Evaluation of Sustainable Zein-Based Barrier Coatings for Cellulose Materials

by

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May 2024

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Acknowledgement

First and foremost, I would like to express my sincere gratitude to my supervisors at Tetra Pak, Maria Petersson and Katarina Jonasson, for their continuous support, guidance and encouragement throughout my research and the writing of the thesis. Your willingness to answer all my questions and provide helpful comments and feedback have been truly invaluable.

A special thanks to Fredrik Linell for explaining different analytical methods and assisting me with the SEM analysis of my coatings. Your time and expertise in discussing the results with me were greatly appreciated. Additionally, I wish to express my gratitude to Cecilia Håkansson for her assistance in conducting FTIR, TGA and DSC analysis on the raw material and for her valuable feedback on the outcomes.

Furthermore, I would like to thank Thomas Schuman for delivering an informative presentation on contact angle measurement and for lending me his PGX meter. I am also grateful to Martin Gunnarsson who helped me conduct WVTR tests and explaining the instrument as well as discussing the results with me.

I extend my appreciation to Andreas Hein for helping me get started in the lab and for his comprehensive oversight of the resources I have required access to.

Special thanks to Jan Wahlberg for his guidance on the blade coater and for our enriching conversations in the lab.

Additionally, I am grateful to my fellow graduate student, Aylin Avcil, for sharing her experiences and providing guidance on the Cobb analysis.

Thanks to Anna Svensson for helping me analyse and understand my results and for providing suggestions on potential areas for further investigations and improvement in this thesis.

Finally, I am deeply grateful for the opportunity to conduct my master´s thesis at Tetra Pak. Thank you for providing me with the necessary resources, including all the delicious coffee that fueled many productive sessions.

Thank you all for your contributions, both big and small, that have made this thesis possible.

Abstract

In recent years, there has been a surge in attention towards biodegradable polymer films, driven by the growing environmental awareness concerning the harmful effects of petroleum-based plastics. The maize protein zein, a by-product from wet milling processes, has shown potential to work as hydrophobic coating. The aim of this study has therefore been to find the most promising process conditions for zein-based liquid barrier coatings, including dissolution, application method, film formation and drying conditions, to achieve maximum barrier efficiency.

Different concentrations of zein were dissolved in various concentrations of ethanol. In some solutions, a plasticizer, either oleic acid (OA) or linoleic acid (LA) was added. The solutions were then coated, using a blade coater, onto printing paper. Several parameters were explored in this study, including the solution´s application temperature, the number of coated layers, the coating´s drying temperature and drying time. Additionally, the significance of the solution´s storage time and the conditioning time (23°C and 50% Relative humidity) for the finished coatings were investigated.

The most promising liquid barrier properties were found for the coating consisting of 10% (g/g EtOH) zein, 30% (g/g zein) OA, and 96% (v/v) EtOH, with the process conditions of; 3 layers with 1h air-drying in between each layer and where the solution has been stored for 8 days before reheated and applied onto the substrate. The coating received a 47% decrease in water uptake compared to reference/ uncoated paper. However, the coatings with 10% (g/g EtOH) zein does not appear to be particularly robust as some of them experience water leakage, suggesting that 20% zein should be used instead for future attempts.

SEM imaging revealed that coatings without a plasticizer displayed sharp cracks, indicating that zein becomes brittle in the absence of a plasticizer. In contrast, coatings with OA exhibited smooth and even surfaces on top of the paper fibers and without any distinct pits.

In this study it became evident that the liquid barrier properties of the coatings were significantly improved by the addition of plasticizer, where coatings with oleic acid yielded lower water uptake than coatings with linoleic acid. It was further found that the ethanol concentration affects both the solution´s rheology and the final coating´s barrier properties. An ethanol concentration of 96% proved to yield the most effective barrier. Rheological changes were also observed when the solution was cooled down to room temperature. However, the storage time of the solution as well as the conditioning time of the coating showed to have no significant effect on the barrier properties. Lastly, air drying yielded a better barrier than drying the coating in oven at 60°C.

From this study it has become evident that the biopolymer zein shows great potential to work as a liquid barrier within a cellulose material. Continued research of these zein-based polymer coatings is highly recommended.

Sammanfattning

Under de senaste åren har intresset för biologiskt nedbrytbara polymerfilmer ökat markant, drivet av den växande miljömedvetenheten kring de skadliga effekterna av fossilbaserade plaster. Majsproteinet zein, en biprodukt från stärkelseproduktion, har visat potential som en hydrofob beläggning. Syftet med denna studie har därför varit att fastställa de mest lovande processförhållandena för zeinbaserade vätskebarriärbeläggningar, inklusive upplösning, appliceringsmetod, filmbildning och torkningsförhållanden, för att uppnå maximal barriäreffektivitet.

Olika koncentrationer av zein löstes i varierande koncentrationer av etanol, och i vissa lösningar tillsattes en mjukgörare, antingen oljesyra (OA) eller linolsyra (LA). Lösningarna applicerades sedan på kopieringspapper med hjälp av en bänkbestrykare. Flera parametrar utforskades i denna studie, inklusive lösningens appliceringstemperatur, antalet belagda lager, beläggningens torkningstemperatur och torkningstid. Dessutom undersöktes betydelsen av lösningens lagringstid och konditioneringstid (23°C och 50% relativ luftfuktighet) för de färdiga beläggningarna.

De mest lovande vätskebarriäregenskaperna hittades för beläggningen bestående av 10% (g/g EtOH) zein, 30% (g/g zein) OA och 96% (v/v) etanol, med processförhållandena: tre lager med en timmes lufttorkning mellan varje lager, där lösningen har lagrats i åtta dagar innan den värmdes upp igen och applicerades på substratet. Beläggningen resulterade i en 47% minskning i vattenupptag jämfört med referens/obelagt papper. Dock verkar beläggningarna med 10% (g/g EtOH) zein inte vara särskilt robusta eftersom några av dem uppvisade vattenläckage, vilket tyder på att 20% zein bör användas istället i framtida försök.

Bilder tagna med svepelektronmikroskopi (SEM) visade att beläggningar utan mjukgörare uppvisade skarpa sprickor, vilket indikerar att zein blir sprött i frånvaro av en mjukgörare. Däremot hade beläggningar med oljesyra släta och jämna ytor ovanpå papperets fibrer utan några tydliga gropar.

I denna studie blev det tydligt att vätskebarriäregenskaperna hos beläggningarna förbättrades avsevärt genom tillsats av mjukgörare, där beläggningar med oljesyra resulterade i lägre vattenupptag än beläggningar med linolsyra. Det visade sig också att etanolkoncentrationen påverkar både lösningens reologi och den slutliga beläggningens barriäregenskaper. En etanolkoncentration på 96% visade sig ge den mest effektiva barriären. Reologiska förändringar observerades även när lösningen kyldes ner till rumstemperatur. Dock visade sig lösningens lagringstid och beläggningens konditioneringstid inte ha någon betydande effekt på barriäregenskaperna. Slutligen gav lufttorkning en bättre barriär än torkning av beläggningen i ugn vid 60°C.

Från denna studie framgår det att biopolymeren zein har stor potential att fungera som en vätskebarriär inom ett cellulosamaterial. Fortsatt forskning på dessa zeinbaserade polymerbeläggningar rekommenderas starkt.

List of Abbreviations

CA – Contact Angle

DSC – Differential Scanning Calorimetry

EtOH – Ethanol

FTIR – Fourier Transform Infrared Spectroscopy

LA – Linoleic Acid

RH – Relative Humidity

TGA – Thermogravimetric Analysis

OA – Oleic Acid

SEM – Scanning Electron Microscopy

WVTR – Water Vapor Transmission Rate

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1. Introduction

The effects of plastics, including their environmental impact has reached more attention in society, especially in the food packaging industry. Petroleum based polymers have for a long time been used in packaging because of its unique properties. However, fossil-based petroleum is a limited resource, simultaneously contributing to increased carbon dioxide emissions. Additionally, toxicity is a major disadvantage for controversial plastics, were some of them are harmful for both human and the environment (Anjali et al. 2023). Requirements from consumers and new EU legislations drive the development towards finding more sustainable packaging alternatives, such as biobased and biodegradable materials, to reduce the environmental impact. The maize protein zein, often obtained as a by-product from wet milling processes, has been studied as a potential biomaterial for coatings and have demonstrated promising barrier properties (Anjali et al. 2023). Moreover, zein is a non-toxic biopolymer, making it a safe choice for food applications (Jaski et al. 2022).

Therefore, the objective of this study was to find promising process conditions for zein-based liquid barrier coatings, including dissolution, application method, film formation and drying conditions, to achieve maximum liquid barrier efficiency.

The barrier effectiveness will be evaluated using various techniques, such as Cobb absorption, water vapor transmission rate, contact angle measurements, and various surface analytical techniques. Printing paper will serve as a reference for comparative analysis.

1.1 Zein

Zein is found in the endosperm of corn and because it is a natural material its molecular weight can vary depending on the specific type. Zein contains four primary amino acids; glutamic acid, leucine, proline, and alanine, and show an overall hydrophobic property making it suitable as a water barrier (Menezes & Athmaselvi, 2018). The molecular structure of the four amino acids is shown in *Figure 1* below:

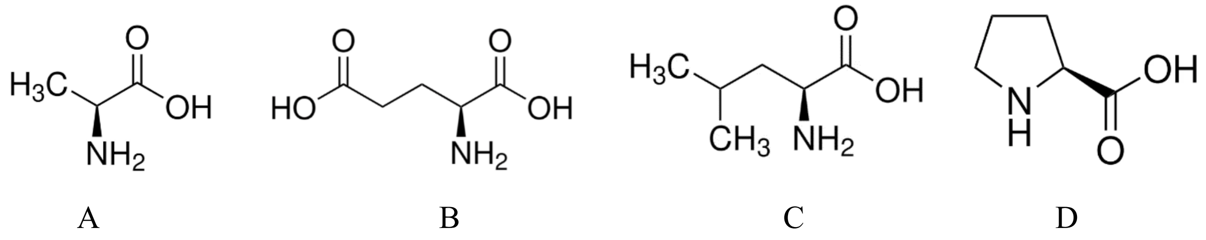


Figure 1. The molecular structures of the four primary amino acids in zein; (A) glutamic acid, (B) leucine, (C) proline, (D) alanine. (Sigma Aldrich, 2024)

The molecular structure of zein is not fully established but previous studies suggest that zein act as globular proteins in nonaqueous solutions. The structure of zein undergoes alterations based on the solvent in which it is dissolved and depends on zein concentration in the solution. The structure is also influenced by the temperature and pH of the solvent. (Lorenzo et al. 2018).

Kim and Xu explain the aggregate formation of zein and its structural changes in aqueous ethanol. They noted that the structural inversion point occurs at an ethanol concentration of 90%, signifying a shift in zein aggregates from a micelle-like configuration with the hydrophilic moiety oriented toward the solvent medium, to an arrangement where the hydrophilic moiety relocates toward the centre of each aggregate, as the EtOH concentration exceeds 90%. (Kim, Xu, 2007)

1.2 Plasticizer

One drawback with zein is that it shows nonhomogeneous and brittle characteristics which can be problematic for film formation (Egea et al. 2022). To overcome brittleness when creating a zein film a plasticizer can be added. By adding a plasticizer, the mechanical properties, such as elongation at break and tensile strength, of the film can be improved. Previous research, however, show that it is most often with a cost of reduced hydrophobicity, water vapor-, and liquid- barrier properties. Wentz and Olofsson (Wentz & Olofsson, 2023), employed glycerol as a plasticizer in their zein films. They observed that glycerol tended to migrate to the surface and contribute to a smoother coating with pinholes present. Pinholes are believed to arise due to evaporation of the solvent droplets (Vieira et al. 2011). Consequently, while the addition of glycerol to a 10% zein film reduced the water vapor transmission rate (WVTR) to a value of 89 g/(m^2day), it also resulted in decreased hydrophobicity, compared to zein films without plasticizer. They suggested that a smoother coating is associated with a lower WVTR. (Wentz & Olofsson, 2023) Moreover, Dong et al. suggest that increased surface roughness indicates higher hydrophobicity (Dong, Padua and Wang, 2013).

Oleic acid emerges as a more promising plasticizer due to its high hydrophobicity, potentially inhibiting moisture absorption (Wang & Padua, 2004). Another plasticizer, which has been compared with oleic acid is linoleic acid. In Vieira et al.´s research it was revealed that linoleic acid surpasses oleic acid in its effectiveness in minimizing water absorption in sheets, when isopropyl alcohol was used as solvent (Vieira et al. 2011). Polymerization of linoleic acid may have sealed off pores in the structure, slowing the water absorption (Santosa & Padua, 1999).

The molecular structure of glycerol, oleic acid and linoleic acid is shown in *Figure 2* below:

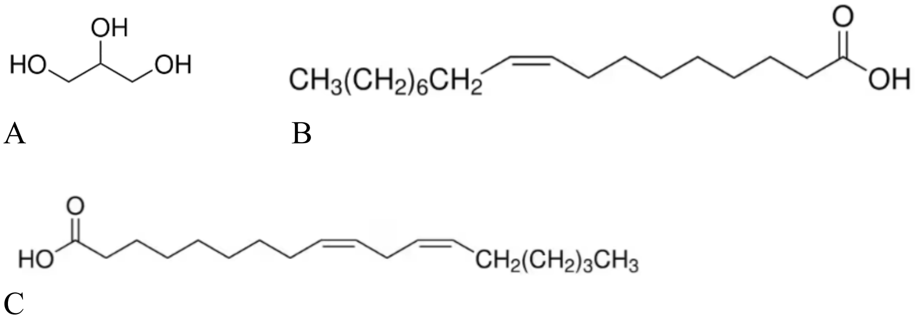


Figure 2. Molecular structures of (A) Glycerol, (B) Oleic acid (C) Linoleic acid. (Sigma Aldrich, 2024)

1.3 Solvent

Due to zein's hydrophobic nature it is insoluble in water, but by adding a sufficient amount of alcohol its solubility improves (Keshanidokht et al. 2022). Ethanol is a suitable and safe solvent choice for producing films intended for food packaging applications.

The solubility properties of zein are important since they affect the final films properties. Dong et al. discovered that the solubility of zein varies with the EtOH concentration in the solvent. Specifically, they noted the highest zein solubility at an EtOH concentration of 80%, with solubility decreasing both above and belove. At 80% they observed a uniform distribution of particle sizes, leading to an increase in the film's hydrophobicity. (Dong et al. 2013) However, Kim and Xu discovered in their study, that zein´s structural inversion point occurs at an EtOH concentration of 90%, meaning that the hydrophobic moiety relocates from an inward to an outward orientation (Kim, Xu, 2007).

1.4 Effect of Zein Concentration

The zein concentration, together with temperature and ethanol concentration, affects the viscosity of the solution. According to Fu and Weller, the viscosity for aqueous ethanol solutions increases with increasing zein concentration at various temperatures (1999).

Wentz and Olofssons research also included the effect of zein concentration on the film's barrier properties. They made two films with concentrations of 10 and 15 wt% zein dissolved in 90% EtOH. The results showed a higher water CA for the film with 15wt% zein, but it also obtained the highest moisture uptake, which they explained by the films uneven surface with pinholes present. (Wentz, Olofsson, 2023)

1.5 Film Formation Methods

There are different film formation methods for creating a polymeric film and which one that is used may determine the final properties of the film (Choudhary et al. 2021). The viscosity of the solution is of importance when developing and creating a film since it determines which technique that can be used. Spray coating is an efficient method when the film solution is less viscous, and it is the most common method used in the application for coating on food products (Menezes & Athmaselvi, 2019). Another coating method, which is more suitable for higher viscosities, is blade coating. It is a technique to form films with well-defined thicknesses (Cherrington and Liang, 2016).

2. Materials and Methods

The experimental work was structured into two parts. The initial part involved creating zein-EtOH solutions and coatings with varying weight percentages of both zein and ethanol. In the subsequent part, two different plasticizers were introduced, and the concentration of all substances was varied. In both parts, a blade coater was used to apply the solutions onto printing paper.

2.1 Materials

Zein, ethanol (3D 96%, CAS-nr: 64-17-5, REF: 1274), oleic acid and linoleic acid (purity between 90% - 99%) was purchased from Sigma-Aldrich.

Deionized water was used throughout the study, including the Cobb measurements.

2.2 Preparation of Zein-EtOH Coatings

Different concentrations of zein were dissolved in aqueous ethanol, in various concentrations, see *Table 1* below for detailed zein-ethanol contents. The mixture was stirred with magnetic stirrer at 70 °C in a water bath for 10 minutes.

Table 1. The concentration of zein is given as weight percentage of solution, and the EtOH concentration in volume-to-volume with water.

Solution	Zein conc. [g/g solution] * 100%	EtOH conc. [v/v] * 100%
1A	5%	70%
2A	15%	75%
3A	10%	80%
4A	20%	80%
5A	30%	80%
6A	10%	96%
7A	20%	96%
8A	30%	96%

The homogeneous mixture was bench- coated onto printing paper using a blade coater. Either 1,2,3 or 4 layers were applied, and the samples were dried in oven at 60°C for 15 minutes in between each layer.

The parameters evaluated for the initial part were the number of layers, as well as the layer thickness, and the solution's temperature when applied onto the paper, either at room temperature or around 70 °C.

A decision was made to continue with an EtOH concentration of 96%, application of warm solution and a layer thickness of 100 µm for the following tests. For detailed explanation to this decision, see section 3.2 under Results & Discussion.

2.3 Preparation of Zein-EtOH + Plasticizer Coatings

Initially, mixtures containing 10% and 30% (w/100% solution) zein were prepared by dissolving it in 96% (v/100% ethanol) EtOH. The mixture was stirred while heated in a water bath at 70°C for 10 or 15 minutes depending on zein concentration, see *Table 2*. Subsequently, 30% (w/100% zein) plasticizer; either oleic acid or linoleic acid, was added, and the solution was stirred for an additional 10 minutes. *Table 2* below provides details of the initial four mixtures created.

Table 2. Initial solutions with varying concentrations of zein, EtOH and plasticizer (OA or LA).

Solution	**Zein conc. [w/100% solution]**	**Plasticizer type**	**Plasticizer conc. [w/ 100% zein]**	**EtOH conc. [v/100% EtOH]**	**Dissolution time**
1B	10%	LA	30%	96%	10 min
2B	30%	LA	30%	96%	15 min
3B	10%	OA	30%	96%	10 min
4B	30%	OA	30%	96%	15 min

The warm solutions were coated onto printing paper using a blade coater, with a coating thickness of 100 µm. In between each layer the films were dried in oven at 60 °C for 15 minutes. The parameters assessed for these samples included type of plasticizer and number of layers, where 2,3 or 4 layers were applied.

A decision to proceed with OA and 3 layers were made, and new solutions were prepared according to *Table 3* below. For detailed explanation for this decision, see section 3.3, under Results & Discussion.

Table 3. New solutions with varying concentrations of zein, plasticizer (OA) and EtOH.

Solution	**Zein conc. [w/100% solution]**	**OA conc. [w/ 100% zein]**	**EtOH conc. [v/100% EtOH]**
3B	10%	30%	96%
4B	30%	30%	96%
5B	20%	30%	96%
6B	10%	30%	80%
7B	10%	70%	96%

The parameters evaluated for the samples, derived from the solutions listed in *Table 3*, included drying time and temperature. The coated papers were subjected to either air drying for 1h or 24h in between each layer, or oven drying at 60 °C for a duration of either 15 or 60 minutes. Additionally, the effect of storage time of solutions and conditioning time of coatings (23°C and 50% RH) were investigated.

2.4 Characterization of Coated Samples

The coated papers along with a reference sample (uncoated printing paper) were characterized by various analytical methods to assess the films water barrier properties. The following analyses were conducted on the samples:

2.4.1 Cobb Analysis

Cobb analysis, a method to determine the amount of absorbed water, was carried out according to ISO standard method ISO 535:2024 Paper and board. Each sample was weighed and 25 cm^2 of the sample was then put under 25 mL of deionized water for 15 minutes. Subsequently, the water was removed, and the samples were reweighed to calculate the amount of absorbed water. An image of the Cobb equipment is found below, see *Figure 3.*

All samples produced throughout both parts were analysed with the Cobb measurement, with a minimum of two replicates each. The Cobb results contributed to the decision of which samples that would also be evaluated in further analysis.

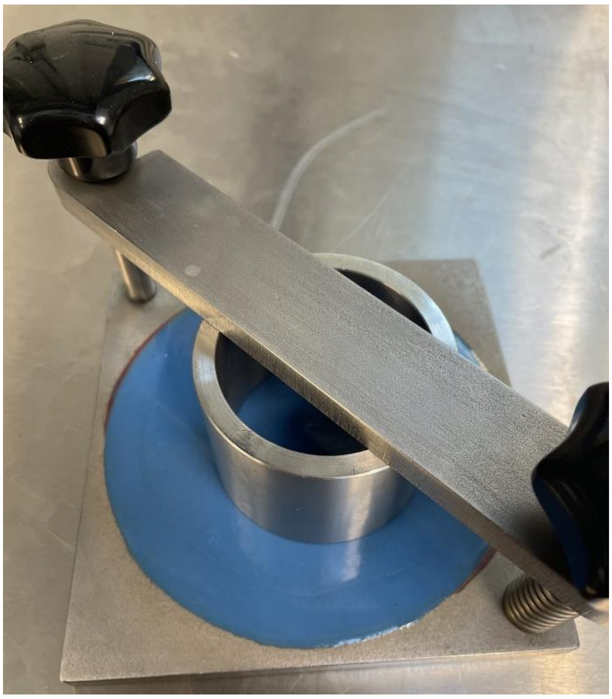


Figure 3. Cobb equipment.

2.4.2 Water Vapor Transmission Rate, WVTR

WVTR analysis was done in a Mocon Permeation Analyzer, according to test method 1025-127 with the barrier facing high humidity. The samples were measured at 23 °C and 50% RH, using a mask to minimize the measuring area of the samples to 5 cm^2. Readings were taken when WVTR was judged to be at equilibrium.

2.4.3 Optical Microscopy

An Olympus BX51 microscope, was used to obtain an initial image of selected samples at a magnification of 5x and 10x.

2.4.4 Scanning Electron Microscopy, SEM

SEM was conducted using a Hitachi TM3030 Microscope, with back scatter electron detector, to examine the surface morphology through both compositional and topographic contrast. SEM imaging was performed both with an overhead and cross-sectional perspective. Images were taken at 100x and 500x magnification.

2.4.5 Contact Angle, CA

CA measurements were conducted using a Krüss MSA instrument and following Krüss own method (*Mobile Surface Analyzer-MSA One-Click SFE, n.d.*) using deionized water. Each sample received 5-10 drops, with the exact number determined by sample variability, and the resulting average were calculated.

2.4.6 Characterization on Raw Material

A general characterization was conducted on zein. The analysis methods included in this characterization were FTIR, TGA, and DSC.

3. Results and Discussion

The aim of this study was to determine the optimal process conditions for zein-based coatings to effectively enhance their water barrier properties. The Cobb analysis was conducted on all samples, while SEM, CA, optical microscopy and WVTR, were performed variably depending on the Cobb results.

3.1 Reference

Uncoated printing paper worked as a reference which all samples were compared to.

3.1.1 Cobb Analysis

Three Cobb measurements on printing paper, were conducted and the average was calculated to 79 g/m^2. All subsequent samples were compared to this value. It was also noted that there was lot of water leaking through the paper, see *Figure 4* and *5* below.

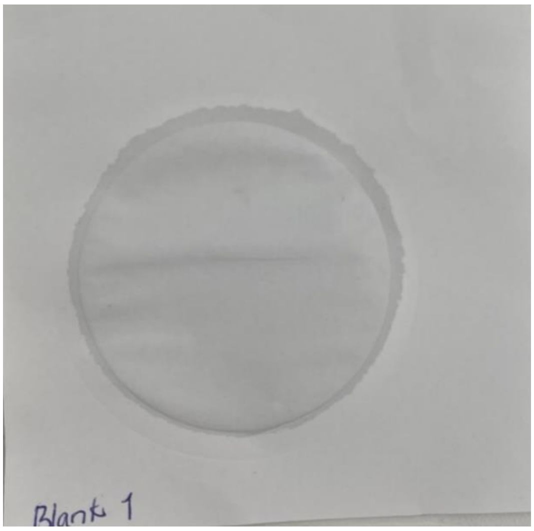


Figure 4. After Cobb measurement.

Figure 5. Cobb equipment after measurement of the reference.

3.2 Zein-EtOH Coatings

Several findings emerged in the first part of the zein-EtOH coatings. Firstly, when the solution was warm, higher zein concentrations increased the viscosity. Secondly, upon cooling the solution to room temperature before application, sedimentation occurred, see *Figure 6* and *7* below for illustration.

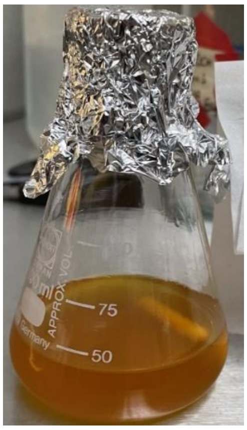


Figure 6. Warm 6A solution (10% zein)

Figure 7. Solution 6A at room temperature

Additionally, solutions with 96% EtOH and 20%, or more zein, exhibited swelling and gel-like behavior, a phenomenon absent in solutions with 70% or 80% EtOH. This observation supports the structural inversion of zein particles, where the hydrophobic part of the micelle is oriented towards the solvent medium, at an EtOH concentration of 96%. For illustration of EtOH´s effect on the rheological change, see *Figure 8* below.

Figure 8. Solution 8A, containing 96% EtOH, to the left and 5A, with 80% EtOH, to the right. Both solutions have cooled down to room temperature.

Furthermore, cracks appeared on the surface of the coatings without plasticizer, becoming more pronounced with higher zein concentrations. This is illustrated in *Figure 9* and *10,* where the finished coatings derived from solution 3A and 5A are displayed together with their respective process conditions.

Figure 9. Coating containing 2 layers with solution 3A, 100µm thick, and warm solution.

Figure 10. Coating containing 4 layers of solution 5A, 100µm thick and warm solution.

3.2.1 Cobb Analysis

In the first part, the effect of number of layers as well as the solutions temperature when applied, were investigated. The Cobb values from solution 1A to 8A, together with reference are compiled in *Figure 11* below.

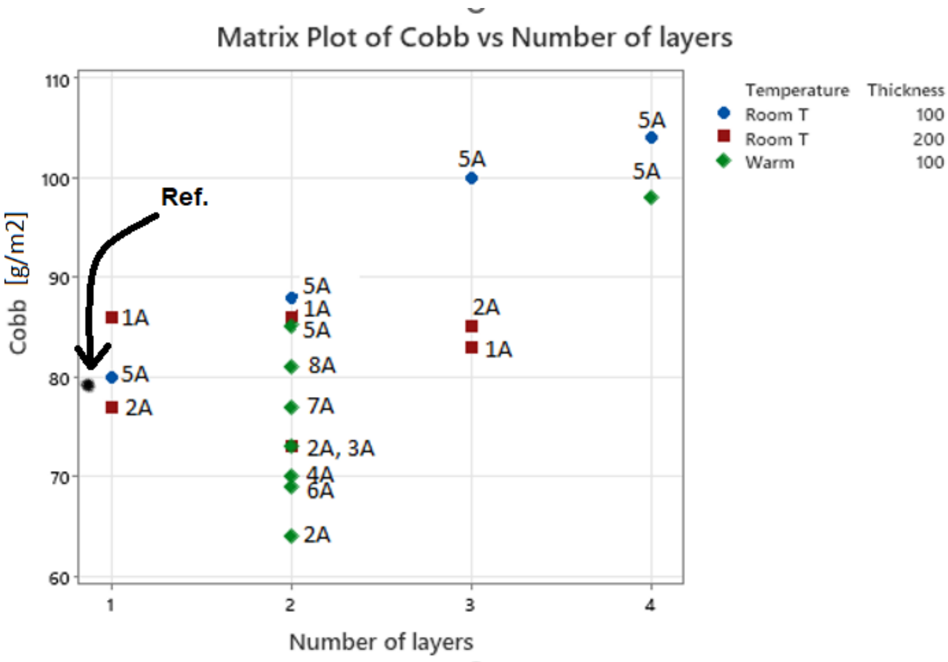


Figure 11. Matrix plot of Cobb values for the solutions with respect to number of layers, thickness, and temperature of solution at application, either at room temperature or around 70ºC. Ref. refers to uncoated paper.

Figure 11 illustrates the effect of the solutions temperature when applied onto the paper, at different number of layers. It indicates a lower Cobb value when the solution is applied while warm. The lowest Cobb value is found for solution 2A and two number of layers. Here, it should be noted that only five samples out of 18 contained more than two layers and therefore the result that 2 layers would be an optimal number may be a bit misleading. *Figure 11* also reveal that 5A absorbs the most, especially with more layers, and 2A the least, indicating that samples with a thicker film and higher zein concentration is not preferable. This could also be misleading since all samples leaked water to some extent. Samples with solution 2A leaked more than samples with 5A, which barely leaked with four layers.

Moreover, the effect of EtOH concentration was investigated, comparing 80% and 96% EtOH at various zein concentrations, see *Figure 12* below.

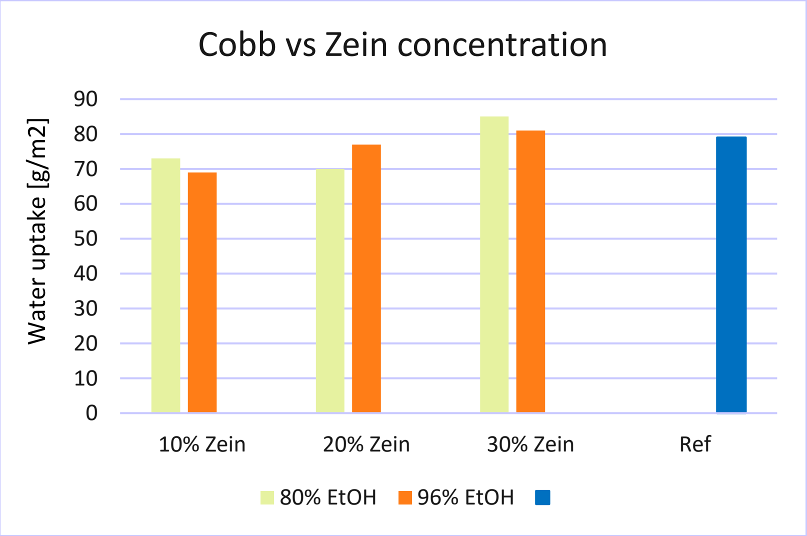


Figure 12. Cobb values for samples containing either 80% or 96% EtOH at various zein concentrations. All samples have been coated with warm solution, 2 x 100 µm thick layers.

There is a slight variance in the Cobb values among different EtOH concentrations, where 80% seems to result in higher Cobb at 10% and 30% zein concentrations, but slightly lower at 20% zein. However, during Cobb measurement, it was observed that samples with 96% EtOH leaked the least, whereas most other samples leaked significantly.

Concerning the layer thickness, a decision was made to proceed with 100 µm, despite the indication in *Figure 11* that 200 µm might be slightly better, as thicker layers were assumed to potentially contribute to surface cracking.

Furthermore, note that the difference between the Cobb values is relatively small and does not differ significantly from the reference, rather some appears to be worse than the reference, even though they leaked a lot less. Cobb analysis should hence be questioned as a decisive characterization method in this case.

Taken together the visual observations made on the solutions and the finished films, along with the Cobb results, a decision was made to proceed with 96% EtOH, a layer thickness of 100 µm, and application of warm solution.

3.3 Zein-EtOH + Plasticizer Coatings

During the initial tests, with 1B-4B solutions, it was investigated how the barrier properties were affected by number of layers as well as type of plasticizer. The samples derived from solution 1B-4B, with related conditions are compiled in *Table 4* below.

Table 4. Summary of the coatings made from solution 1B to 4B.

Sample name	**Zein conc. [w/100% solution]**	**EtOH conc. [v/100% EtOH]**	**Plasticizer conc. [w/100% zein]**	**Plasticizer type**	**Number of layers**	**Coating grammage applied [g/m2]**
1B_1	10%	96%	30%	Linoleic	2	14.6
1B_2	10%	96%	30%	Linoleic	3	17.8
1B_3	10%	96%	30%	Linoleic	4	25.8
2B_1	30%	96%	30%	Linoleic	2	38.2
2B_2	30%	96%	30%	Linoleic	3	61.3
2B_3	30%	96%	30%	Linoleic	4	78.9
3B_1	10%	96%	30%	Oleic Acid	2	12.2
3B_2	10%	96%	30%	Oleic Acid	3	18.9
3B_3	10%	96%	30%	Oleic Acid	4	22.2
4B_1	30%	96%	30%	Oleic Acid	2	40.4
4B_2	30%	96%	30%	Oleic Acid	3	69.8
4B_3	30%	96%	30%	Oleic Acid	4	93.9

3.3.1 Cobb Analysis

The Cobb values from the samples in *Table 4,* together with reference, are compiled in *Figure 13* below.

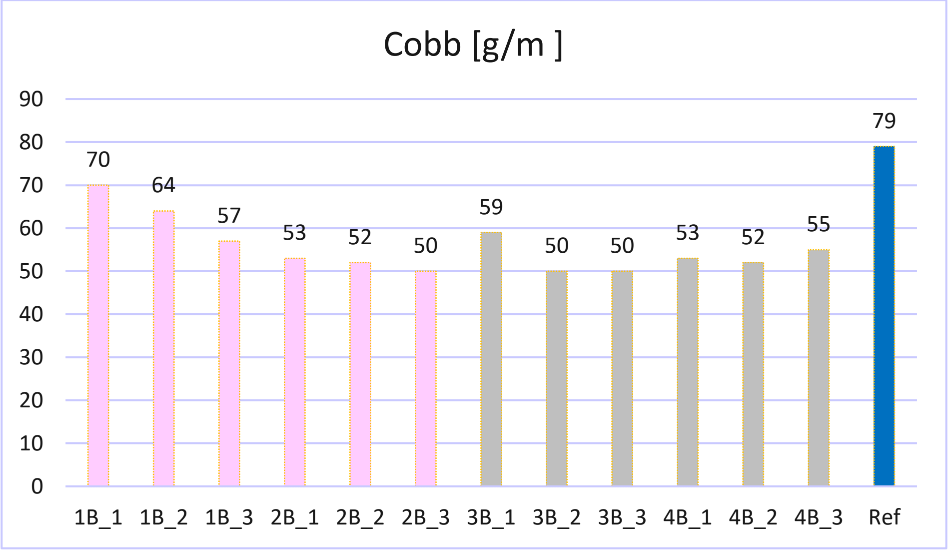


Figure 13. Cobb values for coatings derived from 1B-4B. Pink bars represent LA, gray bars OA, and the blue bar corresponds to the reference.

In *Figure 13* it is evident that all samples derived from solution 1B to 4B exhibit lower Cobb values than reference. However, samples containing LA generally demonstrated higher Cobb values compared to those with OA. This observation is further depicted in *Figure 14*, where the Cobb values are plotted against number of layers.

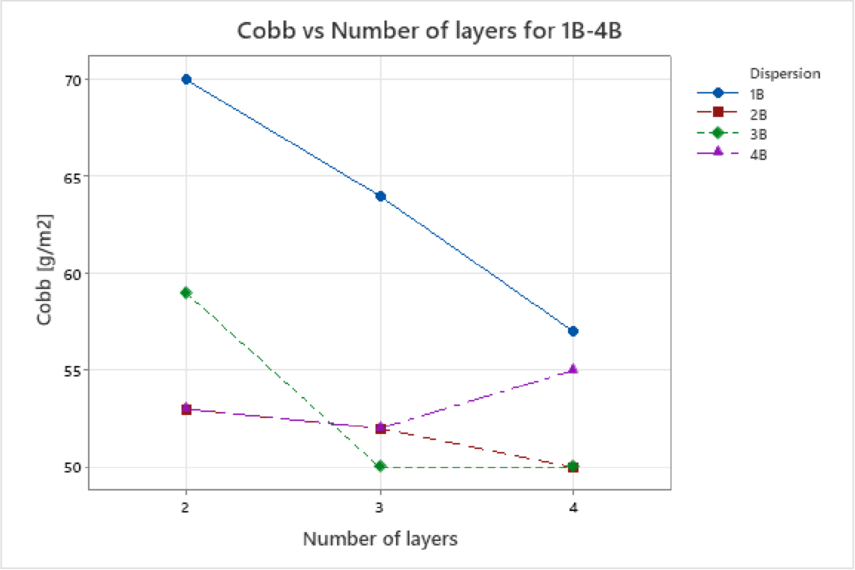


Figure 14. Cobb values for solution 1B to 4B with respect to the number of layers.

Figure 14 reveals that 1B generates the highest Cobb value independently number of layers, indicated that OA surpasses LA in its effectiveness in minimizing water absorption, contrary to what Vieira et al. concluded in their study (2011). Furthermore, number of layers are compared for solution 3B and 4B in *Figure 15* below, suggesting an optimum number of layers of 3.

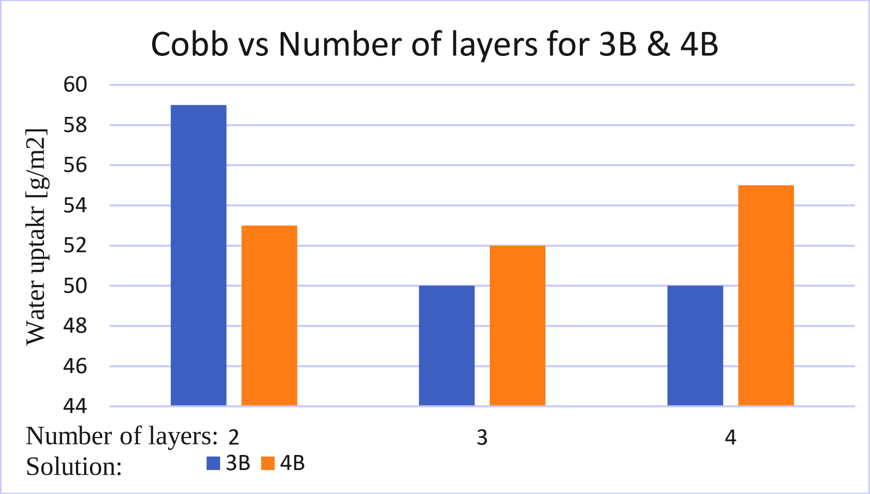


Figure 15. Cobb values for samples derived from solution 3B and 4B with respect to number of layers.

It is a balance between having enough layers to seal all potential pinholes and achieve a complete covering film, while also attempting to minimize the number of layers for reduced material usage, to lower the costs and the environmental impact. *Figure 15* above illustrated a significant difference in water uptake between 2 and 3 layers, but less so between 3 and 4 layers. Therefore, 3 layers was considered the most optimal number and was chosen for the subsequent samples.

In *Table 5* below, a summary of the subsequent coatings and their related conditions can be found.

Sample name	**Zein conc. [w/100% solution]**	**EtOH conc. [v/100% EtOH]**	**OA conc. [w/100% zein]**	**Drying conditions**	**Conditioning time [days]**	**Storage time of solution [days]**	**Coating grammage applied [g/m^2]**
3B_4	10%	96%	30%	R1h	1	0	17.8
3B_5	10%	96%	30%	R1h	7	0	16.9
3B_6	10%	96%	30%	R24h	3	0	17.9
3B_7	10%	96%	30%	R1h	3	0	18.5
3B_8	10%	96%	30%	60C_1h	1	0	13.5
3B_9	10%	96%	30%	60C_15min	1	0	18.9
3B_10	10%	96%	30%	R1h	1	8	20.1
3B_11	10%	96%	30%	R1h	3	8	14.8
3B_12	10%	96%	30%	R24h	5	8	20.9
3B_13	10%	96%	30%	R1h	3	16	22.9
4B_1	30%	96%	30%	R1h	4	0	54.9
4B_2	30%	96%	30%	60C_15min	1	0	69.8
4B_3	30%	96%	30%	R1h	1	0	55.6
5B_1	20%	96%	30%	R1h	2	0	X
5B_2	20%	96%	30%	R1h	1	0	31.8
6B_1	10%	80%	30%	R1h	3	0	23.2
7B_1	10%	96%	70%	R1h	1	0	23.3

Table 5. Summary of coatings derived from solution 3B to 7B. The drying condition R relates to room temperature, 1h or 24h, and 60C to oven drying.

The finished coatings of 3B_4 and 5B_2 are displayed in *Figure 16* and *17* below.

Figure 16. Coating 3B_4

Figure 17. Coating 5B_2

The layers in coating 3B_4 appear to have been applied much more evenly than the layers in 5B_2. Additionally, it was observed that the papers curled during drying. The higher the zein concentration, the more they curled.

A summary of the Cobb values of the coatings, together with reference, is compiled in *Figure 18* below. Sample 3B_4 was also tested at 30 minutes and 48 minutes, resulting in Cobb values of 45 g/m^2 and 52 g/m^2 respectively, without any leakage.

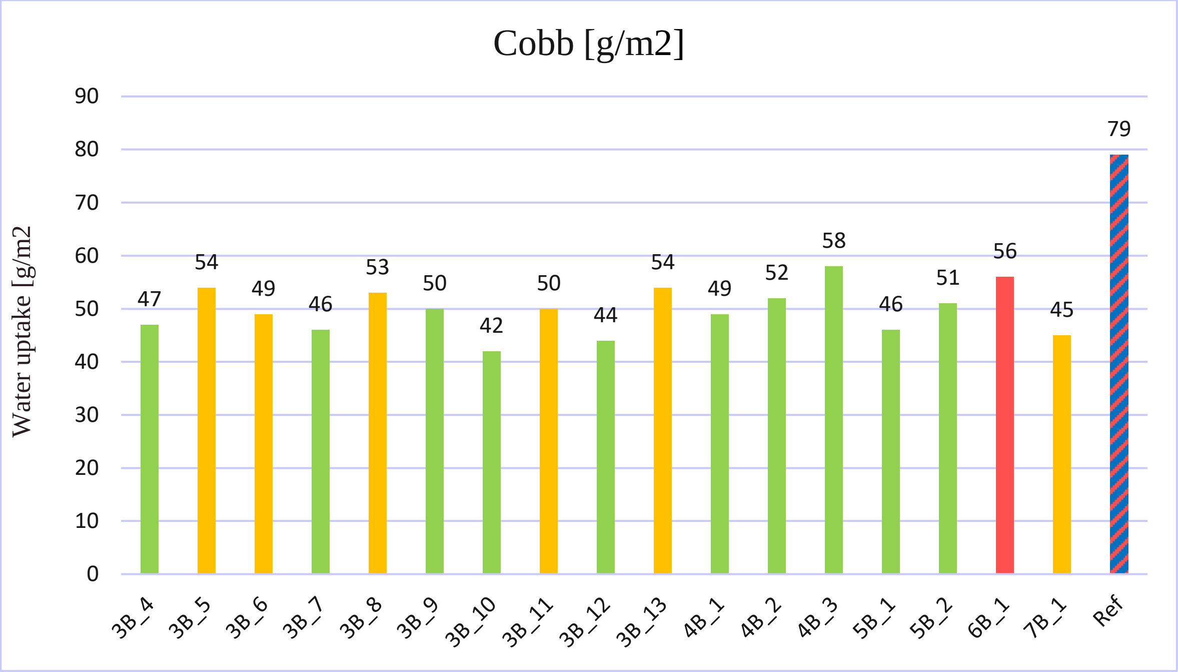


Figure 18. Cobb values for coatings derived from 3B-7B. Green bars indicate no water leaking through the coating, orange bars: almost nothing or a little bit leaking, the red pattern of reference indicates lot of water leakage.

The red bar of 6B_1 suggests that employing 80% EtOH does not impart effective liquid barrier properties, not even with a plasticizer. This aligns with observations from the Zein-EtOH coatings, further validating the structural rearrangement of zein micelles, transitioning from an outward hydrophilic orientation to an inward. Additionally, it can be inferred that despite 7B_1, containing 70% OA, yields relatively low Cobb value, it still exhibits minor leakage through the barrier, indicating inadequacy in providing efficient water barrier properties. This phenomenon could be attributed to the differential polymerization behavior of fatty acids compared to zein, resulting in the inability to form a fully covering film. Moreover, in terms of food safety considerations, excessive amounts of OA may not be ideal as it could with time potentially migrate from the packaging and contaminate the food.

Furthermore, the difference between samples 3B and 5B is not significant, indicating that the zein concentration between 10 and 20% does not generate major differences in Cobb values. However, samples containing 10% zein do not appear to be particularly stable, as some of the coatings have some leakage. The primary cause of this, whether it is the drying condition, conditioning time, storage time of the solution, or drying time, is difficult to determine from data presented in *Figure 18.* Most likely it is primarily due to the low zein concentration, 10% zein may be too low to form a fully covering film.

A closer analysis of how the storage time and conditioning time of samples from solution 3B, as well as the drying conditions and zein concentrations effect on Cobb, can be seen in *Figure 19* and *20* below.

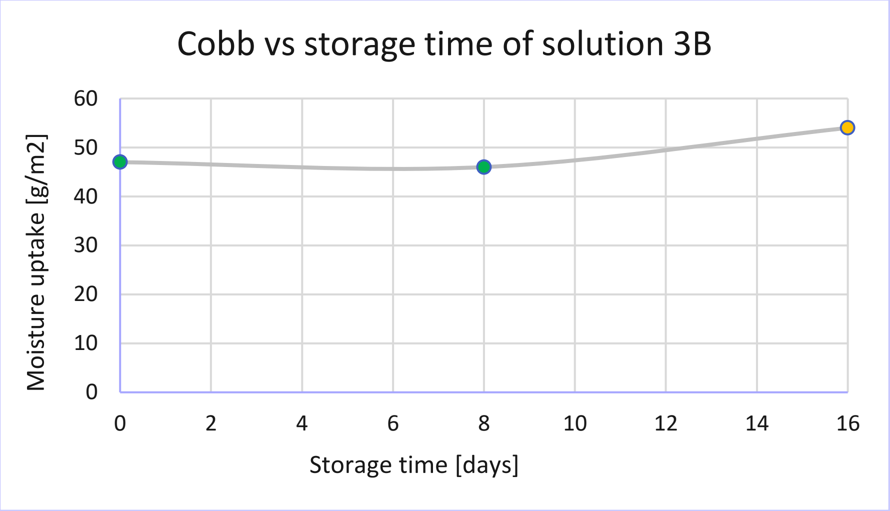


Figure 19. Cobb value with respect to increasing storage time of the solution.

As illustrated in *Figure 19*, storage of the solution has minimal effect on Cobb, meaning it can be prepared as described in the method, cooled down to room temperature, be stored and then be heated and applied onto paper again, with no significant difference in resulting Cobb value. Green dots in the figure indicate no leakage through coating, while yellow dots indicate slight leakage. Further analysis of how the conditioning time (23 °C and 50% RH) affects the coatings is compiled in *Figure 20* below.

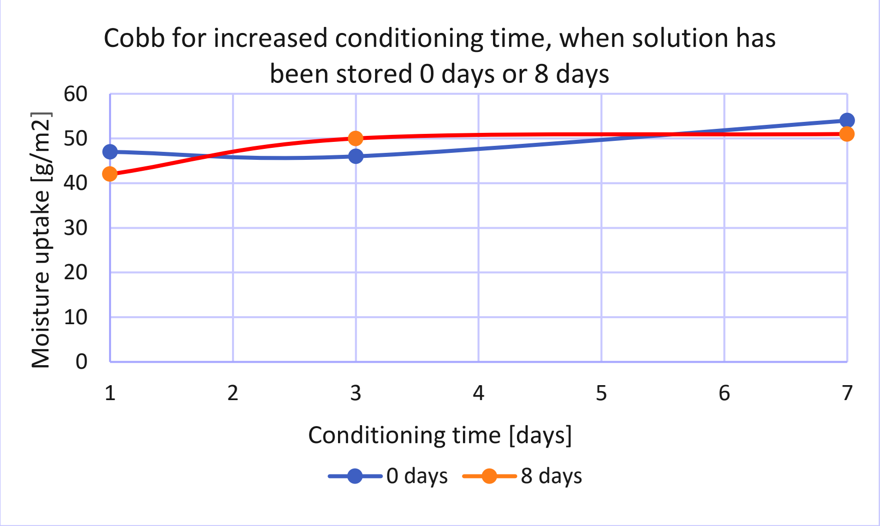


Figure 20. Cobb with increased conditioning time for coatings derived from solution 3B and has been stored for either 0 days (blue curve) or 8 days (red curve).

From the figures above it can be concluded that storage time of solution and conditioning time of the coatings have minimal effect on the final coating's barrier properties.

For deeper analysis of the process conditions affecting zein-based coatings, the impact of drying conditions on water barrier properties was investigated. See *Figure 21* for detailed results.

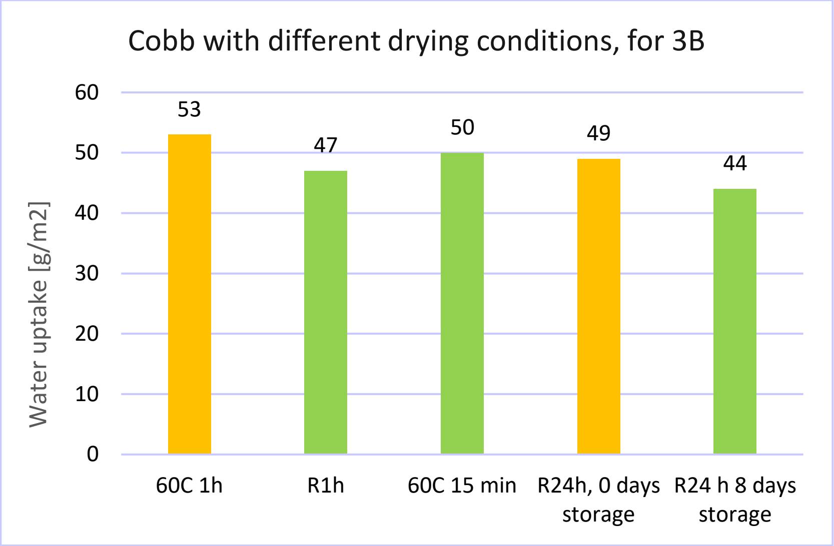


Figure 21. Cobb values for coatings derived from 3B with different drying conditions. Green bars indicate no water leaking through the coating, orange bars: almost nothing or a little bit is leaking.

Drying in air seems to yield better Cobb value. However, the superiority of a longer drying time, such as 24 h at room temperature, is not entirely clear, despite some indications in that direction. Nevertheless, it seems that 1 h drying provides sufficiently good Cobb. From an industrial standpoint, shorter processing times are preferred, thus 1 h should be enough. Oven drying does not yield favorable Cobb values, particularly with extended drying times. From a process perspective, oven drying is not ideal anyways, as it requires more energy and is therefore less economical and environmentally friendly.

From *Figure 18* above, it appears that the zein concentration influences barrier properties, especially in terms of water leakage through the coating. A detailed analysis of how zein concentration affects the Cobb is presented in *Figure 22* below. All coatings contain 96% EtOH (v/100% EtOH) and 30% OA (w/100% zein) and were applied immediately after solution preparation and further dried for 1h at room temperature in between each layer.

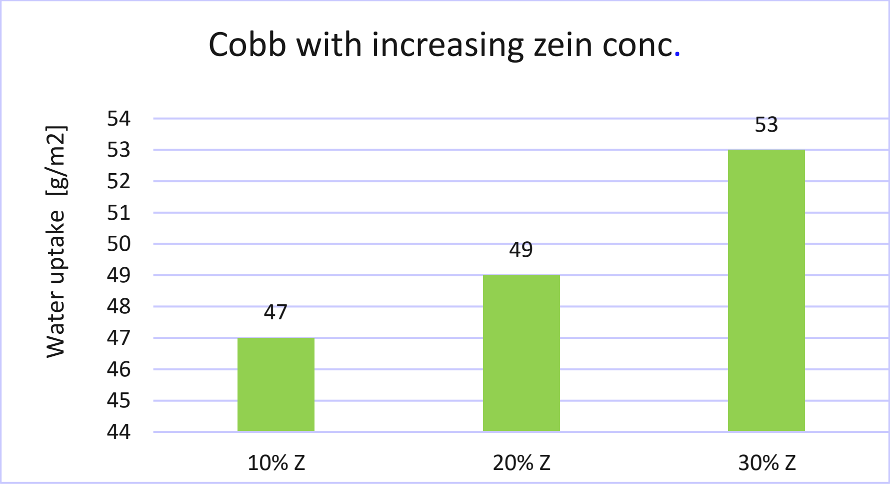


Figure 22. Cobb values for Zein-EtOH + (30%) Plasticizer coatings containing 10%, 20% or 30% zein respectively.

It appears that 10% zein yield the best Cobb value, closely followed by 20% zein. However, it should be noted that all concentrations exhibit lower Cobb than the reference (79 g/m^{2}and none exhibit leakage.

Based on the above discussion regarding the robustness of using lower zein concentration, it can be concluded that 10% zein is not sufficiently reliable and therefore, a minimum concentration of 20% zein should be used to achieve optimal water barrier properties.

3.4 WVTR

Water Vapor Transmission Rate was measured at 23 °C and 50% RH. Sample 3B_4 and 4B_3 was tested, and their average result is compiled in *Table 6.*

Table 6. Water Vapor Transmission Rate values for selected samples.

Sample	**3B_4**	**4B_3**
WVTR [g/ (m^{2}day)]	171	33

The WVTR result for 3B_4 at 171 g/(m^{2}day) is relatively high, compared to previous research of coatings containing 10% zein and glycerol as a plasticizer, which had a WVTR value of 89 g/(m^{2}day) (Wentz & Olofson). This indicates that this coating is not appropriate as a water vapor barrier even though it shows potential barrier for water in its liquid state. A possible explanation for this high value could be the low coating grammage and possibly the uneven application with thin patches. Additionally, limiting the measurement to an area of 5 cm2 and then scaling up to m2 introduces a source of error.

There is a significant decrease in the WVTR value for coating 4B_3, which contains 30% zein, compared to coating 3B_4, with 10% zein. This indicates that coatings with higher concentrations of zein achieve better water-vapor barrier properties.

3.5 Microscopic Analysis

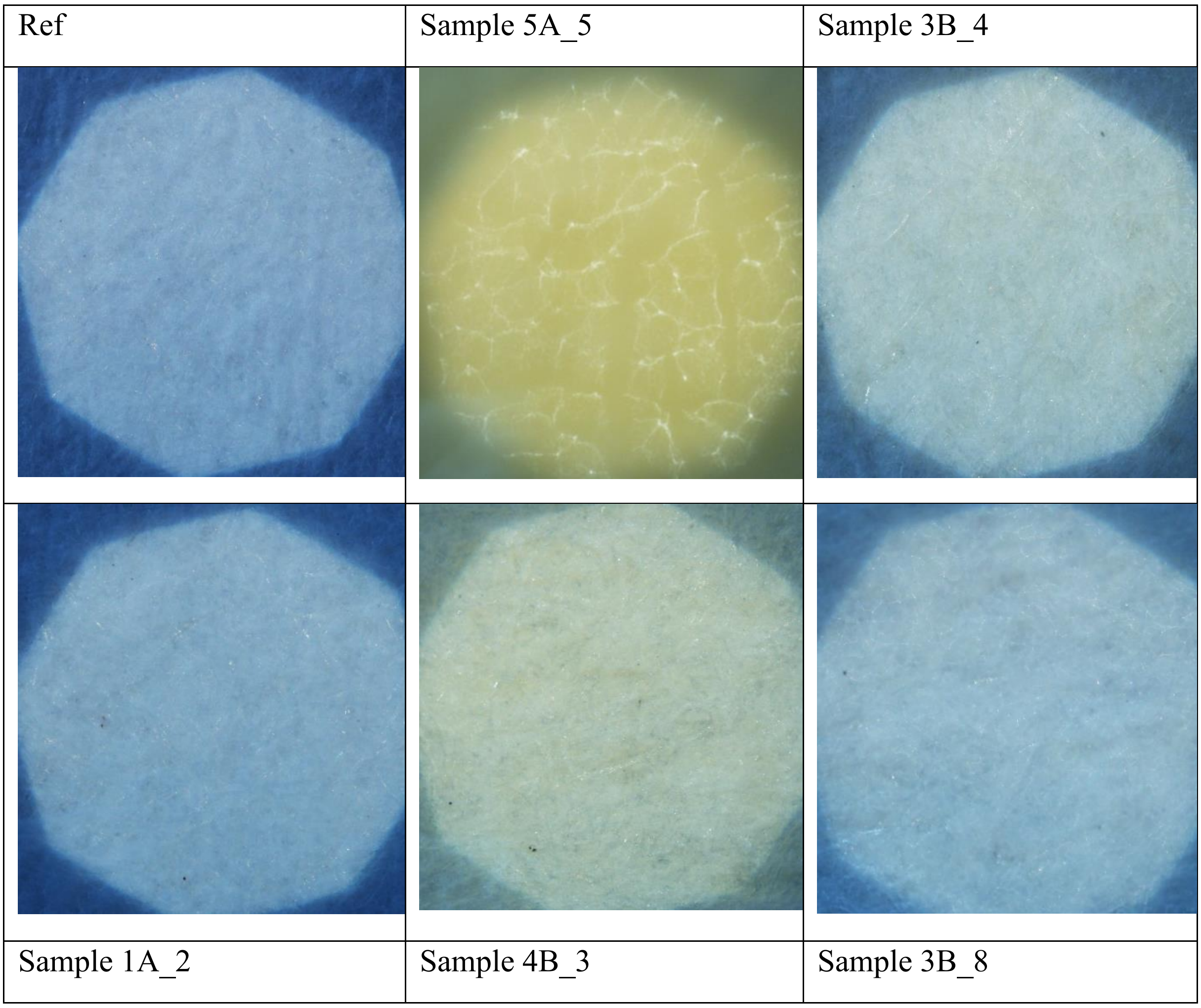
Microscopic analyses, with magnification of 5x and 20x, were conducted on selected samples, based on previous Cobb values. A summary of the selected samples and their water absorption are compiled in *Table 7,* below.

Table 7. Summary of the barrier properties of respective samples that were viewed in optical microscope.

Sample	**Water absorption**
Ref	79 g/m^2. Lot of leakage.
5A_5	104 g/m^2. Some leakage.
3B_4	47 g/mm^2. No leakage.
1A_2	86 g/m^2. Lot of leakage.
4B_3	55 g/m^2. No leakage.
3B_8	53g/m^2. Almost no leakage

The microscopic images taken from the samples in *Table 7* above is compiled in *Figure 23* and *24,* with magnifications of 5x and 20x, respectively.

3.5.1 Optical Microscopy



Ref

Sample 5A_5

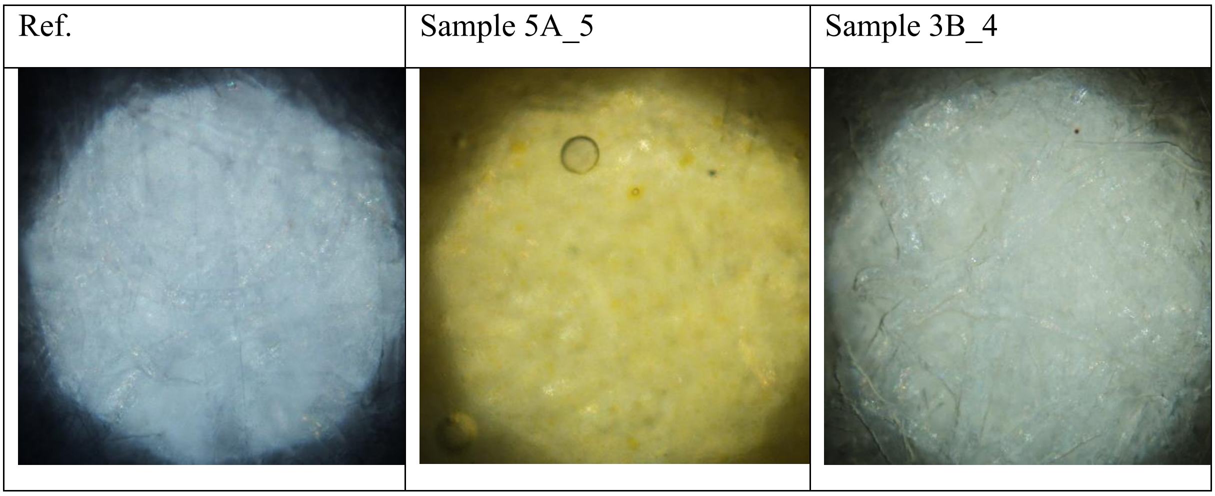
Sample 3B_4

Sample 1A_2

Sample 4B_3

Sample 3B_8

Figure 23. Microscopic imaging with 5x magnification.



Ref.

Sample 5A_5

Sample 3B_4

Sample 1A_2 | Sample 4B_3 | Sample 3B_8

Figure 24. Microscopic imaging with 20x magnification.

Figure 23 clarifies the cracks on the surface of sample 5A_5, coating which lacks plasticizer in its coating. A reasonable explanation for the high Cobb value of the coating could therefore be attributed to water infiltrating these small cracks. While the other samples do not exhibit any remarkable features, the microscopic images of sample 1A_2 closely resemble those of the reference, at both magnifications. Hence, it is reasonable to infer that this is why 1A_2 leaks significantly.

In *Figure 24,* blistering is evident in samples 5A_5 and 3B_8. Both coatings were subjected to oven drying, suggesting that the elevated temperature of 60°C likely caused the formation of these blisters. This may be a source of error resulting in the higher Cobb values.

Drawing further conclusions from the optical microscope images regarding how the coatings surface morphology impact their liquid barrier properties proves challenging.

3.6 SEM

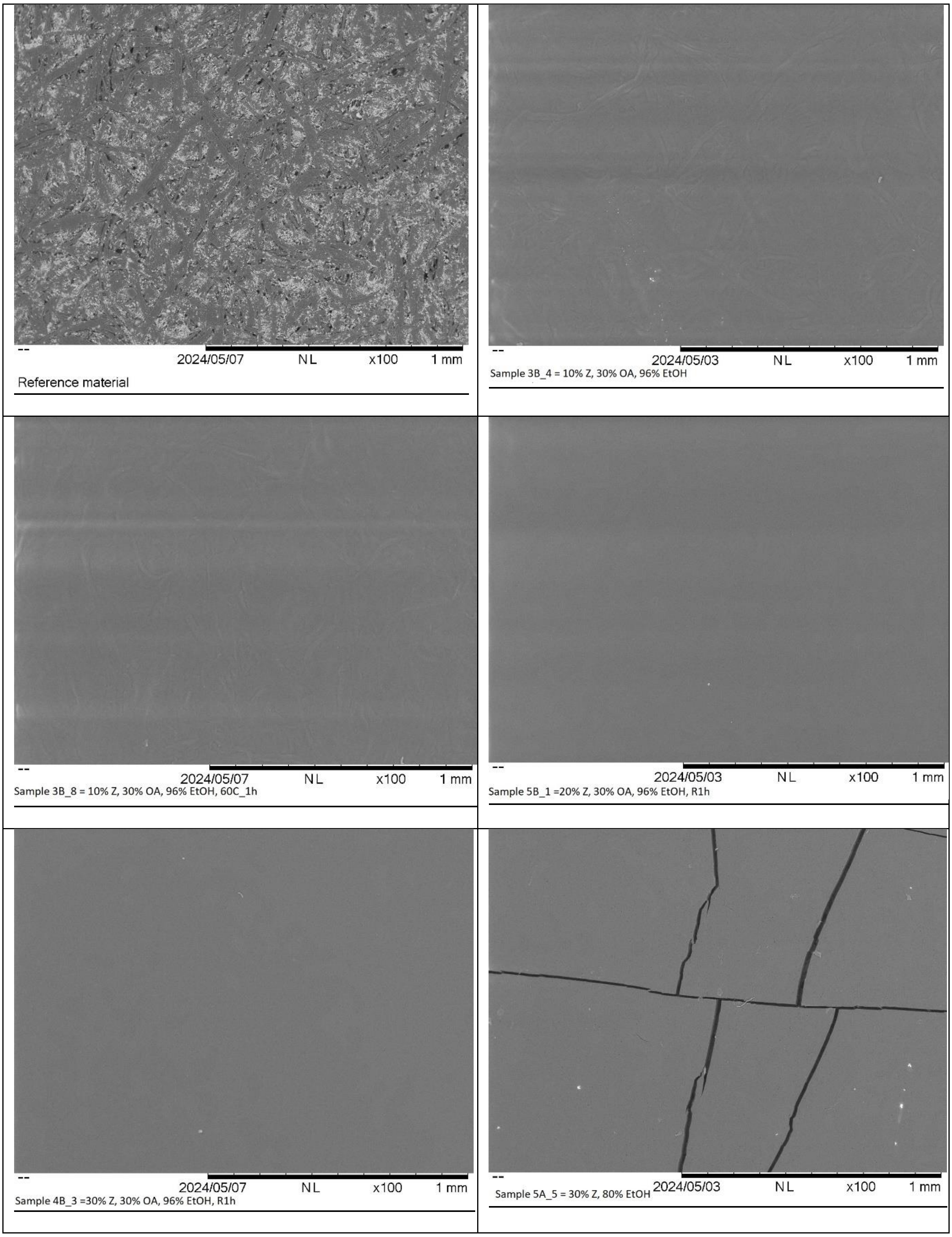
SEM with topographical and compositional contrast was conducted on selected samples, with a magnification of 100x and 500x. A summary of the selected samples and their water absorption are compiled in *Table 8* below. Reference was also viewed at for comparison.

Table 8. Summary of the barrier properties of the samples that were viewed in SEM.

Sample	**Water absorption?**
Ref	79 g/m^2 . Lot of leakage.
5A_5	104 g/m^2. Some leakage.
3B_4	47 g/m^2. No leakage.
3B_8	53g/m^2. Almost no leakage
4B_3	55 g/m^2. No leakage.
5B_1	46 g/m^2. No leakage.

The samples above were viewed from an overhead perspective, but 3B_4 and 4B_3 was also conducted from a cross-sectionally perspective.

3.6.1 Compositional and Topographical Imaging from Overhead Perspective



Reference material

Sample 3B_4 = 10% Z, 30% OA, 96% EtOH

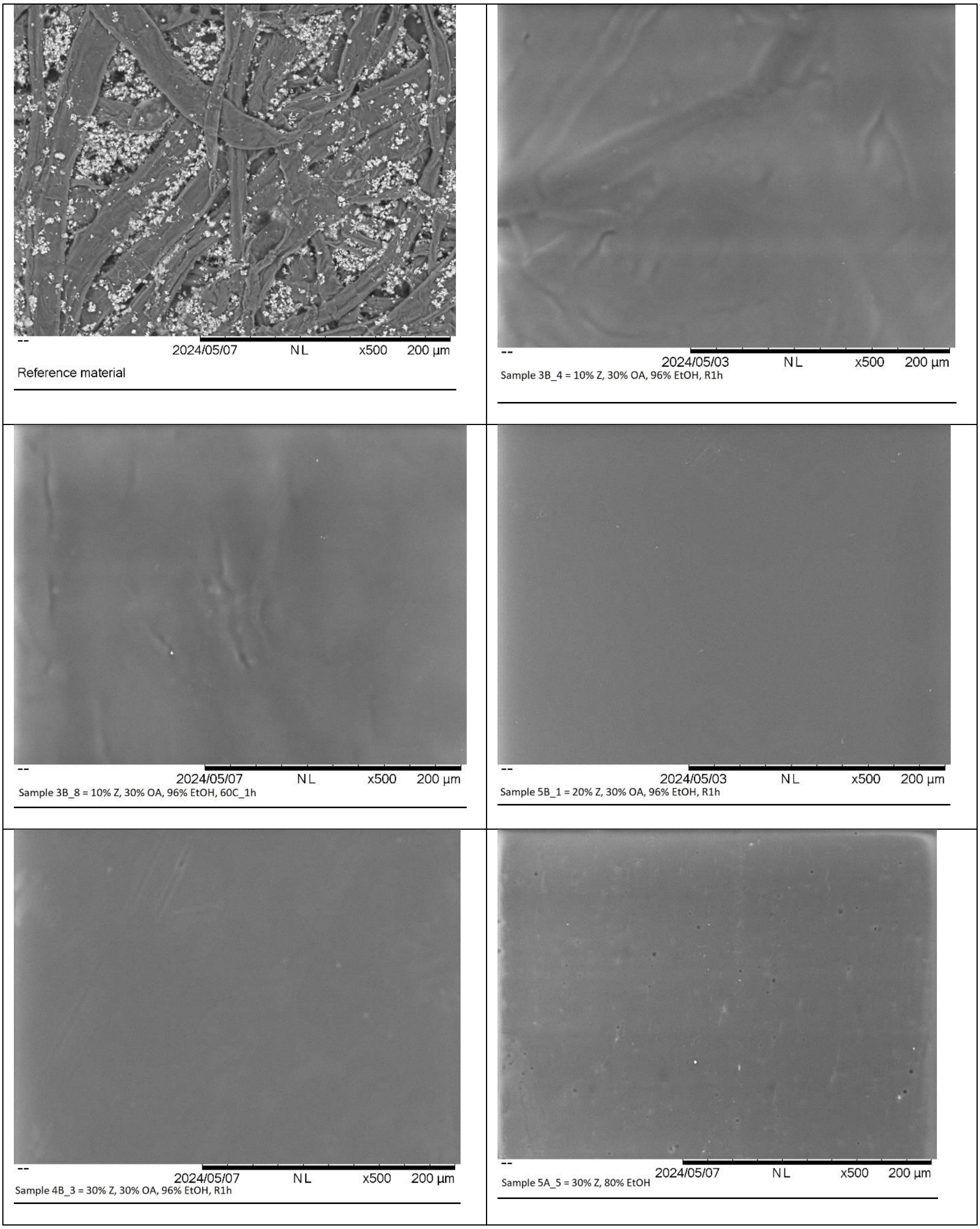
Sample 3B_8 = 10% Z, 30% OA, 96% EtOH, 60C_1h

Sample 5B_1 =20% Z, 30% OA, 96% EtOH, R1h

Sample 4B_3 =30% Z, 30% OA, 96% EtOH, R1h

Sample 5A_5 = 30% Z, 80% EtOH

Figure 25. SEM on selected samples with 100x magnification and compositional contrast.



Reference material

Sample 3B_4 = 10% Z, 30% OA, 96% EtOH, R1h

Sample 3B_8 = 10% Z, 30% OA, 96% EtOH, 60C_1h

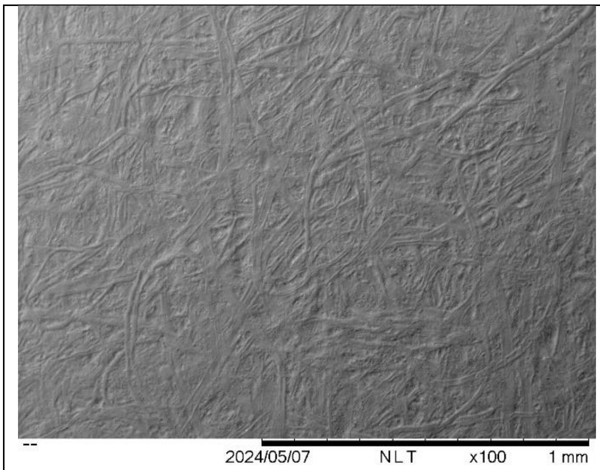
Sample 5B_1 = 20% Z, 30% OA, 96% EtOH, R1h

Sample 4B_3 = 30% Z, 30% OA, 96% EtOH, R1h

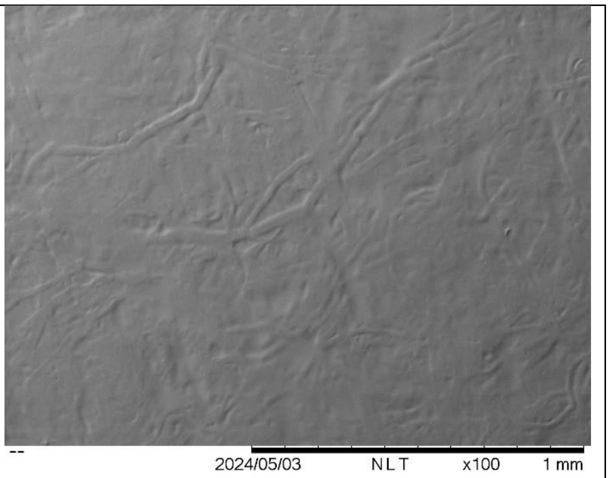
Sample 5A_5 = 30% Z, 80% EtOH

Figure 26. SEM with magnification of 500x and compositional contrast. The image taken for 5A_5 is in between the cracks.

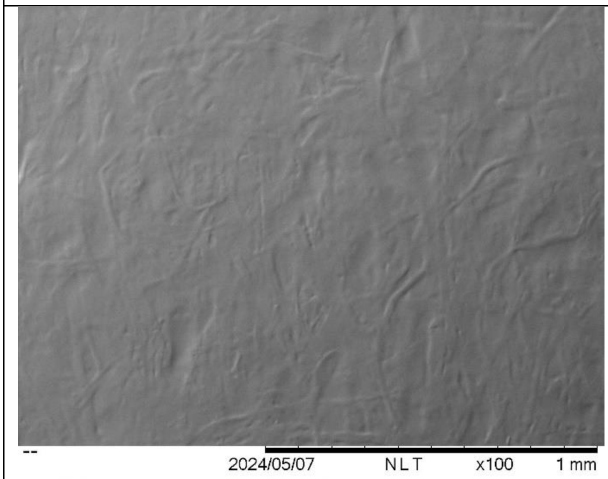
From the compositional contrast images, all samples appear to have a smooth surface, except for sample 5A_5, as clearly shown in *Figure 25,* exhibits sharp cracks. Additionally, in *Figure 26,* representing the surface in between the cracks, small pits can be seen across the surface. Sample 5A_5 is the only sample among those studied that does not contain OA, indicating that this observation aligns with the literature, suggesting that zein exhibits brittleness in the absence of plasticisers (Egea et al. 2022). The pits on the surface could be due to oven-drying, where the polymer chains may not have sufficient time to rearrange as the solvent evaporates. Conversely, sample 3B_8, which also underwent oven-drying, does not display any pits in its structure, challenging the hypothesis that the oven is solely responsible. However, 3B_8 contains OA, unlike 5A_5, which may facilitate polymer rearrangement, potentially sealing off the holes.



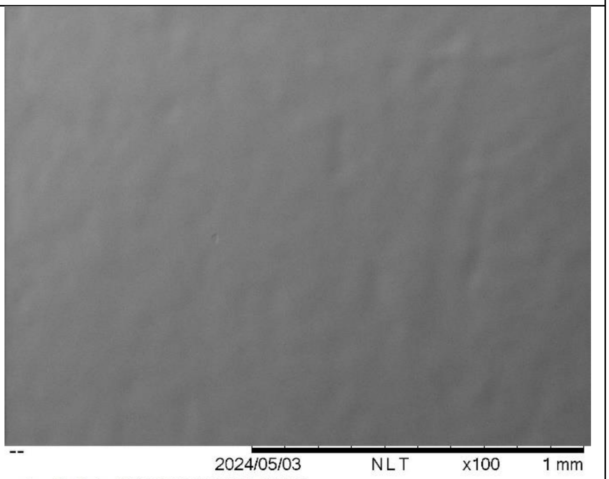
Reference material



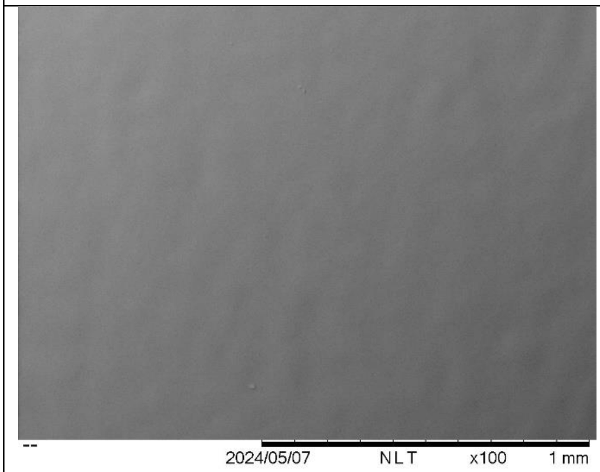
Sample 3B_4 = 10% Z, 30% OA, 96% EtOH, R1h



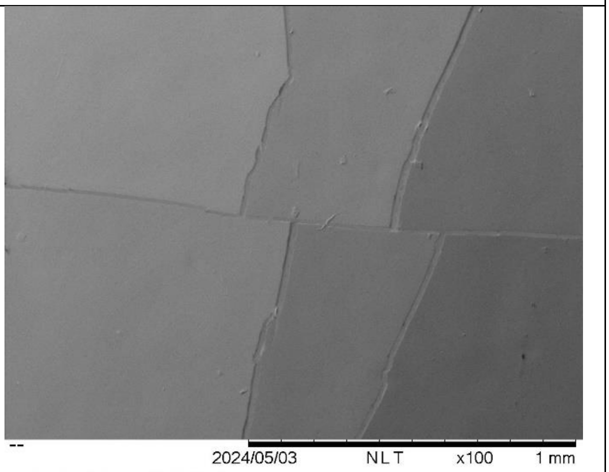
Sample 3B_8 = 10% Z, 30% OA, 96% EtOH, 60C_1h



Sample 5B_1 = 20% Z, 30% OA, 96% EtOH, R1h

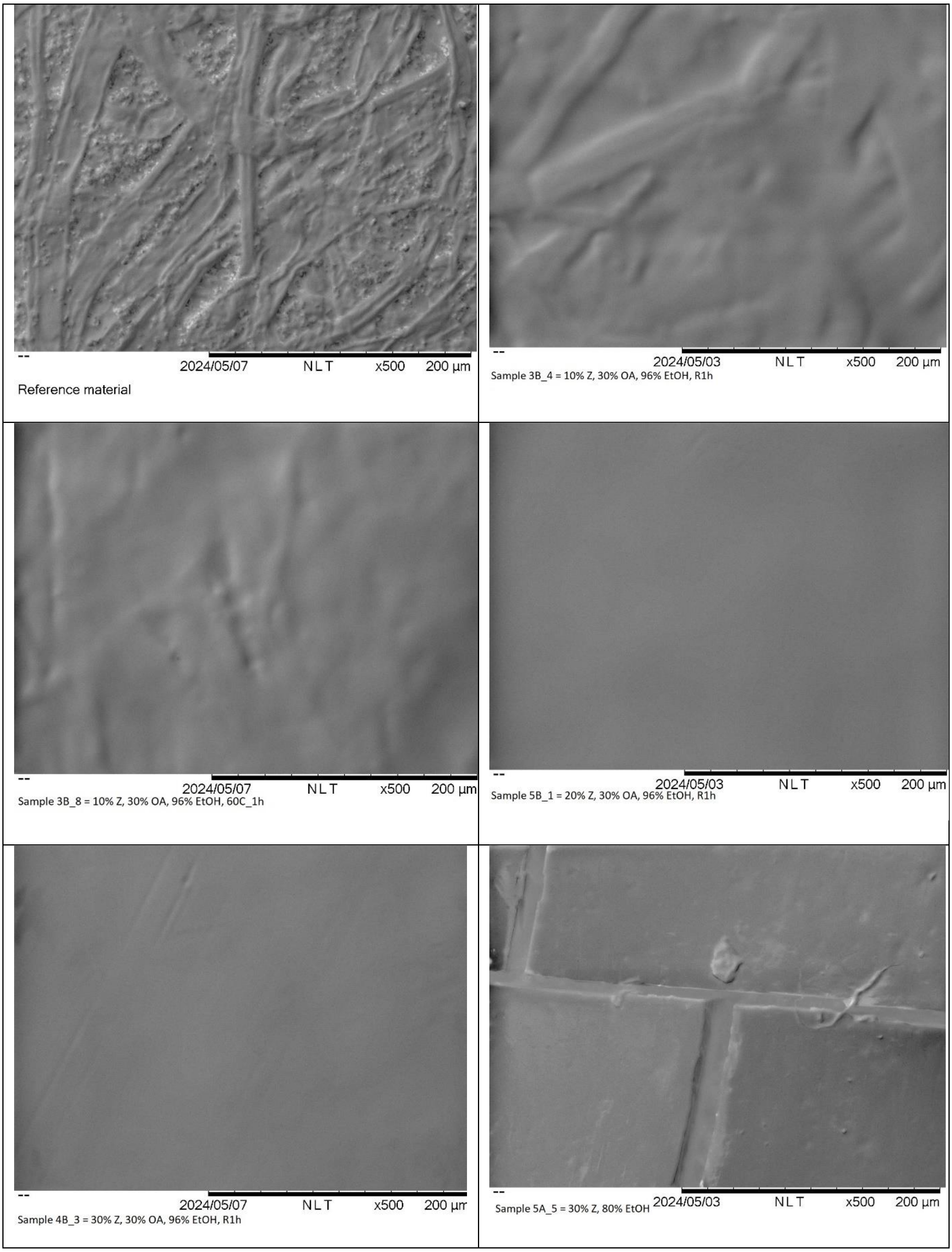


Sample 4B_3 = 30% Z, 30% OA, 96% EtOh, R1h



Sample 5A_5 = 30% Z, 80% EtOH

Figure 27. SEM analysis with magnification of 100 x and topographical contrast. The reference material represents uncoated printing paper.



Reference material

Sample 3B_4 = 10% Z, 30% OA, 96% EtOH, R1h

Sample 3B_8 = 10% Z, 30% OA, 96% EtOH, 60C_1h

Sample 5B_1 = 20% Z, 30% OA, 96% EtOH, R1h

Sample 4B_3 = 30% Z, 30% OA, 96% EtOH, R1h

Sample 5A_5 = 30% Z, 80% EtOH

Figure 28. SEM with 500x magnification and topographical contrast. The reference material represents uncoated printing paper.

In both *Figure 27* and *28,* it can be observed that samples 3B_4 and 3B_8 exhibit significant topographies, likely originating from the paper fibers underneath, as they resemble the topography of the reference material. Furthermore, based on the topographic images, it can be noted that 5B_1 and 4B_3 have a very smooth surface without any distinct pits.

3.6.2 Compositional Imaging from a Cross-Sectional Perspective

Sample 3B_4 and 4B_3 were examined from both an overhead perspective and a cross-sectional perspective, with magnifications of 800x and 2000x. The resulting images are compiled in *Figure 29* below.

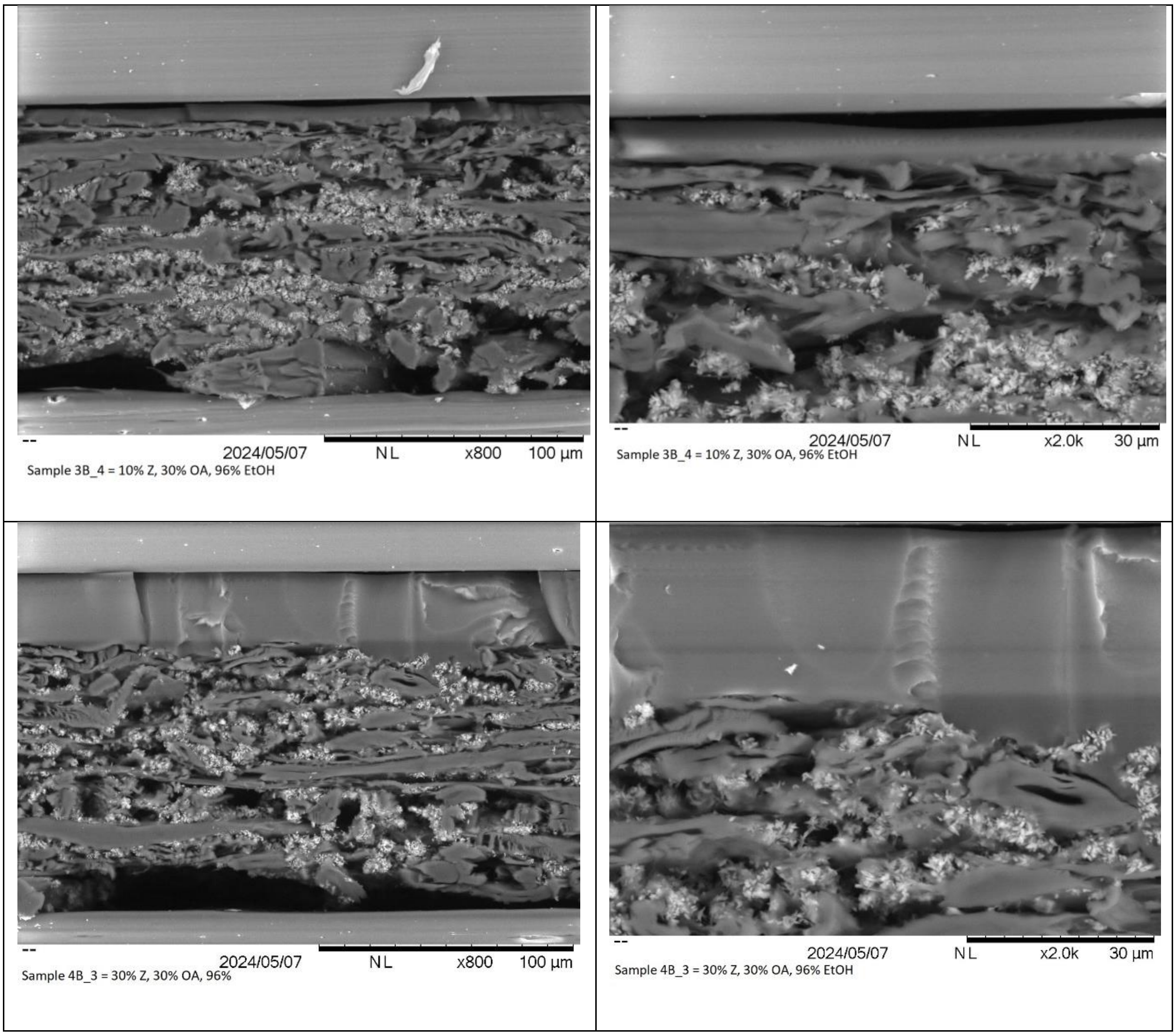


Figure 29. Cross-section images with magnifications of 800x (left) and 2000x (right). The coatings are visible in grey below the air-pockets, which appear as black straight lines.

Sample 3B_4 and 4B_3 both exhibit a smooth and even film on top of the paper fibres, with sample 3B_4 having a slightly thinner film thickness. Rather than penetrating the paper, the coating follows the papers contour to some extent, with larger pits allowing the film to adhere, while smaller pits create air pockets between the paper and the film. In sample 4B_3 vertical cracks are visible, likely originating from the knife during sample preparation. This indicates that 4B_3 appears smooth and even but with brittle characteristics.

3.7 Water Contact Angle Measurements

Water CA measurements were made on some samples but yielded no meaningful results. CA is probably not the optimal analysis method as it seems to be sensitive to uneven and irregular surfaces. Additionally, a film may have high wettability and still serve as an effective liquid barrier, so the CA analysis should be approached with caution.

3.8 Characterization on Raw Material

For the general characterization of zein, see Appendix A1, A2 and A3.

4. Conclusions

The objective of this study was to investigate process conditions to achieve the best liquid barrier properties, including dissolution, application of solutions and drying conditions. The results, primarily derived from the Cobb measurements, have led to numerous significant conclusions:

- An ethanol concentration of 96% is necessary in order to achieve efficient water barrier properties, with no leakage through the paper. Zein films containing either 70 or 80% EtOH results in a non-efficient liquid barrier since water leaks through the coated paper.
- It is recommended to use 20% (w/100% solution) zein concentration for coatings, as they give similar Cobb values to those with 10% zein, but without any water leakage, as observed in some of the 10% zein coatings.
- The solution must be warm and homogenized when applied onto the paper.
- By adding plasticizer, the liquid barrier properties are improved, probably due to minimization of cracks at the surface. In this study, oleic acid proved to be the best choice of plasticizer.
- Several numbers of layers are needed to minimize pinholes that may result in a higher water uptake. In this study, the optimum number was three.
- For drying conditions: Lower Cobb values are obtained for samples dried in air compared to oven-drying. The drying time is of less importance, especially when the samples are dried in air. There is no significant difference in Cobb between samples that has dried for 1 h compared to 24 h. From an economic, as well as environmental perspective, this conclusion is positive as it implies lower energy consumption.

From this study it has become evident that the biopolymer zein shows great potential to work as a liquid barrier within a cellulose material. Continued research of these zein-based polymer coatings is highly recommended. See section 5 below for future recommendations.

5. Future Recommendations

From the conclusions drawn in this report, the following recommendations for future work were reached:

A zein concentration of 20% (w/100% solution) is recommended for future work. Since only a limited number of samples have been tested at this concentration in this study, scaling up production should be done to evaluate its robustness to ensure consistent results. Perhaps an independent film should also be produced to evaluate the mechanical properties, such as tensile and impact strength.

For additional information of the coating's barrier efficiency, OTR measurements could also be done. Furthermore, to minimize the error introduced by scaling from cm2 to m2 , a larger area of 50 cm2 of coating 4B_3 should be measured in WVTR analysis.

It could also be interesting to investigate the combination of linoleic and oleic acid to see if that could generate a coating with even lower water uptake values.

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Appendix A1

FTIR analysis

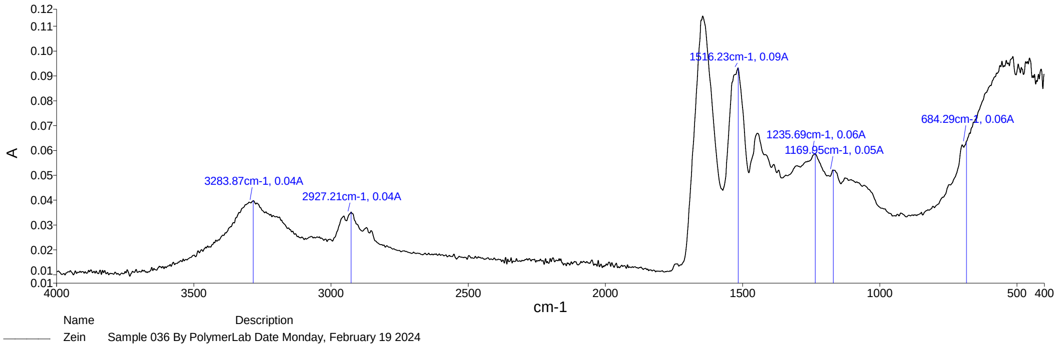

Figure 30. FTIR spectrum showing the chemical composition of pure zein. Peaks indicate the presence of functional groups.

Appendix A2

DSC analysis

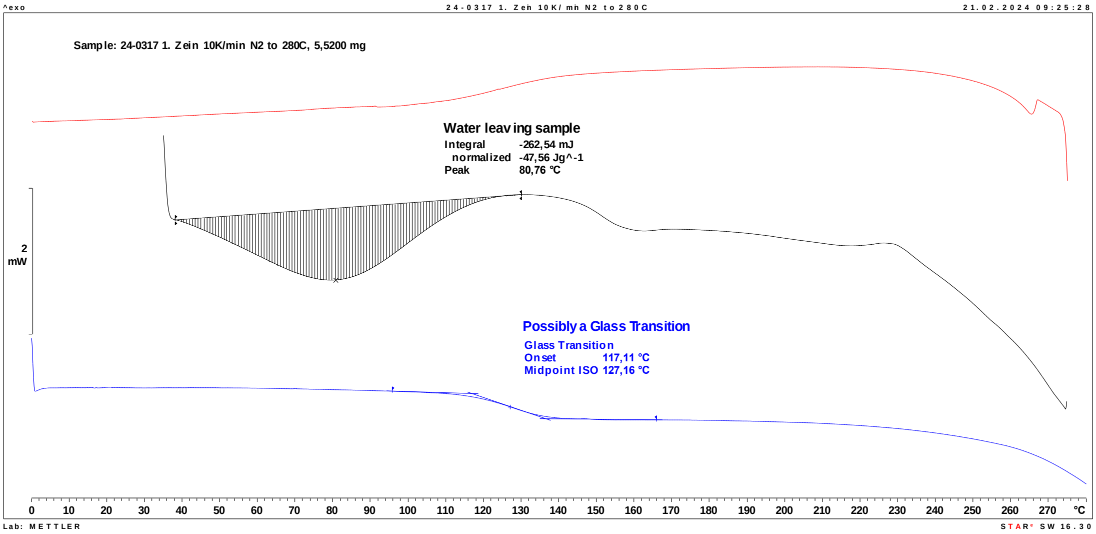

Figure 31. DSC thermogram illustrating the thermal behaviour of pure zein. Peaks indicate phase transitions and changes in heat capacity, providing information on melting points, crystallinity, and thermal stability.

Appendix A3

TGA analysis

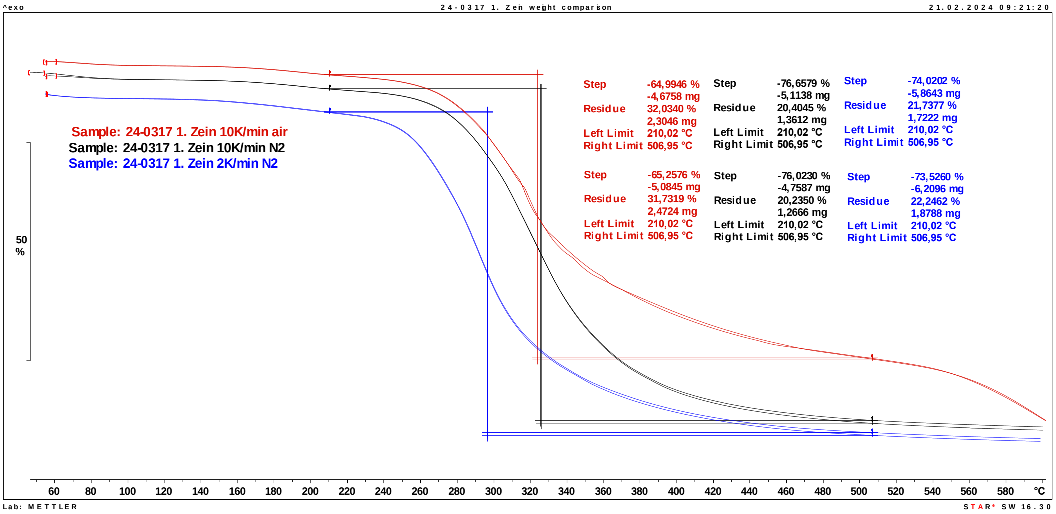

Figure 32. "TGA curve depicting the thermal decomposition behaviour of pure zein. The observed weight loss indicates the temperature at which degradation occurs, offering insights into the sample's thermal stability and composition.