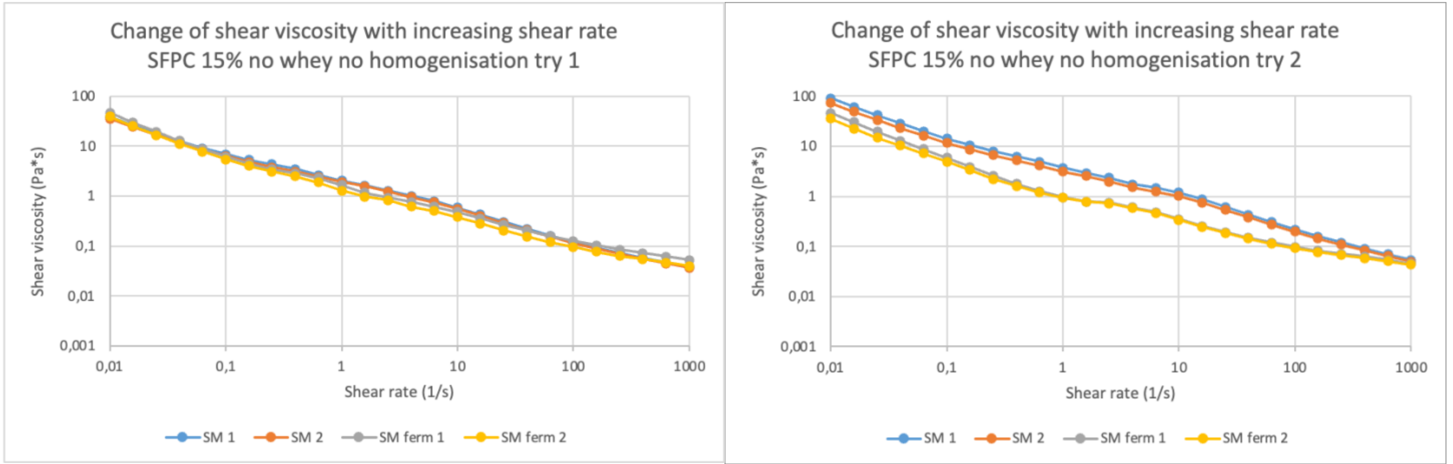


Figure A.23a and b. The shear viscosity plotted against the shear rate for SM (15% w/w) before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, and no whey was added.

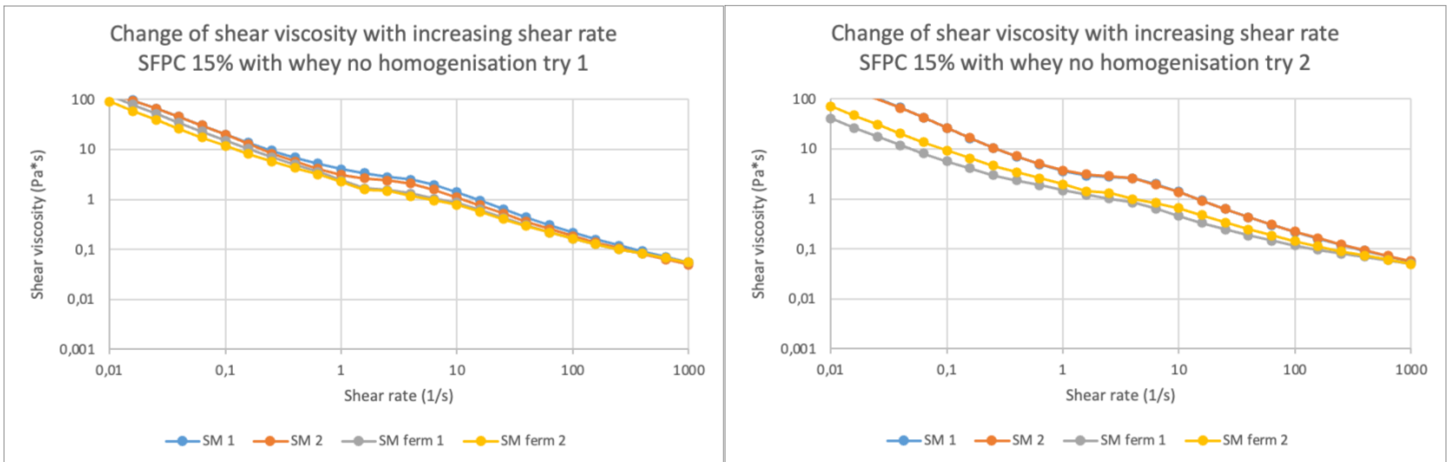


Figure A24a and b. The shear viscosity plotted against the shear rate for SM (15% w/w) before and after fermentation. Figure a is the first try and b is the second. These samples were not homogenised, but whey was added.

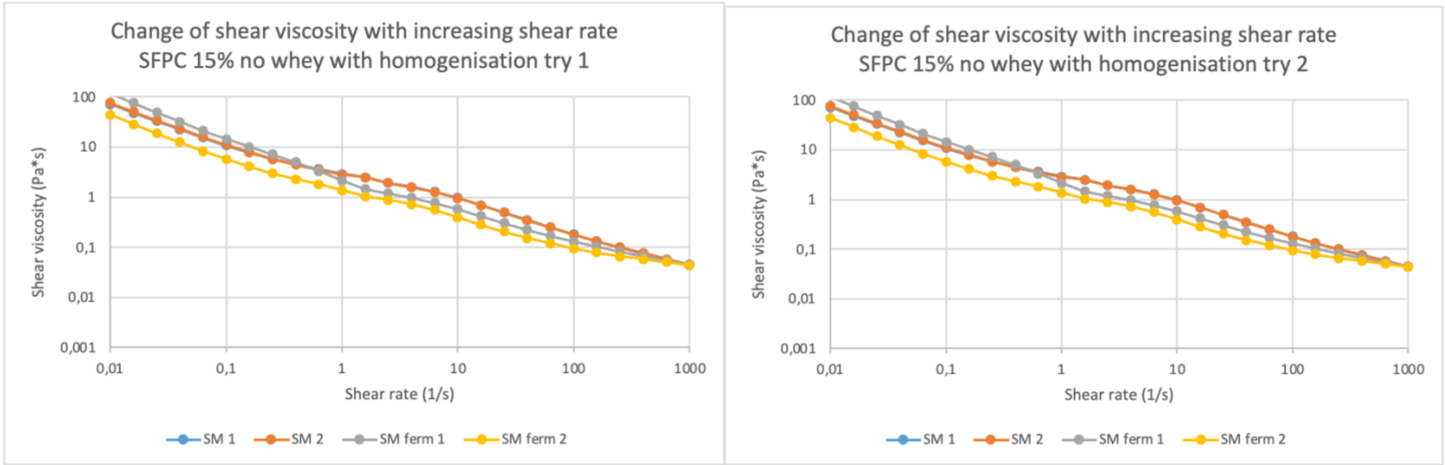


Figure A25a and b. The shear viscosity plotted against the shear rate for SM (15% w/w) before and after fermentation. Figure a is the first try and b is the second. These samples were homogenised, but no whey was added.

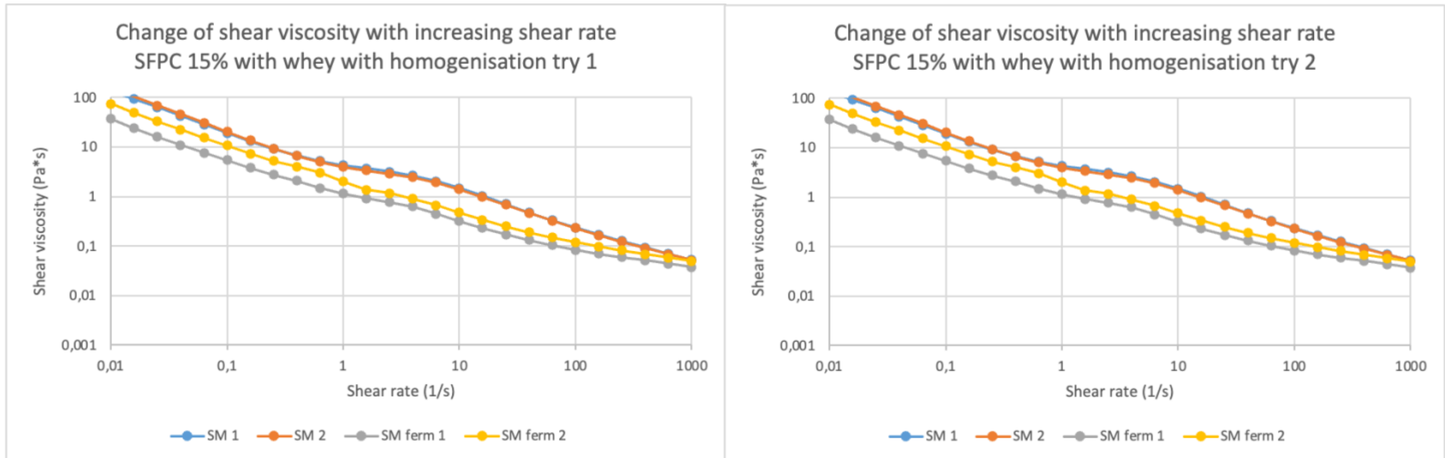


Figure A26a and b. The shear viscosity plotted against the shear rate for SM (15% w/w) before and after fermentation. Figure a is the first try and b is the second. These samples were homogenised, and whey was added.