# Cellulose nanofibrils as rheology modifier in mayonnaise – a pilot scale demonstration

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## Abstract

The applicability of cellulose nanofibrils (CNFs) as viscosifying agent in a starch-reduced low-fat mayonnaise and in an oil-reduced full-fat mayonnaise has been considered. For low-fat mayonnaise a 50 wt% reduction in the ordinary starch content was performed, while for full-fat mayonnaise, the oil content was reduced from 79 to 70 wt%. To study if the stability was affected when CNFs were added, analyses as visual and accelerated stability tests, droplet size measurements and rheology studies, determining the shear viscosity, and the loss and storage moduli, were conducted after 1 day, 1 week and 1 month of storage in room temperature. Even though changes in droplet size distributions and rheological properties indicated some coalescence, the visual stability was not changed after 1 month of storage for any of the samples. The decrease in viscosity and moduli  inflicted by reduction of starch or fat, could be regained by the addition of CNFs at 0.75 wt % and 0.42 wt %, respectively. Based on the results in this work, mayonnaise with reduced starch or fat content can be produced when CNFs are used as a viscosifying agent.

## Keywords

Nanocellulose, cellulose nanofibrils (CNFs), mayonnaise, o/w emulsions, food emulsions

## 1. Introduction

Overweight and obesity is an increasing health challenge in today's society and is probably also a reason for the increase in chronic illnesses as type II diabetes and cardiovascular disease (Despres, 2006). In an attempt to improve public health, many countries have directives for consumption of fat, where the replacement of saturated fatty acids with polyunsaturated fatty acids often is advised to reduce the risk for cardiac infarction (Mann, 2002). Following this advice, the food industry aspires to develop low calorie food and products that have low fat-content. However, this is not straightforward, as fat gives flavor, texture and appearance to the food (Lucca & Tepper, 1994; Ognean, Darie & Ognean, 2006). When fat is removed, it must be replaced with a material that can substitute these properties. One class of fat replacers already on the market is the carbohydrate-based fat mimetics. They mimic the physicochemical properties and desirable eating qualities of fat, such as viscosity, mouthfeel and appearance (Duflot, 1996; Ognean et al., 2006). Dietary fibers, e.g. cellulose-based, are examples of carbohydrate-based fat replacers (Gibis, Schuh & Weiss, 2015). Dietary fibers have numerous beneficial effects on human health, such as improved digestion in the large intestine, reduced risk of cardiovascular diseases, stroke and several diseases in the digestive tract, and of diabetes type II through glycemic control (Anderson et al., 2009; Andrade et al., 2015; Ötles & Ozgoz, 2014).

The most abundant biopolymer in nature is the cellulose fiber, which is found in wood, cotton, plants and vegetables. Cellulose is the source of nanocelluloses, which is obtained after fibrillation of the cellulose fiber. Nanocellulose is a general term for cellulosic materials in nanoscale, which comprises numerous types, including cellulose nanofibrils (CNFs) used in this study. These nanocellulose types are produced from cellulose pulp in top-down processes, such as mechanical fibrillation or enzymatic or chemical treatment in combination with mechanical fibrillation (Habibi, Lucia & Rojas, 2010; Pääkkö et al., 2007; Saito & Isogai, 2004; Wågberg et al., 2008). The CNFs have a high aspect ratio, with widths in the nanometer scale and lengths in the micrometer scale(Klemm et al., 2011). CNFs can be produced with tailored surface groups on the fibril surfaces. Pretreatment methods using 2,2,6,6-tetramethylpiperidinyl-1-oxyl (TEMPO)-mediated oxidation introduce negatively charged carboxyl groups at the fibril surface at physiological pH-values (Saito et al., 2004). Types of CNFs that are produced using enzymatic or mechanical pretreatments have a minimal negatively charge (Henriksson, Henriksson, Berglund & Lindstrom, 2007; Pääkkö et al., 2007).

The morphological properties (aspect ratio, degree of fibrillation, network structure and crystallinity) and surface charge make nanocelluloses highly qualified for use as viscosifying agents (Aaen, Simon, Brodin & Syverud, 2019), and annual citations for publications on the topic «aqueous suspensions of nanocellulose and rheology» have been heavily increasing from year 2010 and onwards (Hubbe et al., 2017).

One highly interesting application where the rheological properties of nanocelluloses play an important role, is when used as a food additive (Gallegos, Franco & Partal, 2004; Turbak, Snyder & Sandberg, 1982). Several different food products are relevant, e.g. mayonnaise (Choublab & Winuprasith, 2018; Golchoobi, Alimi, Shokoohi & Yousefi, 2016), salad dressings (Turbak, Snyder & Sandberg, 1983), ice cream (Okiyama, Motoki & Yamanaka, 1993; Velasquez-Cock et al., 2019) and meat products (Marchetti, Muzzio, Cerrutti, Andres & Califano, 2017). A review summarizing nanocelluloses in food science is written by Gomez and colleagues (Gomez et al., 2016). Some parameters that can affect the performance of nanocelluloses as a rheology modifier in food related applications, are ionic strength, pH and temperature. Tolerance for salt and acidic conditions is essential when CNFs are used in combination with food ingredients as NaCl and acids (Aaen, Brodin, Simon, Heggset & Syverud, 2019; Aaen, Simon, et al., 2019; Fall, Lindstrom, Sundman, Odberg & Wagberg, 2011; Gestranius, Stenius, Kontturi, Sjoblom & Tammelin, 2017; Salas, Nypelo, Rodriguez-Abreu, Carrillo & Rojas, 2014). An increase in ionic strength or decrease in pH can both lead to aggregation of charged fibrils, as the electrostatic repulsion between fibrils is reduced (Fall et al., 2011). CNFs and CNCs can stabilize o/w emulsions through adsorption at the liquid−liquid interfaces, forming emulsions known as Pickering emulsions (Binks, 2002; Cunha, Mougel, Cathala, Berglund & Capron, 2014; Gestranius et al., 2017). In addition, they contribute to an increase in viscosity, and can form networks in the continuous phase, slowing down emulsion destabilization mechanisms, such as creaming and coalescence (Binks, 2002; Quintana, Califano, Zaritzky & Partal, 2002; Saelices & Capron, 2018; Xhanari, Syverud, Chinga-Carrasco, Paso & Stenius, 2011). One of the food products where the emulsion stabilizing effects of nanocelluloses can be applied is mayonnaise. Nanocelluloses as a food additive in mayonnaise has been investigated as a possible way of improving rheological and sensory properties in formulas with reduced fat content, or as emulsion stabilizers in mayonnaise formulas without egg yolk (Choublab et al., 2018; Golchoobi et al., 2016). As previously reported by Aaen and colleagues, a low-charged (enzymatically pretreated) CNF type was best qualified to stabilize o/w model mayonnaise emulsions containing NaCl and acid, compared to a highly negatively charged (TEMPO-oxidized) type, due to its lower sensitivity to salt-induced aggregation (Aaen, Brodin, et al., 2019).

In this study, use of CNFs as a viscosifying agent in a starch-reduced low-fat mayonnaise and in an oil-reduced full-fat mayonnaise has been studied in pilot scale. A long-term storage for up to 1 month was performed, and analyses as visual and accelerated stability tests, droplet size measurements and rheology studies have been conducted.

## 2. Materials and Methods

### *2.1 Cellulose nanofibril production and characterization*

Enzymatically pretreated CNFs were produced at RISE, Division of Bioeconomy and Health, as previously described (Henriksson et al., 2007; Pääkkö et al., 2007). An industrially produced never-dried, bleached softwood sulfite dissolving pulp, obtained from Domsjö Fabriker (Domsjö Mill, Sweden), was used as raw material for the production.

The pretreatment started with a refining step to make the fiber walls more accessible to the endoglucanase enzymes. After the enzyme treatment conducted at neutral pH, the pulp was washed, followed by a second refining step and dilution to 2 % consistency. After dilution, the pretreated pulp was fibrillated by passing the pulp three times through a Microfluidizer (M-110EH-30, Microfluidics Corp.) at 1700 bar pressure. The microfluidizer had two z-shaped interaction chambers (200µm + 100µm).

The morphology of the CNF sample was characterized using scanning electron microscopy (SEM). SEM imaging was performed on CNF films with a grammage of 20 g/m$^2$, with a Hitachi scanning electron microscope (SU3500, Hitachi Scientific Instruments, CA, USA), in secondary electron imaging mode.

### *2.2 Mayonnaise production*

Low-fat and full-fat mayonnaises were produced at Mills pilot plant in Fredrikstad, Norway. The composition of the ingredients in the mayonnaise qualities are shown in Table 1. The first step in the production of the low-fat mayonnaise was the mixing of the starch phase, with a following heating step of the starch at 80 °C for 5 minutes. Subsequently, the solution was cooled to 15 °C. When the appropriate temperature was obtained, the egg yolks and the acidic and oil phases were added, and the phases were emulsified together using emulsification equipment. For the full-fat mayonnaise, that does not contain starch, the heating step was unnecessary, and the different ingredients were therefore directly mixed and emulsified.

Table 1. Composition of low-fat and full-fat mayonnaise, referred to as reference qualities. Some of the formulations and the exact amount of each ingredient are anonymized due to intellectual property rights for the commercial producer Mills.

| Ingredients | Amount (wt%) | |
|---|---|---|
| | Low-fat | Full-fat |
| Acidic phase (acetic acid, citric acid and water) | 6.9 | 15.2 |
| Starch phase (water, sugar, salt, starch A, starch B, potassium sorbate and sodium benzoate) | 48.6 | - |
| Oil phase (rapeseed oil and stabilizers) | 40.0 | 79.2 |
| Egg yolks | 4.5 | 5.6 |

To study if nanocellulose can be used as a texturizer in mayonnaise, addition of CNFs was assessed. Due to the high water content of the CNF sample (98 wt % water), the amount of water in the mayonnaise recipes was reduced accordingly when CNFs were added. For the low-fat mayonnaise, CNFs were added to the starch phase, before mixing with the other ingredients. The final concentration of CNFs in the low-fat mayonnaise was 0.25, 0.50 and 0.75 wt% calculated on dry basis, respectively. For all samples where CNFs were added, a 50 wt% reduction in the starch content was performed. This was done according to initial lab experiments, where reduction in starch content was studied to see if CNFs could compensate for the addition of starch (preliminary results not shown). For full-fat mayonnaise, CNFs were added to the acidic phase, before mixing with the other ingredients. Here, the final concentration of CNFs was 0.25 and 0.42 wt%, respectively. 0.42 wt% CNFs was the maximum amount of fibrils that could be added to the full-fat mayonnaise without changing the total water content. The effect of reduction in oil, from 79 to 70 wt%, was studied.

Samples without CNFs were produced for both mayonnaise qualities. They are referred to as the reference samples. After preparation and for 1 month of incubation, samples were stored at 23 °C in a climate room to study how the stability of the products were affected when stored at room temperature.

### *2.3 Characterization of mayonnaise*

#### *2.3.1. Visual stability and accelerated stability tests*

The appearance of the mayonnaise samples was determined by visual inspection. Changes in color, potential creaming and phase separation were studied. Photos were recorded using a digital camera. Visual stability was evaluated after 1 day, 1 week and 1 month after the mayonnaise was prepared. The samples were stored in vertically placed plastic tubes. Accelerated stability tests were performed to see if the samples underwent creaming or phase separation. Samples were centrifuged (Labofuge 400 R, Heraeus Instruments, Hanau, Germany) with relative centrifugal force (RCF) of 2958×g for 5 min at 23 °C the day after they were prepared. Samples were visually inspected for signs of phase separation and creaming, and photos were taken before and after the centrifugation.

#### *2.3.2. Light microscopy*

To obtain visual information about oil droplet size and homogeneity of the samples, light microscopy analyses were assessed. Samples were prepared taking a small droplet of the mayonnaise between a microscopy glass slide and a cover glass. Images were recorded at 400×magnification using a Leitz DM RXE light microscope (Leica Microsystems, Wetzlar, Germany).

#### *2.3.3. Droplet size measurements*

The size distributions of oil droplets were determined after 1 day, 1 week and 1 month by laser light diffraction in a Mastersizer 3000 (Malvern Panalytical, Worcester, UK). A few drops of mayonnaise sample were added to a 400 mL beaker of distilled water and stirred with the mastersizer propeller at 2590 rpm for dilution, until the obscuration reached 5-10 %. The measurements were run with fifteen replicates for each sample.

#### *2.3.4. pH measurements*

The pH was measured for the mayonnaise samples using a Russell RL060P pH meter (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

### *2.3.5. Rheological measurements*

A Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a concentric cylinders geometry (flow and oscillatory measurements) or a cone and plate geometry (creep tests) was used to evaluate the rheological properties of the mayonnaise samples at 20 ˚C 1 day, 1 week and 1 month after preparation. All samples were subjected to one minute of pre-shearing at 100 $s^{-1}$ followed by two minutes of rest before the measurements started.

Flow curves were obtained in triplicates by increasing the shear rate from 0.1 to 1000 $s^{-1}$ over a 10 minutes period, followed by a decrease to 0.1 $s^{-1}$ over the next 10 minutes. The procedure was then repeated immediately on the same sample, giving two up-sweeps and two down-sweeps for each of the triplicates. To determine the linear viscoelastic region of the samples, strain sweeps (0.1 to 100%)were performed in duplicate, with the frequency set at 0.01 Hz. Frequency sweeps (0.01 - 10 Hz) were performed in triplicate with the strain set at 1%, which was well within the linear viscoelastic region for all the samples.

For the starch-reduced low-fat mayonnaise samples, creep tests were performed. A constant stress in the range of 5 Pa to 55 Pa, with a stepwise increase of 10 Pa between measurements, was applied for 5 minutes, and subsequently removed, while the resulting strain of the mayonnaise was measured.

## 3. Results and Discussion

### *3.1 Cellulose nanofibril characterization*

SEM images were recorded to study the morphology of the CNF sample. A picture taken at high magnification level (10 000x) is shown in Figure 1. The sample has a coarse structure and consists of a tight entanglement network of thinner fibrils interspersed with fibrils with larger fibril diameters.

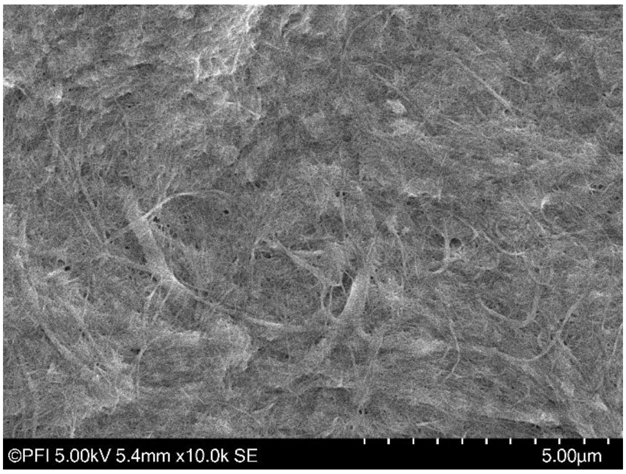

*Figure 1: SEM image of a CNF film with a grammage of 20 g/m$^2$ obtained at 10 000x magnification.*

### 3.2 Starch-reduced low-fat mayonnaise

#### 3.2.1. Visual and physical stability

Stability of the starch-reduced low-fat mayonnaise samples was observed visually on a regular basis, and pictures were taken 1 day, 1 week and 1 month after preparation. The pictures of samples stored for 1 day and 1 month are shown in supplementary material (Figure S1). All of the samples were stable, even after one month of storage. For the reference sample, trapped air was observed in the bottom of the tube after the first day of incubation. After one month, the sample was unchanged. The samples were not affected by the centrifugation as no phase separation could be observed (Figure S2 in the supplementary material section).

Micrographs of the reference low-fat mayonnaise and the starch-reduced low-fat mayonnaise samples with various CNF content, taken after 1 day, 1 week and 1 month of storage, are shown in Figure 2. The largest droplets observed are in the range of 50 µm for all the samples, both the reference low-fat mayonnaise and the starch-reduced low-fat mayonnaises containing CNFs. This qualitative analysis of the droplets indicates that all the emulsions are stable, even after incubation for 1 month.

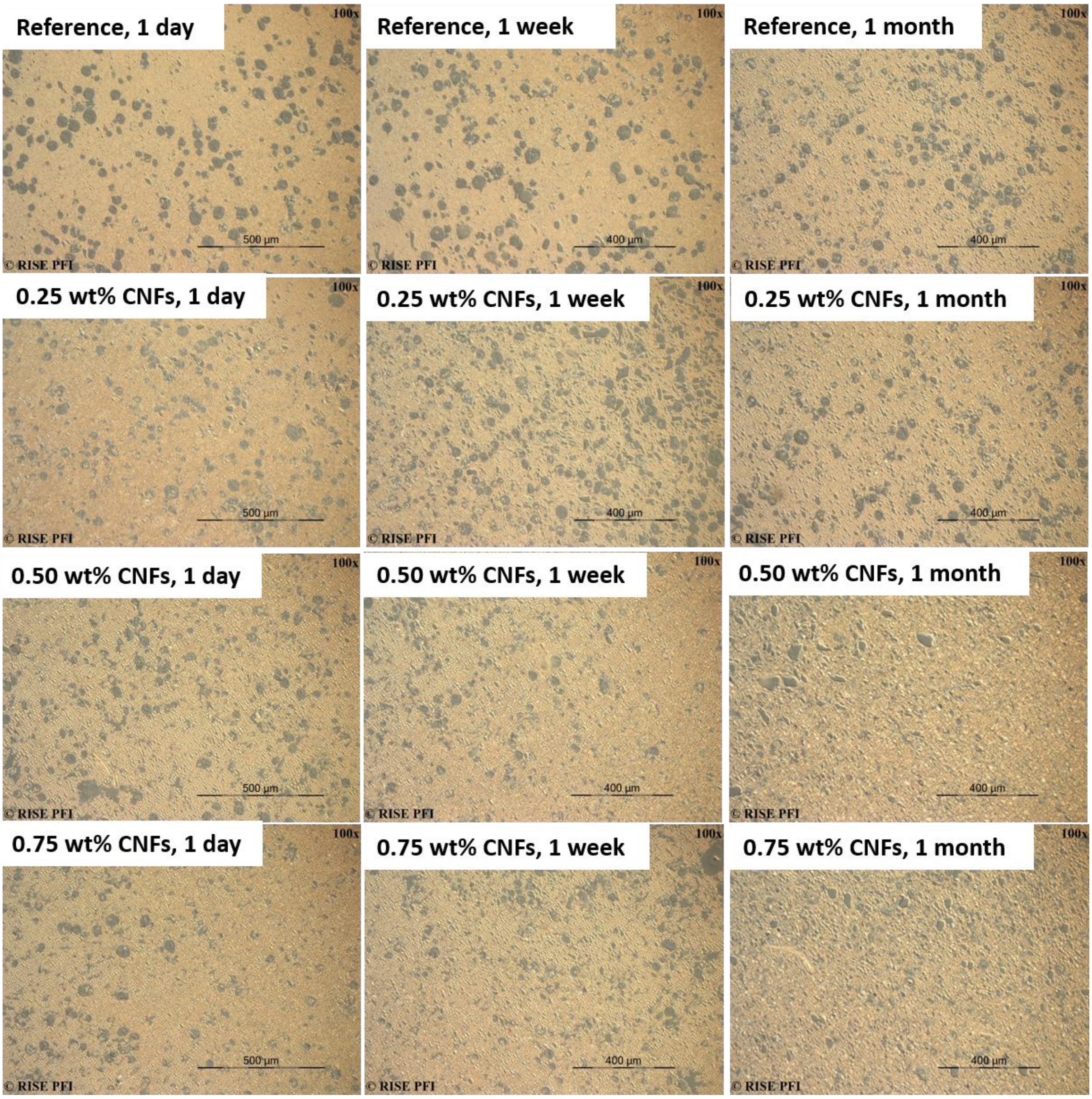

*Figure 2. Light microscopy images of low-fat mayonnaise; Reference (top) and starch-reduced low-fat mayonnaise with 0.25, 0.50 and 0.75 wt% CNFs (row 2-4). Images on left are after 1 day of incubation, in the middle after 1 week of incubation, while images on the right are taken after 1 month of incubation in room temperature.*

The droplet size distributions over time for starch-reduced low-fat mayonnaise are shown in Figure 3. The reference sample and the sample containing 0.25 wt % CNFs had clearly divided bimodal droplet size distributions. At higher CNF concentrations, the division between the two peaks is much smaller, and the distributions develop towards unimodal distributions during the storage time. Over the one month of storage, the change in droplet size was relatively small, as confirmed by the average droplet size, $d_{4;3}$ shown in Figure S3 in supplementary. The droplet sizes estimated from the micrographs, are within the range covered by the droplet size distributions (Figure 2).

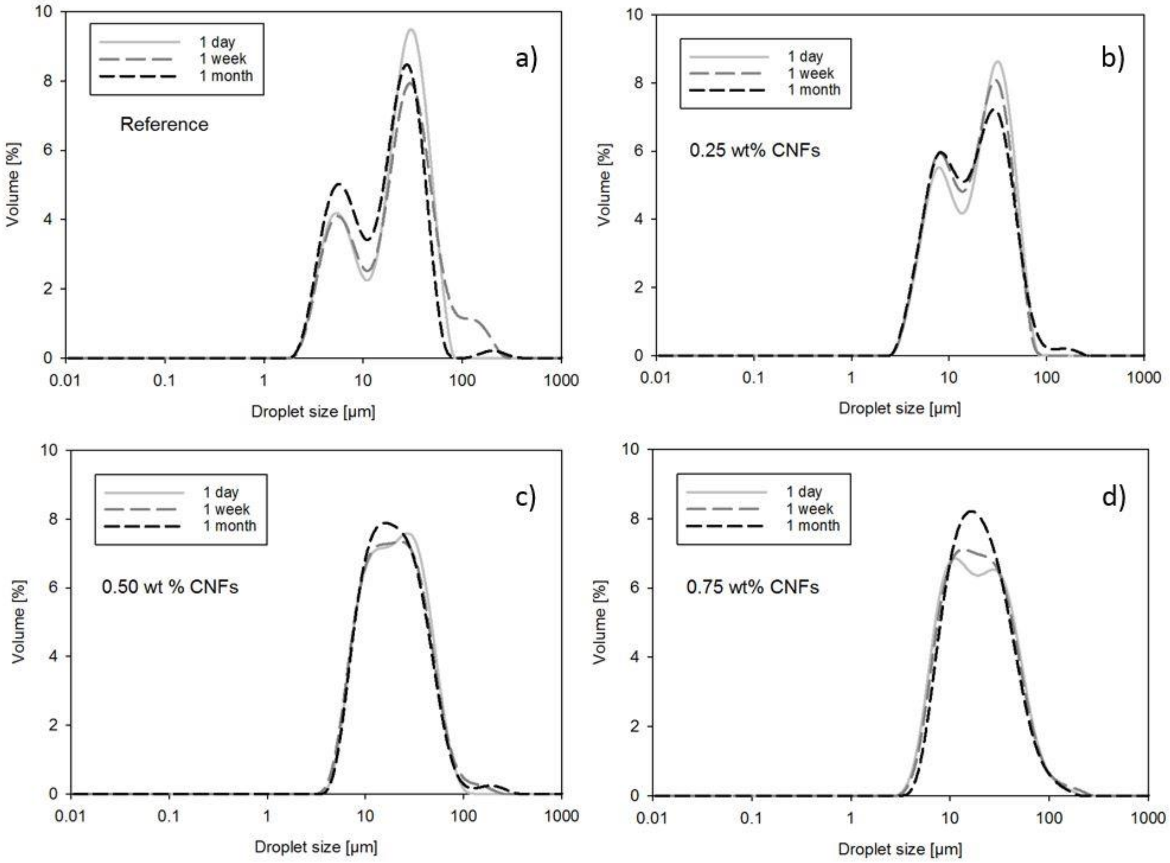

*Figure 3: Droplet size distributions after 1 day, 1 week and 1 month for (a) a reference low-fat mayonnaise, and for starch-reduced low-fat mayonnaise with 50 % reduced starch content and CNF contents of (b) 0.25 (c), 0.50 or (d) 0.75 wt % .*

pH was measured for all the samples after 1 day, 1 week and 1 month of storage. As for visual stability, micrographs and droplet sizes, the pH values were stable during the storage, around pH 4 to 4.5, for both the reference sample and the samples with CNFs added, with no major differences between the mayonnaise samples. The measured pH values are within the range commonly approved for commercial mayonnaise in Europe (Lund, Baird-Parker & Gould, 2000). A pH value around 4 is low enough to avoid microbial growth, and is close to the isoelectric point of the stabilizing proteins from the egg yolk, which ensures the best conditions for emulsion stabilization (Depree & Savage, 2001). Results are shown in Figure S4 in supplementary materials.

### *3.2.2. Rheological measurements*

To supplement the observations of visual and physical stability, flow and oscillatory measurements were conducted after 1 day, 1 week and 1 month of storage. The flow curves obtained for the starch-reduced low-fat mayonnaise samples are shown in Figure S5 in the supplementary material, together with the moduli from the frequency sweep. The flow curves show similar behavior for all the starch-reduced low-fat mayonnaise samples, which were all shear thinning, in agreement with other studies on the rheology of mayonnaise (Moros, Franco & Gallegos, 2002; Peressini, Sensidoni & de Cindio, 1998). The shear thinning effect can be related to deformation and disruption of aggregated droplets as the shear rate increases (McClements, 2015; Mun et al., 2009). For all the mayonnaise samples, the shape of the curve for the first up-sweep differs somewhat from the shape of the three following curves (Figure S5), indicating thixotropic behavior (Razavi & Karazhiyan, 2009; Steffe, 1996). The evolvement of the viscosity at a low shear rate of 1 $s^{-1}$ over the storage time of 1 month, is shown in Figure 4.

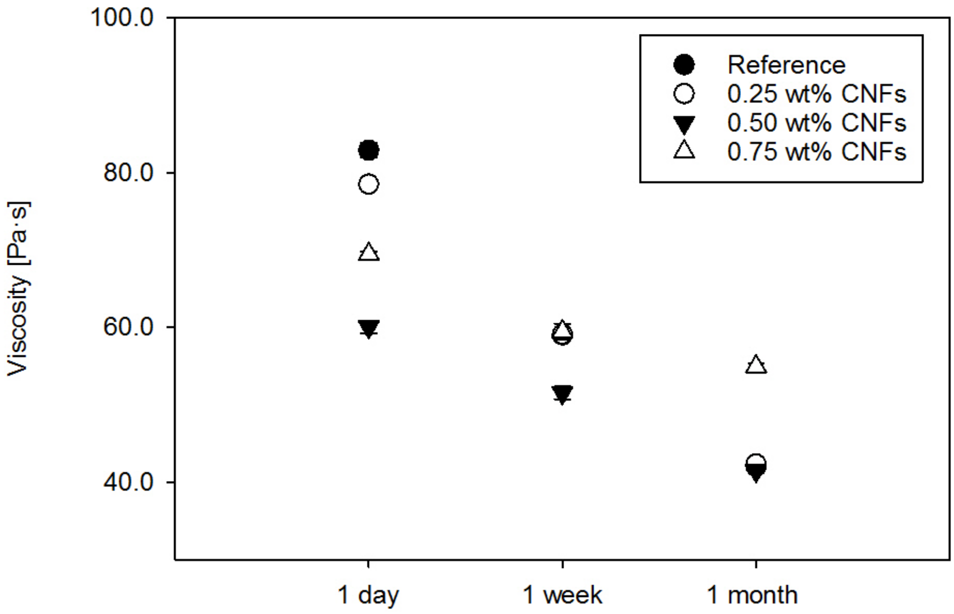

*Figure 4: The development of the viscosity throughout the storage period, with data from the first up-sweep, at a shear rate of 1* $s^{-1}$ *for a reference of low-fat mayonnaise, and for starch-reduced low-fat mayonnaise at three different levels of CNF addition. The error bars included in the figure are smaller than the symbols.*

Figure 4 shows that the starch-reduced low-fat mayonnaises containing CNFs had viscosities in the same range as the reference low-fat mayonnaise. Golchoobi et al. have previously shown that CNFs as a fat replacer in a low-fat mayonnaise formulation could provide a viscosity, yield stress and moduli comparable to the rheological properties of a commercial low-fat mayonnaise (Golchoobi et al., 2016). Looking at the development of apparent viscosity over the storage time of 1 month (Figure 4), there was a decrease over time for all samples, including the reference sample. This implies that some change is taking place in the samples, although this is not visible from the visual inspection and the droplet size measurements.

All samples had higher storage than loss modulus (Figure S5), over the whole frequency range measured, in accordance with results obtained for mayonnaise by other groups (Gallegos, Berjano & Choplin, 1992; Moros et al., 2002). This shows the predominant elastic character of mayonnaise samples over the viscous one. The storage modulus at a selected frequency of 1.17 Hz over the 1 month of storage shown in Figure 5 is, as the viscosity, decreasing with time for all the mayonnaise samples. The 0.75 wt % CNF sample differs from the other mayonnaise samples, with its higher storage modulus, especially after one month of storage. An ability of CNFs to increase the storage modulus of low-fat mayonnaise has previously also been observed for 1 wt % CNFs in a 30 wt % oil mayonnaise system (Golchoobi et al., 2016). This effect may be due to the network-forming ability of CNFs, where the fibrils form a gel-like viscoelastic network in the continuous phase (Ougiya, Watanabe, Morinaga & Yoshinaga, 1997; Paximada, Tsouko, Kopsahelis, Koutinas & Mandala, 2016; Winuprasith & Suphantharika, 2013).

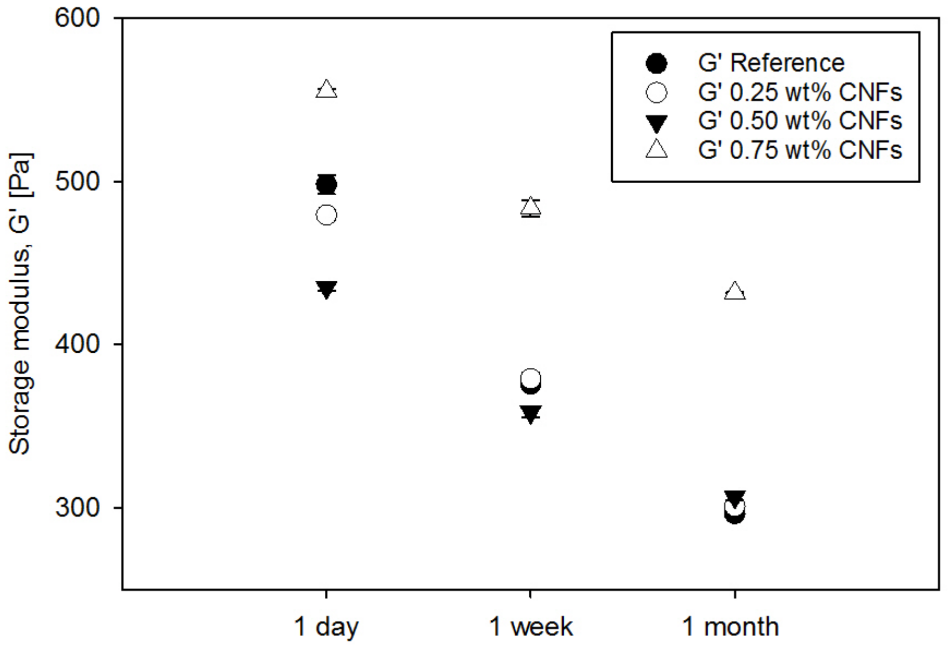

*Figure 5 : The development of G' with storage time, for a low-fat mayonnaise reference, and three CNF-containing starch-reduced low-fat mayonnaise samples, taken at a frequency of 1.17 Hz (right). Error bars for the figure are smaller than the symbols.*

The creep curves in Figure 6, and in Figure S6 in the supplementary material, showed a transition from a viscoelastic response (partial recovery of the strain) to a more viscous response (no recovery of strain) as the applied stress was increased, which indicates a yield stress for the mayonnaise. A yield stress is commonly observed for mayonnaise, with yield stress values for full-fat mayonnaise around 24.8-26.9 Pa and 24-46 Pa for a low-fat mayonnaise, depending on the amount of fat replacer (Mun et al., 2009; Steffe, 1996). For the starch-reduced low-fat mayonnaise with 0.75 wt % CNFs, shown in Figure 6, this transition appears to happen between 35 and 55 Pa. For the reference low-fat mayonnaise and the other two starch-reduced low-fat mayonnaises, this transition has an onset from 25 Pa. In a low-fat mayonnaise with 30 wt% oil and 1 wt % CNFs, the yield stress was found to be about 30 Pa, placing it in the same area as our findings (Golchoobi et al., 2016). For all the samples, there is an increase in compliance and an earlier onset of viscous response after 1 month compared to after 1 week of storage (Figure S6). This means that the ability to resist flow decreases with storage time, as the other rheological properties.

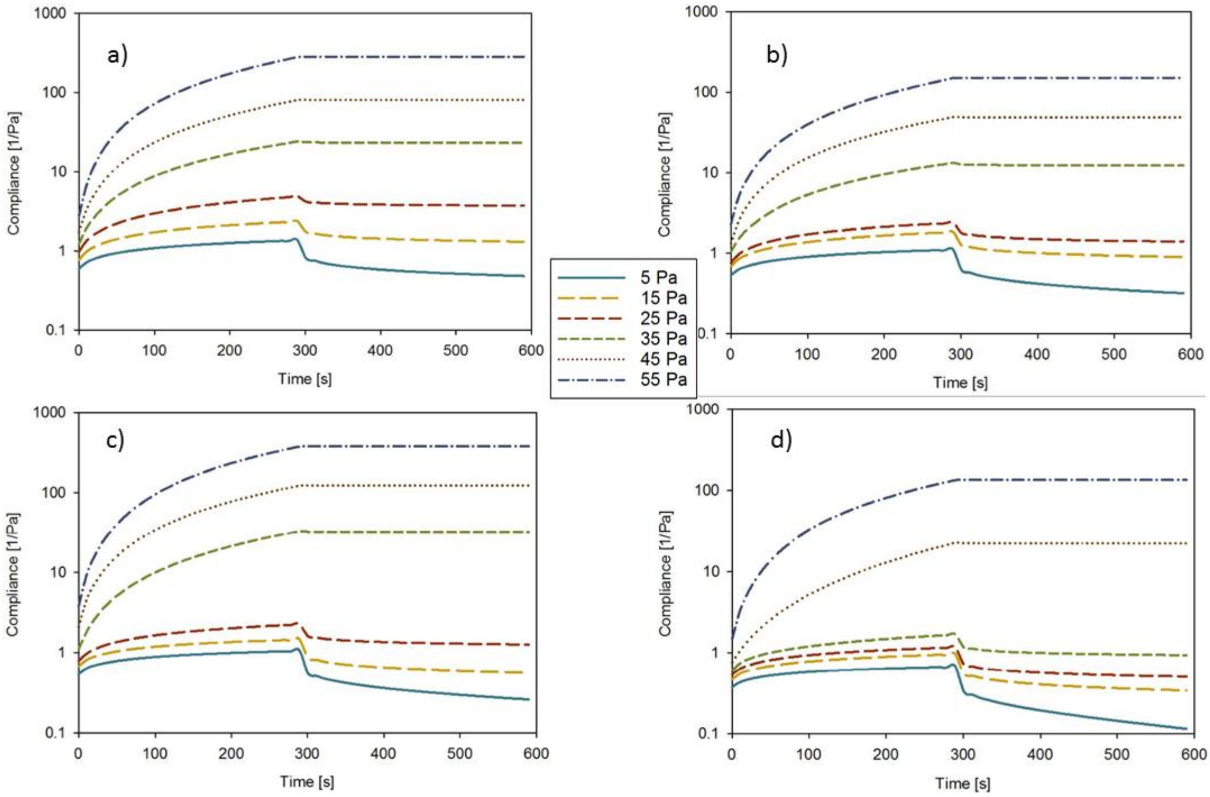

*Figure 6 : Curves obtained from creep relaxation tests with increasing stress, for a) a reference low-fat mayonnaise, and starch- reduced low-fat mayonnaise with b) 0.25 wt %, c) 0.50 wt % and d) 0.75 wt % CNFs, after 1 week. .*

Even with some changes in rheological properties with storage time, the mayonnaise appears to be rather stable, with no change in appearance, minor changes in droplet sizes, and with no visible coalescence. In addition, CNFs can compensate the loss of the rheological properties of mayonnaise induced by the reduction in starch content.

## *3.3. Oil-reduced full-fat mayonnaise*

### *3.3.1. Visual and physical stability*

The oil-reduced full-fat mayonnaise samples were observed visually to obtain information about stability. Pictures were taken 1 day, 1 week and 1 month after preparation, with pictures of samples stored for 1 day and 1 month  shown in supplementary material (Figure S7). Both reference samples and the samples with CNFs added appeared stable, even after one month of storage.

Micrographs of the mayonnaise samples taken after 1 day, 1 week and 1 month of storage, are shown in Figure 7. The reference mayonnaise with 70 wt % oil differs from the other samples with a greater number of larger droplets. Changes in droplet size are observed for both the reference samples after 1 month of storage. The samples where CNFs are added, show less changes over the storage period, and is therefore suggested to be slightly more stable after 1 month of storage in room temperature than the reference samples. The droplets observed after 1 day of storage were smaller than 20 µm, while after 1 month the largest droplets in the reference mayonnaise had diameters up to 60 µm. For the other mayonnaise samples, with 70 wt% oil and various amounts of CNFs, the largest droplets were around 30 µm.

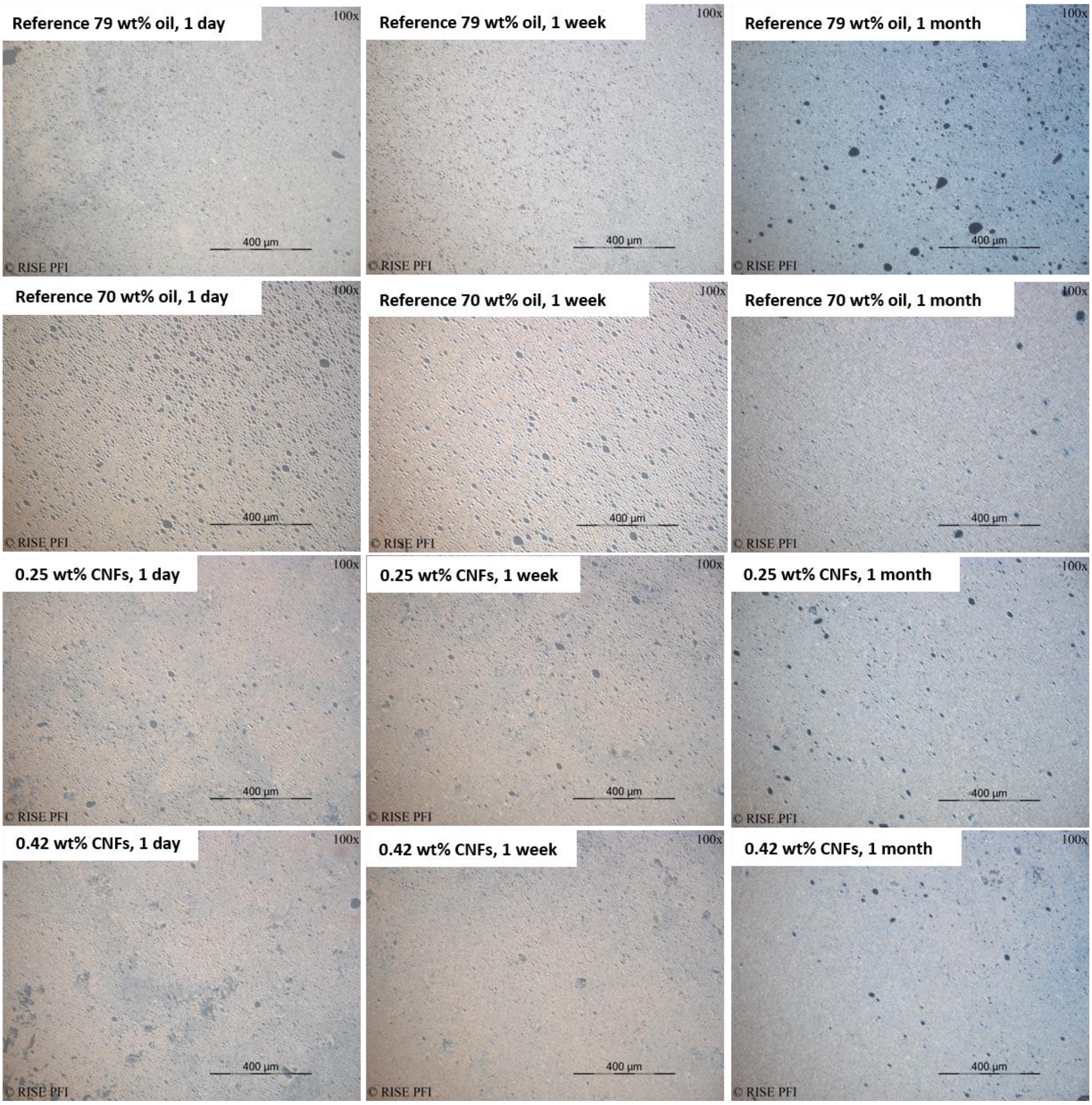

*Figure 7. Light microscopy images of full-fat mayonnaise; Reference (with 79 wt% oil; top) oil-reduced full-fat mayonnaise with 70 wt% oil (row 2) and oil-reduced full-fat mayonnaise with 70 wt% oil and 0.25 and 0.42 wt% CNFs added (row 3 and 4). Images on left are after 1 day of incubation, in the middle after 1 week of incubation, while images on the right are taken after 1 month of incubation in room temperature.*

The droplet size distributions over time for a reference full-fat mayonnaise, and oil-reduced full-fat mayonnaises with various additions of CNFs, are shown in Figure 8. The reference full-fat mayonnaise displayed a very clear bimodal distribution, as was also shown for full-fat mayonnaise samples prepared by Di Mattia et al., with various kinds of oil (Di Mattia et al., 2015). The distributions of the oil-reduced full-fat mayonnaise samples show another shape, with a more marked main peak of droplet sizes. A higher volume fraction of oil leads to an increase in viscosity and moduli, and is known to lead to larger droplets, due to less turbulent flow in the emulsification process (Tadros, 2013). Thus, the bimodal size distribution of the 79 wt% oil mayonnaise reference is probably caused by the less efficient mixing during emulsification. Some changes in the droplet size distributions can be observed between 1 day and 1 week, while between 1 week and 1 month of storage there is almost no change in the droplet sizes. This is also evident from the average droplet sizes ($d_{4;3}$) shown in Figure S8 in supplementary material. However, from the micrographs (Figure 7), the largest alterations in the emulsions can be seen between 1 week and 1 month, making it hard to draw any conclusions on this matter. As the volume-based distribution is very sensitive to the presence of a few large droplets in the sample, the observed differences can stem from local inhomogeneity in the samples, where the few droplets with diameter over 100 µm, might not have been present in the sample volume used for the micrographs. Another possibility is that the mastersizer has detected aggregated droplets, giving the impression of larger droplets in the mayonnaise. . For the 79 wt % reference sample, the population of large droplets decreased between 1 day and 1 week, either through coalescence, giving droplets or local areas of oil with diameters larger than detected by the mastersizer, or through the de-aggregation of droplets. As there were no changes in composition or pH likely to alter the aggregation state of the oil droplets, coalescence is the most likely of these two. The decrease in larger droplets leaves the smaller oil droplets with a larger volume fraction than in the freshly prepared samples, causing a decrease in the a average droplet size (Figure S8). The sample containing 0.25 wt % CNFs also shows similar patterns of coalescence, but to a smaller extent than the reference mayonnaise. For both these samples, the coalescence is not to an extent that it affects the visual appearance of the mayonnaise.

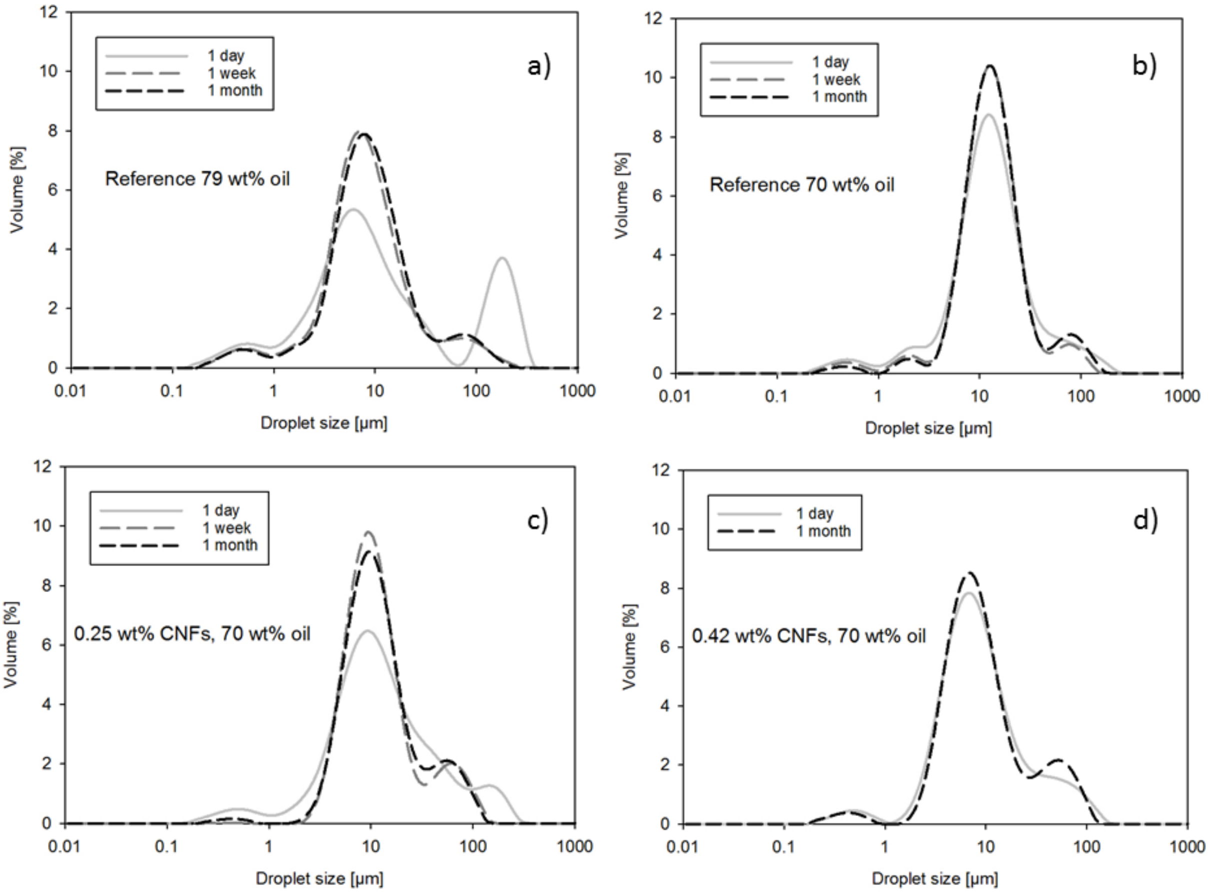

*Figure 8: Droplet size distributions after 1 day, 1 week and 1 month for (a) a reference full-fat mayonnaise with 79 wt% oil, (b) one reference with 70 wt% oil, and two samples with (c) 0.25 or (d) 0.42 wt % of CNFs and 70 wt % oil. Due to technical issues during measurements, the droplet size distribution after 1 week is not included for the sample containing 0.42 wt% CNFs.*

As for the starch-reduced low-fat mayonnaise samples, the pH values for the oil-reduced full-fat mayonnaise samples were stable after storage for 1 day, 1 week and 1 month. The measured pH values were from pH 3.85 to pH 4.06, with no major differences between the mayonnaise samples. Results are shown in Figure S9 in supplementary materials.

### 3.3.2. Rheological measurements

Flow and oscillatory measurements for oil-reduced full-fat mayonnaise were conducted after one day, one week and one month of storage, with results shown in Figure 9 and 10, as well as in Figure S10 in supplementary.

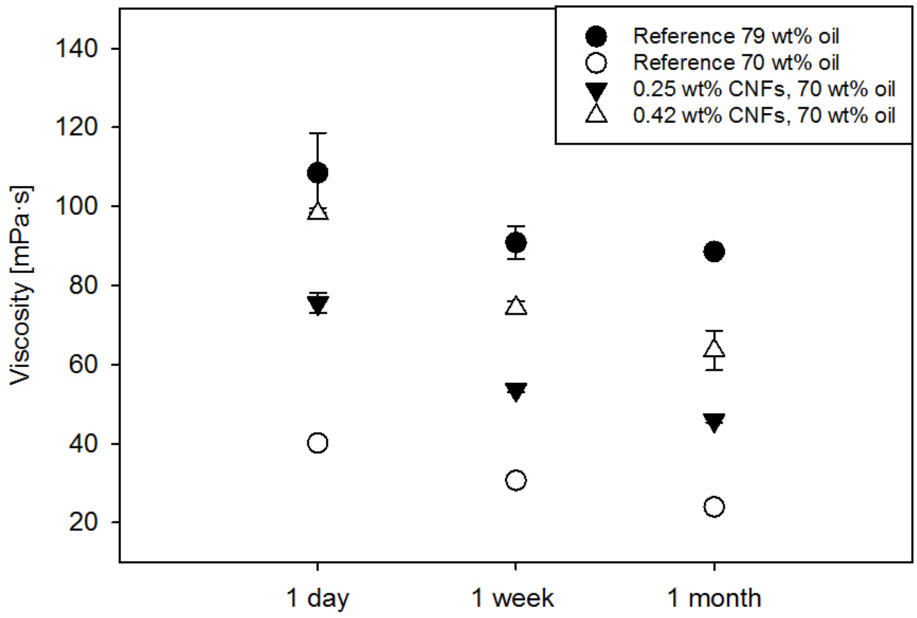

*Figure 9 : The development of the viscosity throughout the storage period, with data from the first up-sweep, at a shear rate of 1* $s^{-1}$ *for a reference of full-fat mayonnaise, a mayonnaise produced with a reduced oil content, and two different levels of CNF addition.*

As observed by other groups, the reduction of oil content from 79 to 70 wt % in the mayonnaise, led to a decrease in viscosity and moduli (Figure 9 and 10) (Lee, Lee, Lee & Ko, 2013; Ma & Barbosa-Canovas, 1995). The addition of CNFs could contribute to restore the rheological effect of the oil reduction, as has previously been shown for xanthan gum and oil-reduced mayonnaise (Ma et al., 1995). As for the starch-reduced low-fat mayonnaise, these samples were also shear-thinning and thixotropic, had higher storage than loss modulus (Figure S10), and experienced a decrease in apparent viscosity and storage modulus over time. A decrease in the apparent viscosity of mayonnaise with storage time, both control samples and mayonnaise stabilized solely with CNFs, have been observed previously(Choublab et al., 2018). Choublab and Winuprasith explained this change in viscosity with some coalescence of oil droplets in the mayonnaise. For the storage and loss moduli shown in Figure 10, a large drop in both moduli can be observed for the oil-reduced reference compared to the full-fat reference. The storage modulus has previously been correlated to the texture of mayonnaise, and can thus be an important parameter to control when changes are made to mayonnaise recipes (Maruyama, Sakashita, Hagura & Suzuki, 2007). The moduli of the full-fat mayonnaise with reduced oil content, reached the same level as the original full fat mayonnaise when 0.42 wt% CNFs were added.

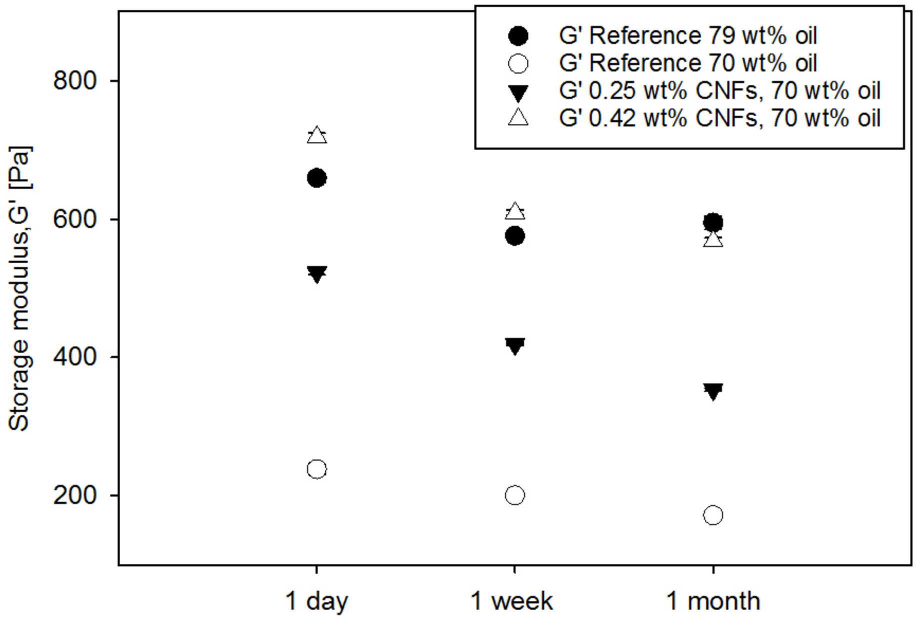

*Figure 10 : The development of G' for a full fat mayonnaise reference, the reference with reduced oil content, and the two CNF-containing mayonnaise samples with reduced oil content with storage time, taken at a frequency of 1.17 Hz (right). The error bars are smaller than the symbols in the graph.*

Addition of CNFs to mayonnaise with 70 wt% oil content cause the mayonnaise to behave more like the mayonnaise with 79 wt% oil when it comes to rheological properties and stability. The concentrations of CNFs added to the mayonnaise samples is above values for critical overlap concentration for CNFs found in literature (values between 0.04 - 0.23 %, varying with fibrillation degree, aspect ratio and fibril quality) (Lasseuguette, Roux & Nishiyama, 2008; Naderi, Lindstrom & Pettersson, 2014). It is thus very likely that a percolation network is formed for the fibrils, causing a texturizing effect. This effect, together with possible interactions between the CNF network and the emulsion droplets, may explain the observed increase in rheological properties when CNFs are present.

## 4. Conclusion

Two different types of mayonnaise were produced at a pilot-scale in this study, a low-fat and a full-fat type. The effect of addition of CNFs was studied, to see if the CNFs could compensate for the reduction in starch and oil, respectively. Analyses were performed after 1 day, 1 week and 1 month of storage in room temperature, to observe how the stability was affected. Changes in droplet size distributions and rheological properties during storage indicated some coalescence both for reference mayonnaises and CNF containing samples, but not to a degree that the visual stability was affected. For starch-reduced low-fat mayonnaise, mayonnaise of good stability, and similar viscosity and moduli as the reference low-fat mayonnaise, was obtained when reduced starch content (to 50 wt%) was compensated by adding 0.75 wt % CNFs. As starch needs heating and cooling before addition to the other mayonnaise components, a reduction in starch content can help reduce energy costs. For full-fat mayonnaise, the oil content was reduced from 79 to 70 wt%. The reduction in fat content led to lower viscosity and moduli compared to the ordinary full-fat reference, but this could be compensated by addition of 0.42 wt % CNFs. This suggests that addition of CNFs to mayonnaise can allow for reduction in starch or fat content without reducing properties, such as viscosity and moduli.

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