ABSTRACT

GUTIERREZ PENA, JOSEPH NICOLAS. Evaluation and Characterization of Biopolymers to Reduce the Usage of Synthetic Polymers in Single-use Disposable Materials, Coating, and Packaging Applications. (Under the direction of Dr. Lokendra Pal, Dr. Richard Venditti).

The accumulation of single-use disposable products is a huge problem that needs an immediate solution. This work addressed two different approaches to this problem. The first part of this study aimed to develop a testing methodology to benchmark the properties of paper straws already in the market with common single-use plastic straws to identify which of their properties need improvement. The physical, mechanical, and compositional characteristics, as well as the liquid interaction properties of the straws, were determined. As the most important remark, the mechanical tests indicated that all the evaluated paper straws lost 70% to 90% of their compressive strength after being in contact with liquid for less than 30 min. This research provides baseline data for future work to develop a better paper or biopolymers for straw and other single-used food products. The second part of this study used RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) concentrates, the most abundant plant protein, from tobacco and alfalfa in a coating formulation as a co-binder for packaging applications. A comparison was made with two standard options in the industry: latex as the synthetic option and soy protein as the current bio-based alternative. The tobacco protein produced a shear-thinning behavior similar to soy protein, with lower water retention due to the lesser water holding capacity of tobacco. The properties of the coated paper are comparable to the ones obtained with soy protein and latex as a synthetic binder. However, the porosity and the glueability were enhanced with the tobacco protein, which is important in converting paperboard to boxes. This work shows that tobacco proteins have the potential to be used as a co-binder in coating applications and reduce the use of latex as a renewable and sustainable material.
Evaluation and Characterization of Biopolymers to Reduce the Usage of Synthetic Polymers in Single-use Disposable Materials, Coating, and Packaging Applications

by
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DEDICATION

I dedicate this accomplishment to my family and friends; sometimes, we cannot distinguish between them; most of the time is not necessary. To my parents and my sister, I keep pushing for you. Marielis, I will always be grateful for your kindness and love. I know that no matter what lays ahead, I can count on you. To Darlene and Adriana, the sisters that I found in my life.

Joseph Gutierrez

“Try not to become a man of success, but rather try to become a man of value.”

— Albert Einstein

“The greater danger for most of us is not that our aim is too high and we miss it, but that it is too low and we reach it”

— Michelangelo
BIOGRAPHY

Joseph was born and raised in Merida, Venezuela a small city up in the mountains in Los Andes. He received his B. S. in Chemical Engineering from the Universidad de Los Andes, Merida, Venezuela. The focus of his bachelor dissertation was on the study of natural extractives from the bark of softwoods and hardwoods applied as inhibitors of precipitation of the asphaltenes of the heaviest fraction of Venezuelan heavy crude oil.

He decided to continue the pursuit of sustainable alternatives, helping to reduce the environmental impact of current practices in the industry, taking the opportunity as a research assistant at NC State University as a Master student. Currently, he is working on evaluating different suitable alternatives to single-use plastics, using renewable materials in coating and packaging applications. He is part of the USDA/SBBP project that is teaching students and teachers of different communities how to be involved in the bioeconomy, to encourage new generations to recognize the importance of environmentally friendly practices.
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CHAPTER 1. INTRODUCTION

Today’s world is demanding better management of its resources. Society needs to make the transition to a more sustainable approach to consumption, requiring efforts from consumers, industries, and academia. The pollution that is generated mainly from single-use disposable products due to waste accumulation is a huge problem that needs an immediate solution. Thus, it is important to limit or reduce the use of non-degradable synthetic plastic, replacing it with biopolymers that can be either recycled or degraded naturally or in a composting facility. However, for biomaterials based on cellulose or proteins, for example, the mechanical or functional properties are often behind in comparison to plastics. The other constraints to fully replacing synthetic plastics is the generally higher cost of these materials.

The production and usage of synthetic plastics in packaging applications and disposable straws have grown in the last few decades, bringing environmental concerns due to the accumulation of these non-degradable materials [1]. Current trends in the bioeconomy, sustainability, and a growing public concerned about marine pollution due to the breakdown of material into little pieces and waste contamination littered in the environment are demanding big action to minimize the single-use of non-degradable or non-recyclable materials such as synthetic plastics.

Plastic is cheap and offers great mechanical and functional properties. However, it will not be degraded by any natural means, therefore accumulating and worsening contamination problems and endangering both humans and wildlife. The massive use of petroleum-based products will eventually decrease the availability of raw materials, increasing the necessity of sustainable and renewable alternatives. In this sense, a great amount of effort is focused on biopolymeric materials, which can be derived from a range of sources, such as proteins, lipids, or polysaccharides. These
materials can be obtained as by-products and industrial waste from the agroindustry, wood and paper production, and food waste materials. [2], [3].

All the biomass available can supply the packaging industry as well as straws manufacturing, helping to reduce the contamination and the carbon footprint of the industry. However, the challenge is to produce materials having the same or similar physical or functional properties, at a similar cost, with the convenience of being disposable material with sustainable characteristics [4].

Several academic works have been dedicated to the development of protein-based coatings as high-performance renewable materials in the packaging industry [5]. Taking advantage of the unique structure of proteins that confer to these biopolymers a wide range of functional properties, it can be used as a component in barrier films, with antimicrobial properties, oil, moisture or gas barriers, etc. [3], [6].

A protein with unexplored potential, such as RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase), can be used, since it is the most abundant protein on Earth, and is easily obtained from almost any leafy plants with photosynthetic characteristics [7]. Proteins from plants, such as soy protein, are used in the coating applications as co-binders to improve several properties of the paper and as alternatives to synthetic co-binders. The goal is to test RuBisCO in this application as a novel alternative for local producers and potentially create a new market in the coating and packaging industry.

This work explores the advantages and disadvantages of current bio-based alternatives to products made of single-use disposable plastic and a sustainable alternative to the use of synthetic polymers in the coating and packaging industry. Two research proposals are presented. The first
one is the evaluation of paper straws versus plastic straws, and the other is a study of tobacco proteins as sustainable co-binders in paper and paperboard coatings.

On the one hand, in the second chapter, the foundations for a technical comparison of paper straws against plastic straws are set. Several tests were made under controlled conditions to replicate typical usage, monitoring how papers straws mechanical properties decay over time after being in contact with common beverages. The physical, mechanical, and compositional characteristics, as well as the liquid interaction properties of the straws, were determined. This information will be essential for future development of a better paper-based version of paper straws.

On the other hand, the third chapter of this research focuses on the study of the leaf-protein RuBisCO as a renewable alternative to synthetic latex in coating applications. Coating formulations containing tobacco or alfalfa proteins concentrate with formulas with soy isolates, and latex only are compared. Several fundamental properties and characteristics of the proteins are determined, describing the protocols for the preparation, application, and testing of the coated paper. This allows comparison of the most critical properties for the industry obtained to discern whether these kinds of proteins indeed have a future in the coating industry for packaging applications.
CHAPTER 2. EVALUATION OF PAPER STRAWS VERSUS PLASTIC STRAWS: DEVELOPMENT OF A METHODOLOGY FOR TESTING AND UNDERSTANDING CHALLENGES FOR PAPER STRAWS

2.1 ABSTRACT

New alternatives to plastic straws are being considered due to consumer demands for sustainability and recent changes in government policies and regulations, such as bans on single-use plastic products. There are concerns regarding paper straw quality and stability over time when in contact with beverages. This study evaluated the performance and properties of commercially available paper straws and their counterpart plastic straws in various intended applications. The physical, mechanical, and compositional characteristics, as well as the liquid interaction properties of the straws, were determined. The paper straws were composed mainly of hardwood fibers that were hard sized with a hydrophobic sizing agent to achieve a water contact angle of 102° to 125°. The results indicated that all the evaluated paper straws lost 70% to 90% of their compressive strength after being in contact with the liquid for less than 30 min. Furthermore, the paper straws absorbed liquid at approximately 30% of the straw weight after liquid exposure for 30 min. Increased liquid temperatures caused lower compressive strengths and higher liquid uptake in the paper straws. This report provides directions and methods for testing paper straws and defines current property limitations of paper straws relative to plastic straws.

1 The material of this chapter has been published as:
2.2 INTRODUCTION

Straws provide a simple solution for drinking beverages more conveniently, which makes straws an excellent example of an item people take for granted. Currently, straws are massively consumed. The estimated disposable plastic straw consumption in the US is between 170 million to 490 million straws per day or 63 billion to 142 billion straws per year [8].

Since the use of straws dates so far back, an accurate time and place of the first usage are impossible to determine. The earliest evidence of straw use was found in a Sumerian tomb dating back to 3000 B.C. The tomb seal showed two men drinking beer from a jar using a tube made of gold [9]. In the 1800s, straws became popular and were made of rye grass, a biodegradable material, which tended to change the flavor and disintegrate into the drink, leaving sediment at the bottom [10]. Paper, another biodegradable material, replaced rye grass to solve these issues. Paper straws were the best option for several decades, but the straws still had one problem: they were not durable enough and lost their physical integrity and compressive strength. Thus, they easily collapsed once wet.

In the 1960s, the usage of plastic as a novel material changed the paper straw market to a point where no paper straws were produced after 1970 [10]. Plastics are remarkable materials with a wide variety of properties and are durable, inert, and moldable. The problem arose when plastic became a single-use, disposable material on a daily basis. The world produces more than 400 million tons of plastics every year, and 36% is destined for single-use materials, such as packaging, which in turn generates 300 million tons of waste [11]. Of that amount, only 9% is recycled, 12% is incinerated, and the remaining 79% accumulates in landfills and dumps or is littered in the environment, with half of this amount coming from packaging waste [12].
This amount of waste generates pollution and other environmental problems. Plastic pollution in oceans chokes and entangles sea life. It is also linked to diseases on coral reefs, as well as decreases in the reproduction and population growth of zooplankton [13]. Plastic products do not biodegrade, and instead, these materials break down into smaller pieces that can be consumed by organisms, putting them at risk [14], [15]. Seabirds, marine turtles, and cetaceans are included among the 267 species most affected by plastic ingestion [15], [16], [17].

Plastic litter in the ocean has been reported since the early 1970s, but it only started to draw attention from the scientific community in the last 25 years [18]. Activism against single-use plastic, particularly plastic straws, started in 2015 after videos arose of a turtle with a plastic straw in its nose and because of media interest in the garbage patch in the Pacific Ocean [19]. Because of this, cities like Seattle, WA and Berkley, CA and big companies like Starbucks have announced the elimination of plastic straw use in the next few years [20], [21], [22]. In addition, Starbucks has announced a $10 million grant intended for the development of a global solution of a recyclable and compostable cup, claiming that the technology will be open to the public after its development [23].

It is important to point out that the bans need to take into account (and it is not always the case) people with disabilities, notably if the bendy (plastic) straws are banned, since many of the people depend on bendy straws to drink any beverage [24], [25]. For this reason, a disposable plastic straw ban cannot merely be the solution to this problem. It is then necessary to have a viable alternative to plastic straws.

These market consumption changes and the increasing demand for more sustainable and environmentally friendly options to plastic have generated several alternative materials in the production of drinking straws [10]. Metal, glass, or silicon are some of the best alternatives for
reusable straws. However, single-use straws made of paper are returning to the market. Even bendable straws made of paper are now available [26]. Several brands, mostly in China, the UK, and the US, have returned to products not seen in more than four decades [10], [27], [28].

Paper straws are once again the best option for a disposable straw to drink a beverage, while avoiding the plastic waste that can last for over 500 years in the environment [11]. Nevertheless, paper straws are still not durable enough and typically cost more than their plastic counterparts. They lose their mechanical integrity once they are in contact with a typical beverage, and some brands’ straws can change the taste of the drink [29].

The aim of this study was to benchmark properties of paper straws already on the market with common, single-use plastic straws and by this means to identify which properties need improvement. The tensile and compressive properties as well as their interactions with liquids of commercial paper straws were compared with plastic versions.

2.3 MATERIALS

Four commercial brands of plastic straws and three commercial brands of paper straws were used for this research (names of the brands were excluded). All plastic and paper straws were acquired through Amazon.com, Inc. (Seattle, WA, USA). Common drinking (fountain) water, Coca-Cola (*i.e.*, Coke) (Atlanta, GA, USA), and Chick-fil-A (Atlanta, GA, USA) sweet tea were used as the beverages for the longevity tests. Table 1 describes each sample used.
Table 1. Straw Sample Description

<table>
<thead>
<tr>
<th>Material</th>
<th>Sample ID</th>
<th>Color/Characteristic</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Plastic1</td>
<td>Orange</td>
<td><img src="image" alt="Plastic1" /></td>
</tr>
<tr>
<td>Plastic</td>
<td>Plastic2</td>
<td>White/Bendable</td>
<td><img src="image" alt="Plastic2" /></td>
</tr>
<tr>
<td>Plastic</td>
<td>Plastic3</td>
<td>Green</td>
<td><img src="image" alt="Plastic3" /></td>
</tr>
<tr>
<td>Plastic</td>
<td>Plastic4</td>
<td>Multi-color</td>
<td><img src="image" alt="Plastic4" /></td>
</tr>
<tr>
<td>Paper</td>
<td>Paper1</td>
<td>White</td>
<td><img src="image" alt="Paper1" /></td>
</tr>
<tr>
<td>Paper</td>
<td>Paper2</td>
<td>Brown</td>
<td><img src="image" alt="Paper2" /></td>
</tr>
<tr>
<td>Paper</td>
<td>Paper3</td>
<td>Multi-color</td>
<td><img src="image" alt="Paper3" /></td>
</tr>
</tbody>
</table>

2.4 Methods

The determination of the weight and dimensions of the straw samples was necessary to make a proper comparison between the paper and plastic straws. The weight, length, external diameter, and thickness were measured. In addition, the internal diameter, external area, basis weight, and density of the samples were calculated. A Mettler Toledo analytical balance (PB303-S; Columbus, OH, USA) was used for the weight measurements. All tests and measurements were made under standard conditions (23°C and 50% relative humidity (RH)) using conditioned samples according to the TAPPI T402 sp-08 standard [30]. The fold endurance test was made with an MIT #1 Folding Endurance Tester (Tinius Olsen Testing Machine Co., Horsham, PA, USA) in accordance with the TAPPI T511 om-08 standard [31].
The fiber length of the paper in the straws was measured with a fiber quality analyzer (FQA) (FQA-360; OpTest Equipment Inc., Hawkesbury, Ontario, Canada) according to the TAPPI T271 om-07 standard [32]. The sample disintegration was completed using a pulp disintegrator (TMI 73-18; Testing Machines, Inc., New Castle, DE, USA) using 0.5 g of paper straw sample in 1 L of water at 15000 rpm for 10 min.

The mechanical and longevity tests compared the plastic straws with the paper straws. The longevity test replicated a typical usage of these products under controlled conditions. The samples were placed in water at four different initial temperatures (0 °C, 21 °C, 48 °C, and 82 °C) and in a cold, carbonated beverage (0 °C). The liquid height was fixed at 2/3 of the paper straw’s height. The longevity test exposed samples for different time lengths (0.5, 1.0, 2.0, 3.0, 4.0, and 6.0 h). For each time length, the entire sample was immediately weighed, and an axial and compression test was performed with the wet samples using the bottom part of the straw.

The paper straw tensile strength was measured based on the TAPPI T494 om-01 standard [33], using a horizontal tensile tester (TMI 84-56; Testing Machines, Inc., New Castle, DE, USA) with an initial gap of 30 mm. The plastic straw tensile strength was measured based on ASTM D882-12 [34] with a tensile testing machine (4443; Instron, Norwood, MA, USA) with an initial gap of 25 mm.

The compressive strength for both plastic and paper samples was measured based on ASTM D695-15 and ASTM D2412-11 [35] using a tensile testing machine (4443; Instron, Norwood, MA, USA). The axial and radial configuration for compression was tested. A compression speed test of 10 mm/min was used for the axial configuration using samples with a length/diameter ratio of 2. A compression speed test of 1 mm/min was used for the radial configuration with a length/diameter ratio greater than 8.
A surface electro-optics (SEO) contact angle analyzer (Phoenix 300; Surface Electro-Optics Co., Ltd., Suwon City, Gyeonggi-do, Korea) was used to determine the water contact angle and the surface tendency of the paper straws to absorb liquid. The angle was measured 10 s after the drop touched the surface. To control the formation speed of the drop, the software equipment defined the fast speed at 47 and the slow speed at 32. The drop that formed on the samples took 10 s for each trial before touching the surface. An industrial needle with a gauge of 27 was used for all tests.

Thirty paper straws of each brand were soaked in 1 L of water for 24 h to determine whether materials leached from the paper straws. The water was then analyzed with a portable turbidity meter (2020wi; LaMotte, Chestertown, MD, USA). Turbidity is the measurement of water cloudiness caused by particles suspended in the liquid [36], although each measurement method uses different units. For example, the nephelometric turbidity unit (NTU) is used by the Environmental Protection Agency (EPA) standard [37] and the formazin nephelometric unit (FNU) is used by the ISO standard [38]. However, these units can be considered equivalent, according to ASTM D6855-17 [39].

2.5 RESULTS AND DISCUSSION

The length, external diameter, and internal diameter were measured for all straws tested. The thickness shown in Table 2 was calculated. The caliper was measured using a Vernier (Traceable Digital Calipers, Fischer Scientific, Hampton, NH, USA). The results shown in Table 3 were calculated based on these measurements. Despite the different dimensions of the plastic straw samples, the thickness of each sample was similar. The dimensions of the paper straw samples were similar as well, but the apparent density changed between the brands and had a relative difference of approximately 12%.
Table 2. Dimensions and Calculated Properties of Plastic and Paper Straws. Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Material</th>
<th>Sample ID</th>
<th>Length (mm)</th>
<th>External Diameter (mm)</th>
<th>Internal Diameter (mm)</th>
<th>Thickness (calculated) (mm)</th>
<th>Caliper (Vernier) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Plastic1</td>
<td>250±0</td>
<td>7.57±0.12</td>
<td>7.04±0.13</td>
<td>0.27</td>
<td>0.21±0</td>
</tr>
<tr>
<td></td>
<td>Plastic2</td>
<td>190±0.47</td>
<td>6.12±0.17</td>
<td>6±0.15</td>
<td>0.06</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td></td>
<td>Plastic3</td>
<td>198±0</td>
<td>6.29±0.12</td>
<td>6.16±0.06</td>
<td>0.07</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td></td>
<td>Plastic4</td>
<td>299±0</td>
<td>9.92±0.15</td>
<td>9.49±0.16</td>
<td>0.21</td>
<td>0.21±0</td>
</tr>
<tr>
<td>Paper</td>
<td>Paper1 (White)</td>
<td>192±0.47</td>
<td>6.13±0.03</td>
<td>5.33±0.05</td>
<td>0.40</td>
<td>0.52±0.07</td>
</tr>
<tr>
<td></td>
<td>Paper2 (Brown)</td>
<td>195±0</td>
<td>6.14±0.02</td>
<td>5.22±0.08</td>
<td>0.46</td>
<td>0.5±0.07</td>
</tr>
<tr>
<td></td>
<td>Paper3 (Color)</td>
<td>195±0</td>
<td>5.96±0.07</td>
<td>5.04±0.04</td>
<td>0.46</td>
<td>0.49±0.05</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Material</th>
<th>Sample ID</th>
<th>Area π × D × L (mm²)</th>
<th>Weight (g)</th>
<th>Basis Weight (g/m²)</th>
<th>Density (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Plastic1</td>
<td>5946</td>
<td>1.06±0.01</td>
<td>179.7</td>
<td>855.6</td>
</tr>
<tr>
<td></td>
<td>Plastic2</td>
<td>3660</td>
<td>0.46±0.01</td>
<td>128.3</td>
<td>855.0</td>
</tr>
<tr>
<td></td>
<td>Plastic3</td>
<td>3911</td>
<td>0.55±0.01</td>
<td>142.2</td>
<td>812.5</td>
</tr>
<tr>
<td></td>
<td>Plastic4</td>
<td>9320</td>
<td>1.38±0.09</td>
<td>148.2</td>
<td>705.8</td>
</tr>
<tr>
<td>Paper</td>
<td>Paper1 (White)</td>
<td>3690</td>
<td>1.1±0.01</td>
<td>298.1</td>
<td>569.2</td>
</tr>
<tr>
<td></td>
<td>Paper2 (Brown)</td>
<td>3759</td>
<td>1.08±0.01</td>
<td>289.1</td>
<td>579.8</td>
</tr>
<tr>
<td></td>
<td>Paper3 (Color)</td>
<td>3650</td>
<td>1.138±0.03</td>
<td>311.8</td>
<td>637.7</td>
</tr>
</tbody>
</table>

2.5.1 Fiber Quality Analyzer (FQA) Results

The FQA results are shown in Table 4. The fiber length and the coarseness indicated that all paper straws were made mainly of hardwood fibers. Hardwood fibers are approximately 1 mm in length with a coarseness of 0.08 mg/m [40]. Hardwood fibers are shorter, giving to the paper better stiffness and higher density.

2.5.2 Water Contact Angle

The water contact angle test reflects the relative hydrophobicity of the straws, as shown in Table 5. Generally, a contact angle with water larger than 90° forms with hydrophobic surfaces, and less than 90° for hydrophilic surfaces [41]. The water contact angle for the plastic samples
was between 80° and 98°. For all paper straws, the tested surface was considered hydrophobic because the angles were between 102° and 125°. The surface of the paper straws was more hydrophobic than that of the plastic straws; this is an indication of surface treatment made on the paper. The untreated paper will have a lower water contact angle, and will absorb the liquid, additionally reducing the water contact angle noticeably over time [42], [43]. On the other hand, the plastic straws are not expected to take up any significant amount of water, as the paper straws might, and the water contact angle is expected to stay constant with time.

Table 4. Paper Straw Fiber Quality Analysis (FQA). Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Paper1 (White)</th>
<th>Paper2 (Brown)</th>
<th>Paper3 (Color)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber Length $L_a$ (mm)</td>
<td>0.75 ± 0.05</td>
<td>0.72 ± 0.01</td>
<td>0.75 ± 0.00</td>
</tr>
<tr>
<td>Fiber Length $L_w$ (mm)</td>
<td>1.12 ± 0.06</td>
<td>0.92 ± 0.01</td>
<td>0.99 ± 0.01</td>
</tr>
<tr>
<td>Fines (%) (Length Weighted)</td>
<td>14.43 ± 0.04</td>
<td>8.21 ± 0.53</td>
<td>7.50 ± 0.3</td>
</tr>
<tr>
<td>Curl Index (Length Weighted)</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.00</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>Mean Width (µm)</td>
<td>20.3 ± 0.85</td>
<td>16.9 ± 0.00</td>
<td>17.2 ± 0.00</td>
</tr>
<tr>
<td>Kink Index (1/mm)</td>
<td>1.6 ± 0.01</td>
<td>1.83 ± 0.06</td>
<td>1.75 ± 0.06</td>
</tr>
<tr>
<td>Coarseness (mg/m)</td>
<td>0.08 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

Note: $L_a$ = Arithmetic average of fiber length and $L_w$ = weighted average fiber length

Table 5. Water Contact Angle for Paper and Plastic Straws (10 s). Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Water Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner Layer</td>
</tr>
<tr>
<td>Plastic1</td>
<td>85.10</td>
</tr>
<tr>
<td>Plastic2</td>
<td>95.38</td>
</tr>
<tr>
<td>Plastic3</td>
<td>80.62</td>
</tr>
<tr>
<td>Plastic4</td>
<td>89.26</td>
</tr>
<tr>
<td>Paper1 (White)</td>
<td>112.83</td>
</tr>
<tr>
<td>Paper2 (Brown)</td>
<td>124.65</td>
</tr>
<tr>
<td>Paper3 (Color)</td>
<td>117.24</td>
</tr>
</tbody>
</table>

2.5.3 Dry Tensile Strength

The tensile strength was measured for the plastic and paper straws, Table 6 and Table 7. Because of the inherent difference between these two materials, a fair comparison between the
tensile properties was established using the specific strength and calculated by dividing the tensile strength by the density of the respective material. In this manner, the paper straws had a similar value between them, but the value changed considerably for the plastic straws. However, the paper straws had a higher specific strength with the exception of one of the plastic samples.

2.5.4 Folding Endurance

The folding endurance test determined the capacity of the straw to withstand repeated bending. The plastic straws offered a considerably higher resistance to this stress (Table 6). However, the paper straws were strong enough to resist the stresses of typical usage and were unbroken after being bent multiple times (Table 7).

Table 6. Mechanical Measurements for the Plastic Straws. Tested under standard TAPPI conditions (23 °C and 50% relative humidity (RH)). Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Plastic</th>
<th>Plastic1</th>
<th>Plastic2 (Normal)</th>
<th>Plastic2 (Flexible Zone)</th>
<th>Plastic3</th>
<th>Plastic4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load at Break (N)</td>
<td>192±0</td>
<td>94±0.01</td>
<td>99±0</td>
<td>164±0</td>
<td>212±0.07</td>
</tr>
<tr>
<td>Tensile Strength (MPa)</td>
<td>47.98±1.05</td>
<td>15.58±1.6</td>
<td>16.57±0.14</td>
<td>27.44±9.98</td>
<td>14.11±4.7</td>
</tr>
<tr>
<td>Specific Strength (KN × m/Kg)</td>
<td>56.08</td>
<td>18.22</td>
<td>19.38</td>
<td>33.78</td>
<td>19.99</td>
</tr>
<tr>
<td>Stretch (%)</td>
<td>1226.46±35.4</td>
<td>953.89±48.8</td>
<td>996.04±18.08</td>
<td>1278.96±37.27</td>
<td>1262.95±27.79</td>
</tr>
<tr>
<td>Young’s Modulus (MPa)</td>
<td>850.93±96.15</td>
<td>242.2±16.2</td>
<td>71.67±0.52</td>
<td>457.55±201.88</td>
<td>256.11±11.96</td>
</tr>
<tr>
<td>Stiffness (kN/m)</td>
<td>709.11</td>
<td>201.83</td>
<td>59.73</td>
<td>381.30</td>
<td>320.13</td>
</tr>
<tr>
<td>Fold Endurance</td>
<td>&gt; 5000</td>
<td>&gt; 5000</td>
<td>&gt; 5000</td>
<td>&gt; 5000</td>
<td>&gt; 5000</td>
</tr>
</tbody>
</table>
Table 7. Mechanical Measurements for the Paper Straws. Tested under standard TAPPI conditions (23 °C and 50% relative humidity (RH)). Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Paper1 (White)</th>
<th>Paper2 (Brown)</th>
<th>Paper3 (Color)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load at Break (N)</td>
<td>99.97</td>
<td>113.10</td>
<td>132.90</td>
</tr>
<tr>
<td>Tensile Strength (kN/m)</td>
<td>10±3.67</td>
<td>13.29±0.47</td>
<td>11.31±0.27</td>
</tr>
<tr>
<td>Specific Strength (KN × m/Kg)</td>
<td>33.53</td>
<td>39.13</td>
<td>42.62</td>
</tr>
<tr>
<td>Stretch (%)</td>
<td>3.66±0.86</td>
<td>4.13±0.8</td>
<td>4.01±1</td>
</tr>
<tr>
<td>Stiffness (kN/m)</td>
<td>1219.2±76.97</td>
<td>1285.2±19.76</td>
<td>1252.2±19.49</td>
</tr>
<tr>
<td>Fold Endurance</td>
<td>2582±1294</td>
<td>1681±753</td>
<td>1984.67±1057</td>
</tr>
</tbody>
</table>

Figure 1 shows the plots of the strongest plastic and paper straws during the tensile test and displays the expected behavior of these types of polymeric materials.

![Average tensile strength curves for paper and plastic straws; strongest plastic (plastic1) and paper1, paper2, and paper3. Enlargement of range to 0.1 mm/mm.](image)

Figure 1. Average tensile strength curves for paper and plastic straws; strongest plastic (plastic1) and paper1, paper2, and paper3. Enlargement of range to 0.1 mm/mm.

Overall, the plastic tended to be stronger than the paper. The force needed to break the paper samples was 60% of the force needed to break the plastic straw, and the plastic straws could stretch roughly ten times or more before failure. In addition, the paper straws failed at 3.6% to 4.1% of strain, and the paper was stiffer than the plastic.
Figure 1 shows a typical tensile curve for polymers (green trend), with a Hookean or elastic region with a linear response (Young’s modulus) of strain and increasing stress. The tensile yield strength was the first point where the linear trend ceased, and the plastic deformation started. The ultimate stress was the reported tensile strength and is the maximum load the material can stand before it breaks, divided by the initial transversal area.

2.5.5 Axial Compression

Table 8 shows the compressive strength for the plastic and paper straws for the axial configuration. The Paper3 straws achieved the highest compressive strength of the paper straws, but when compared with plastic, the paper could only withstand half of the force of the strongest plastic straw.

<table>
<thead>
<tr>
<th>Material</th>
<th>Sample ID</th>
<th>Compressive Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Plastic1</td>
<td>21.6±3.2</td>
</tr>
<tr>
<td></td>
<td>Plastic2</td>
<td>12±2.2</td>
</tr>
<tr>
<td></td>
<td>Plastic3</td>
<td>17.9±3.4</td>
</tr>
<tr>
<td></td>
<td>Plastic4</td>
<td>15.9±0.9</td>
</tr>
<tr>
<td>Paper</td>
<td>Paper1 (White)</td>
<td>9.26±1.69</td>
</tr>
<tr>
<td></td>
<td>Paper2 (Brown)</td>
<td>7.96±1.1</td>
</tr>
<tr>
<td></td>
<td>Paper3 (Color)</td>
<td>9.99±2.25</td>
</tr>
</tbody>
</table>

Table 8. Dry Axial Compressive Strength for Plastic and Paper. Mean value ± Standard deviation.

Figure 2 shows the strongest of the plastic straws in contrast with the three brands of paper straws. The plastic cylindrical structure presented a narrower peak and the highest compression stress before it collapsed. In contrast, the paper showed a broader curve and reached the maximum load at a higher strain before it failed.
Figure 2. Average compressive strength curves in the axial direction for plastic straws and the three brands of paper straws

2.5.6 Radial Compression

The results of the radial configuration compressive strength are shown in Table 9. The paper straws exhibited more strength and a higher modulus than the plastic straws. The plastic deformed more easily, with more obvious elastic (reversible deformation) behavior. People often enjoy this property, as they sometimes like to repeatedly bite the straw and have it come back to its original state. This reversible deformation is not as likely with paper straws.


<table>
<thead>
<tr>
<th>Material</th>
<th>Sample</th>
<th>Maximum Load(N)</th>
<th>Compressive Strength (kPa)</th>
<th>Young's Modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Plastic1</td>
<td>29.7±7.6</td>
<td>39.6±10.2</td>
<td>92.28</td>
</tr>
<tr>
<td></td>
<td>Plastic2</td>
<td>13.9±0.4</td>
<td>19.4±0.5</td>
<td>51.19</td>
</tr>
<tr>
<td></td>
<td>Plastic2 (Flexible Zone)</td>
<td>22.1±0.9</td>
<td>40.1±1.7</td>
<td>110.01</td>
</tr>
<tr>
<td></td>
<td>Plastic3</td>
<td>12.1±0.7</td>
<td>21.9±1.9</td>
<td>97.52</td>
</tr>
<tr>
<td></td>
<td>Plastic4</td>
<td>36±2.2</td>
<td>36±2.2</td>
<td>83.24</td>
</tr>
<tr>
<td>Paper</td>
<td>Paper1 (White)</td>
<td>8.75±1.92</td>
<td>124.75±24.63</td>
<td>517.25</td>
</tr>
<tr>
<td></td>
<td>Paper2 (Brown)</td>
<td>6±1.41</td>
<td>83±21.92</td>
<td>438</td>
</tr>
<tr>
<td></td>
<td>Paper3 (Color)</td>
<td>9.5±2.06</td>
<td>132±27.87</td>
<td>776.5</td>
</tr>
</tbody>
</table>
Paper2 (Brown) displayed the lowest strengths for both the axial and radial configurations. The axial configuration had a more considerable load tolerance than the radial configuration due to a geometrical configuration that allows an even distribution of the stress through the entire structure.

Figure 3 shows the radial compression curves of the plastic and paper straws. The transition between the elastic and plastic regions was not easy to distinguish in the plastic curve but was more easily shown in the paper curve. This means that the plastic acted as an elastic material without the plastic region for the strain tested. In addition, the paper could not recover the initial geometry of the material after the test, as did the plastic samples.

![Image of stress-strain curves](image)

**Figure 3.** Average compressive strength curves in the radial direction for plastic straws and the three brands of paper straws

### 2.5.7 Weight Gain for Different Liquids

The first part of the longevity test was to measure how much liquid the straws in the test conditions were retained as a function of time. Figure 4 shows the weight gain of the straws due to water absorption versus time at room temperature. The weight gain increased at a high rate for the first 20 min, and then at a slower rate after that. Plastic straws showed negligible weight gain in all cases.
Figure 4. Weight gain for paper straws in water at 21 °C in 5 min intervals
Figure 5. Weight gained for (a) Paper1 (White), (b) Paper2 (Brown), and (c) Paper3 (Color) in three different beverages with an initial temperature of 0 °C

Figure 5 shows the weight gain as a percentage for Paper1 (White) (Figure 5a), Paper2 (Brown) (Figure 5b), and Paper3 (Color) (Figure 5c) for some common beverages. This test was performed using the same initial temperature and liquid height in three different but common cold beverages: water, a carbonated beverage (Coke), and sweet tea. The most noticeable difference was between paper straw types rather than liquids. The Paper1 (White) straws gained up to 75% of their weight after four h of testing, while the other two brands only gained approximately 30% each. These results suggest that the brand of Paper1 (White) lacked the coating or protective material or had less internal sizing than did the other paper straws.
2.5.8 Weight Gain for Different Temperatures

Similarly, the three brands of paper straws were tested using water at three different temperatures (i.e., 0 °C, 21 °C, and 48 °C). Paper1 (White) retained more liquid than the other paper straws. For Paper2 and Paper3 straws, the weight gain was higher for the higher liquid temperature, as expected. However, for Paper1 the opposite was true (Figure 6).

Figure 6. Weight gained for (a) Paper1 (White), (b) Paper2 (Brown), and (c) Paper3 (Color) in water at three different initial temperatures
2.5.9 Effects of the Paper Straw and Liquid Interactions

A concern for using paper straws is how the appearance of the liquid and the straws are affected by the interaction between them. The appearance of the paper straws after 30 min and 6 h after being in contact with Coke is shown in Table 10. It is observed that the paper straws all showed a distinct darkening due to the absorption of the Coke.

Table 10. Paper Straw Appearance After Longevity Test

<table>
<thead>
<tr>
<th></th>
<th>Paper1 (White)</th>
<th>Paper2 (Brown)</th>
<th>Paper3 (Color)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coke</td>
<td>Coke</td>
<td>Coke</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>6 h</td>
<td>30 min</td>
<td>6 h</td>
</tr>
<tr>
<td>Coke</td>
<td>Coke</td>
<td>Coke</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>6 h</td>
<td>30 min</td>
<td>6 h</td>
</tr>
<tr>
<td>Water</td>
<td>Water</td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>24 h</td>
<td>24 h</td>
<td></td>
</tr>
</tbody>
</table>

To investigate whether the straws released any material/particles during the contact with the liquids, the turbidity of the water was measured after 6 and 24 h of the straws being in contact with the water. The appearance of the straws after 24 h in water is also shown in Table 10. The results indicated that the paper straws acquired the color of the liquid, but the straws did not visually change the appearance of the liquid and did not release any solids into the liquid, even under periods considerably larger than the average for these single-use disposable materials.
2.5.10 Material Transfer from Straw to Liquid Measured by Turbidity

The turbidity values obtained from the paper straws being in contact with water for 24 h are shown in Table 11. The liquid was completely clear to the human eye with an average value of 0.56 FNU (formazin nephelometric unit).

Table 11. Turbidity Measurement after 24 h of Direct Contact between Water and Paper Straws. Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Turbidity (FNU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking Water</td>
<td>0</td>
</tr>
<tr>
<td>Water + Paper1 (White)</td>
<td>0.52 ± 0.11</td>
</tr>
<tr>
<td>Water + Paper2 (Brown)</td>
<td>0.63 ± 0.12</td>
</tr>
<tr>
<td>Water + Paper3 (Color)</td>
<td>0.53 ± 0.09</td>
</tr>
</tbody>
</table>
2.5.11 Compression of Paper Straws Exposed to Liquid

Figure 7. Axial compressive strength vs. time for straws immersed in: (a) Coke at an initial temperature of 0 °C (temperature remained at 0 °C for 3 h, and after 6 h, the temperature reached 15 °C); (b) water at an initial temperature of 0 °C (temperature remained at 0 °C for 3 h, and after 6 h, the temperature reached 15 °C); (c) water at an initial temperature of 21 °C; (d) water at an initial temperature of 48 °C; and (e) water at an initial temperature of 82 °C.
To be seen by the naked eye, the value must be at least 4 FNU [36], 55 FNU for a cloudy suspension, and 515 FNU for an opaque suspension [44]. The values indicated that no relevant amount of solid migrated in the liquid. Considering that drinking water needs to have a value under 10 FNU to be acceptable, the paper straws did not noticeably contaminate the liquids after 24 h of direct contact [45].

2.5.11.1 Axial Compression

In this section, the second part of the longevity test is discussed. The compressive tests of the paper straws in wet conditions as a function of time, with an initial liquid temperature and a fixed liquid height, was conducted. The axial and radial configurations were evaluated.

Figure 7a shows the compressive strength of the paper straws relative to the dry condition (point zero) as a function of time. The strength decreases by about 80% within the first 30 min and then retained that level throughout the rest of the test. The reduction of force was approximately 90% in some cases. The Paper3 (Color) straws remained as the brand with the highest compressive strength at every condition, in all liquids tested and at all temperatures. As shown in Figure 7a and 7b, when Coke and water were at the same initial temperature (0 °C), there was no relevant difference in compressive strength between the two beverages. As shown in Figure 7b through 7e, initial temperature increases reduced the compressive strength of the paper straws even further. Several samples completely lost their structural integrity in the water at 82 °C, making the compression test not possible for these samples.
2.5.11.2 Radial Compression

The longevity tests of the paper straw compressive strength in the axial direction of the straws are discussed in this section. The results for water at 0 °C and 21 °C, and Coke at 0 °C after two h in direct contact with the liquid are shown in Tables 12 to 14 for the paper straws.

Table 12. Radial Compression Strength for Paper1 (White) Straws out of the box* and after 2 h immersed in liquid

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Load (N)</th>
<th>Compressive Strength (kPa)</th>
<th>Young's Modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out of the box</td>
<td>8.75</td>
<td>124.75</td>
<td>517.25</td>
</tr>
<tr>
<td>Water at 21 °C</td>
<td>3.77</td>
<td>10.48</td>
<td>28.00</td>
</tr>
<tr>
<td>Ice Water</td>
<td>4.00</td>
<td>11.11</td>
<td>29.00</td>
</tr>
<tr>
<td>Ice Coke</td>
<td>3.80</td>
<td>10.55</td>
<td>27.00</td>
</tr>
</tbody>
</table>

*Out of the box refers to the results of Table 9, the compressive test of the straws before contact with any liquid

The Paper3 (Color) straws remained the strongest in terms of the load they could sustain. Similar to the axial configuration, the paper straws lost 80% to 90% of their compressive strength after exposure to the liquids for 30 min.

Table 13. Radial Compression Strength for Paper2 (Brown) Straws out of the box* and after 2 h immersed in liquid

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Load (N)</th>
<th>Compressive Strength (kPa)</th>
<th>Young's Modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out of the box</td>
<td>6.00</td>
<td>83.00</td>
<td>438</td>
</tr>
<tr>
<td>Water at 21 °C</td>
<td>5.50</td>
<td>15.29</td>
<td>41</td>
</tr>
<tr>
<td>Ice Water</td>
<td>4.81</td>
<td>13.38</td>
<td>33</td>
</tr>
<tr>
<td>Ice Coke</td>
<td>5.09</td>
<td>14.13</td>
<td>39</td>
</tr>
</tbody>
</table>

*Out of the box refers to the results of Table 9, the compressive test of the straws before contact with any liquid

Table 14. Radial Compression Strength for Paper3 (Color) Straws out of the box* and after 2 h immersed in liquid

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Load (N)</th>
<th>Compressive Strength (kPa)</th>
<th>Young's Modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out of the box</td>
<td>9.50</td>
<td>132.00</td>
<td>776.5</td>
</tr>
<tr>
<td>Water at 21 °C</td>
<td>12.00</td>
<td>33.33</td>
<td>92.0</td>
</tr>
<tr>
<td>Ice Water</td>
<td>9.49</td>
<td>26.36</td>
<td>64.0</td>
</tr>
<tr>
<td>Ice Coke</td>
<td>8.78</td>
<td>24.39</td>
<td>53.0</td>
</tr>
</tbody>
</table>

*Out of the box refers to the results of Table 9, the compressive test of the straws before contact with any liquid
The longevity test was also performed with water at different initial temperatures, as shown in Figure 8. The results show how the compressive strength was reduced by 80% to 85% after 30 min and remained in this range during the remainder of the test. Like the axial configuration, a higher temperature negatively affected the radial compressive strength and further reduced the results to close to 90% of the dry value.

![Figure 8. Radial compressive strength vs. time for straws immersed in water at (a) an initial temperature of 48 °C and (b) an initial temperature of 82 °C](image)

### 2.6 CONCLUSIONS

1. The paper straws were made mainly of hardwood fibers and had been treated to increase their hydrophobicity. Their surfaces formed initial water contact angles with water in the range of 100° to 125°, indicative of hard sized paper surfaces.

2. The paper straws experienced weight gain almost immediately after exposure to liquids and gained weights of 30% to 50% within 60 min.

3. The plastic straws were generally stronger than the paper straws in the dry state and did not gain weight when immersed in fluids. Plastic straws did not display any decreases in mechanical properties upon immersion in liquids.
4. Paper straws displayed higher compressive strength in the radial configuration under dry conditions than the plastic straws; however, the plastic straws returned to the original shape after release of the force, and the paper straws did not.

5. The type of fluid did not have a noticeable impact on the weight gain or wet strength of the paper straws.

6. An increase in the liquid temperature increased the weight gained for the paper straws and reduced the wet strength.

7. The paper straws did not release appreciable particle solids into the liquids as evidenced by liquid turbidity measurements before and after exposure to the paper straws.

8. None of the paper straws evaluated had considerable stability after 30 min in liquids, losing 80% to 90% of their strength within 30 min of exposure to liquid.

9. Tracking of the time-dependent weight gain and compressive strength of paper straws under immersion of liquids was an insightful way to evaluate paper straw product performance.

2.7 ACKNOWLEDGMENTS

The authors are thankful to Dr. Ved Naithani for assisting in conducting the FQA testing.
CHAPTER 3. STUDY OF TOBACCO PROTEINS AS A SUSTAINABLE CO-BINDER IN PAPER AND PAPERBOARD COATINGS

3.1 ABSTRACT

This study aimed to demonstrate that biopolymers such as proteins are viable options to reduce the usage of synthetic polymers in the coating and packaging industry. Tobacco Derived Protein (TDP) and Alfalfa Protein Concentrates (APC) were characterized and tested as co-binders in a typical coating formulation and compared with existing commercially available Styrene Butadiene Latex (SBR) and Soy Protein Isolate (SPI) binders.

The results indicate that the tobacco samples had a low purity (20 and 67%). The alfalfa protein was found to be trapped in the plants' cells and needed further isolation processing for it to be used in coating applications. The rheological testing of the coating colors showed that the tobacco protein produced a shear-thinning behavior similar to soy protein, with lower water retention due to the lesser water bonding capabilities of this protein. The measurements of the protein-coated samples indicated similar properties between soy and tobacco.

The properties of the coated paper are comparable to those obtained with soy protein and latex as a synthetic binder, making the tobacco protein a suitable option in coating for paper and packaging applications, driving us to the conclusion that tobacco proteins have the potential to be used as co-binders in coating applications and reduce the use of latex.
3.2 INTRODUCTION

The world today is concerned about sustainability and biodegradability and is claiming innovative approaches looking for the reduction of petroleum-based materials in the packaging industry. In this research, the aim is to demonstrate that biopolymers such as proteins are viable options to reduce the usage of synthetic polymers in the coating and packaging industry. With this purpose in mind, Tobacco Derived Protein (TDP), and Alfalfa Protein Concentrates (APC) were characterized, tested, and compared as co-binders in a coating formulation versus formulations with Styrene Butadiene Latex (SBR) and Soy Protein Isolates (SPI).

This characterization consists of the determination of the actual protein content (purity of the protein), molecular weight, isoelectric point, and solubility. For testing the proteins, coating formulations were made and applied to paper samples. Rheology and water retention measurements were made to the fluids, and the properties of the paper were obtained. The properties of the coated paper were compared, using the protein-coated sample properties against the latex-coated samples.

Commercial samples of soy and alfalfa proteins were compared with two tobacco protein samples extracted from the tobacco leaves. The performance of TDP and APC as co-binders was determined by incorporating the biopolymers into a typical pigmented coating formulation. The coating formulation consists of clay and calcium carbonate as pigments, with the binder (SBR Latex, SPI, TDP, or APC) and additives (dispersant, and alkali as needed) dispersed in water. The density, pH, rheology at low and high shear, and water retention of each coating color were measured. The coatings were applied to linerboard and whiteboard as base papers, using Mayer rods followed by hot air drying and calendering.


3.3 BACKGROUND

3.3.1 Tobacco Outlook

The tobacco industry started in the 15th century when Christopher Columbus became the first importer of tobacco into Europe from the Americas. Afterward, cultivation and commercialization took place within decades. After the mechanical process of making cigarettes was established in the 1880s, tobacco consumption increased the demand for tobacco leaves [46]. Now, the tobacco crop industry is worth around US$ 20 billion [47].

Tobacco production in North Carolina started around 1663 when settlers moved from Virginia, and one of the only suitable crops to grow in the dry and sandy soil was tobacco. The new colonists saw this as an opportunity to enter the European market. With the passage of centuries, the tobacco business became an integral part of North Carolina's culture [60].

For tobacco production, it is necessary to have a mild and sunny climate. The leading tobacco-producing states in the USA include North Carolina, Kentucky, and Virginia. North Carolina lies in the Virginia-Carolina tobacco belt, which in 2016 was the top producer with more than 331 million pounds [59].

Between 2007 and 2011, rising consumption and increased production quality worldwide kept the demand for the tobacco strong [46]. Although consumption rates have declined in developed countries, the demand has grown in emerging markets, particularly in China [48]. The compound growth between 2012 and 2017 was 1.1% in total. Moreover, the world price of tobacco is anticipated to fall, with an estimated value of -0.6% in total for the 2017-2022 period [48]. Figure 9 shows the changes in the price of tobacco.
Historically, the tobacco harvested in the United States used to be considered the product of the highest quality, conferring a premium status. However, improvements in tobacco quality from other countries are closing the quality gap, making the profit margins smaller [48]. For a given quality, it is cheaper to import tobacco. This has an impact on its trading. During the years 2008 to 2012, although North Carolina’s total imports had fallen by 36.7%, the total U.S. imports of tobacco products increased by 25.4% (from $650.49 – $871.35 million). [60].

Because of US has increased import of low-quality tobacco, the premium-graded tobacco from NC has a surplus, even though, as mentioned before, China and other Asian nations are growing the demand for tobacco from the USA, particularly for flue-cured products [58]. However, market changes and price fluctuations make it hard to predict the demand for the product.

Also, the demand for tobacco will be reduced as smoking rates continue to fall in developing countries. Just in the USA, cigarette sales declined at an average of 2.3% per year from 2011 to 2016 [48]. In order to surpass this decline in demand, is necessary to find alternative uses for the NC tobacco surplus.

All these factors, less local consumption, more imports of cheaper tobacco, and lower prices of premium local tobacco are generating a decline on tobacco leaves growth in North Carolina. For that reason, this research aims to create a new market for the tobacco leaves in the coating industry, using its proteins, preventing the production drop.
3.3.2 Tobacco Protein

Proteins are polymers of amino acids with a wide range of chain lengths, from about 50 amino acids to large complexes of more than 100,000 amino acids. Two main groups of proteins can be distinguished: firstly, proteins showing a physiological function, such as enzymes and hormones and secondly, structural or storage proteins such as collagen and pea seed proteins [49].

The tobacco protein used in this project is Ribulose-1,5-Bisphosphate Carboxylase-Oxygenase (RuBisCO), which is present not only in tobacco but in many other photosynthetic plants. This protein acts as an enzyme in the photosynthesis process [50]. This protein comprises 50-60% of the leaf extract and is probably the most abundant protein in the biosphere [51].
RuBisCO has a molecular weight of 560 kDa and consists of eight small (14 kDa each) and eight large (56 kDa each) subunits arranged as eight heterodimers [52].

The factors just mentioned make the protein structure very complex compared with a synthetic polymer with similar applications. Additionally, proteins not only have a primary and a secondary structure, but they also have tertiary and quaternary structures. Such structural and chemical complexities associated with protein polymers affect their dispersion, solubilization, viscosity, curing, and moisture resistance. Thus, much of the current research and development (R&D) concerning protein-based adhesives is devoted to improving such parameters via protein modification [53].

RuBisCO is an enzyme involved in the Calvin-Benson Cycle for the CO₂ fixation or Dark Reactions of the photosynthesis process [2]. This process is the primary source of sugar and other energy-storage molecules used for autotrophs, such as plants [2]. Overall, the process consists in the capture of sunlight and CO₂, and using water, which produces complex molecules useful for other biochemical processes needed for the growth and function of the plant, leaving O₂ as a by-product [50].

The structure of the protein can be seen in Figure 10. Blue represents the primary structure, purple represents alpha-helixes, and yellow represents beta-sheets [54].
Traditionally tobacco leaves have mainly been used in the smoking industry, although some researchers have pointed out the potential for other applications. It was suggested by Sheen et al. (1985) that the Tobacco protein extract named F-1-p could be a suitable option for food formulations, capable of replacing other proteins, such as soy proteins in some foods. The protein crystals obtained after the extraction process are soluble in water, tasteless, and odorless [55].

Later, Sheen et al. (1988) made modifications of the F-1-protein and were able to modify several properties, including foaming and emulsion viscosity. On the other hand, the degraded protein lost some ability to absorb water and fat, fat-binding, and hydrophobicity, but these properties were enhanced with N-chlorosuccinimide (NCS)/urea treatment [56].

The same author did another study comparing properties of the same protein (F-1-p) with different leaf extracts: alfalfa, soybean, sugar beet, and tobacco. It was determined that this protein has very similar properties, no matter its origin. In general, the amino acid composition, both essential and nonessential, is comparable to the plants studied [57].
Also, according to the results, properties including solubility index vs. pH, foaming capacity and stability, water absorption, fat binding capacity, emulsion activity index, and emulsion capacity are very similar, with the (F-1-p) tobacco protein showing better results on the emulsions-related properties[57]. Therefore, in a specific application, it will be expected to have a similar performance when using proteins extracted from different plants with the same protocol.

3.3.3 Alfalfa Outlook

Alfalfa (*Medicago sativa*) or Lucerne is one of the most important forage crops in the world. It has high yield potential without the need for nitrogen. It has a high content of protein. Making this crop a versatile option for livestock feeding [58]. Alfalfa is a source of inexpensive and easy to assimilate protein; human interest in its consumption has increased [59]. It is mainly consumed as sprouts, in salads, or sandwiches. However, it is available as a nutritional supplement, such as protein concentrates (powder), tablets, or capsules, targeting consumers with a diary or soy consumption restrictions [60]. Commercial Alfalfa protein concentrates (APC) consists of 45-60 % proteins, 9-11 % fats, 11-15 % polysaccharides (insoluble fiber), and 8-13 % minerals, and also contains many vitamins [59].

As tobacco, alfalfa is a photosynthetic plant, loaded with large amounts of RuBisCO protein, considered the most abundant and available-spread protein in the world. Moreover, alfalfa can produce the highest amount of protein per hectare, and it is the most widespread crop in the world [59], [61]. These facts make alfalfa protein concentrates a potential source of protein for both food and non-food applications, given that leaf protein concentrates can be produced from either leafy plant crops or plant by-products, making the product relatively cheap [62].
There are small differences in the amino acid sequence of RuBisCO protein between tobacco and alfalfa, which do not change greatly the enzymatic activity, the extraction process, or the properties of the protein. There are other proteins of plant origin extracted from the bean instead of the leaves like RuBisCO. Soybean is the best example.

3.3.4 Soy Protein Outlook

Since the arrival of soy (Glycine max) to the US from China in 1765, until the 1920s, the purpose of its crops was mainly as forage. Osborne & Mendel, with their researches, demonstrated the nutritional value of soybean and its protein, highlighting the importance of its use as food. [63]. Currently, derivates from soybeans, like soy protein, are used widely in the food industry, given the high nutritional value, flavor, and functional properties. Soybean protein is consumed as an alternative to the animal one.

Soy protein offers several functional properties, such as good optical (gloss), fat barrier, moderate mechanical abilities, and great binding abilities [5]. Products derived from soybean are also used in the manufacture of non-food applications, including paint and coatings, lubricants, adhesives, solvents, surfactants, printing inks, polyols, thermoset plastics, elastomers, rubber compounds, plasticizers, grease-resistant paper, fibers, hydrogels, in addition to the well-established use of soy oil to make biodiesel [64]. Thanks to functional properties of soy protein, such as good optical (gloss), fat barrier, moderate mechanical, and great binding abilities [5], products derived from soy bean are also used in manufacture of non-food applications, including paint and coatings, lubricants, adhesives, solvents, surfactants, printing inks, polyols, thermoset plastics, elastomers, rubber compounds, plasticizers, grease-resistant paper, fibers, hydrogels, in addition to the well-established use of soy oil to make biodiesel [64].
The use and development of natural or biodegradable polymers started with the need to reduce society’s dependence on petroleum-based polymers, looking to eliminate the adverse environmental effects and to employ less expensive alternatives [65]. Over the last few years, the use of barrier coating on packaging materials based on natural materials has increased [66]. Food packaging, in particular, represents a big market for such materials. The focus has been on non-toxic, environmentally friendly, renewable polymers, mostly as edible films, barriers, and coatings [66], with the purpose of preservation and protection of processed food and raw materials during processing, manufacturing, handling, and storage [65].

Soy products can be categorized into three groups based on the protein contents, and this content can vary from 40% to 90%. These groups are flours, soy protein concentrates, and soy protein isolates (SPI), with isolates having the highest protein content [67]. The structure of the main fractions of the soy protein extracted from soybean can be seen in Figure 11. Blue represents the primary structure, purple represents alpha-helixes, and yellow represents beta-sheets. [68], [69].

![Glycinin (7S) and Beta-Conglycinin (11S)](image)

**Figure 11.** Soy protein main protein structures. (a) 7S fraction, Glycinin. (b) 11S fraction, Beta-Conglycinin. Blue is the primary structure, purple represents alpha helixes, and yellow represents beta sheets [68], [69].
SPI, is a significant by-product of soybean oil extraction; it is a cheap biopolymer that is competitive with the commonly used packaging material polyethylene [66], [70]. SPI has great film-forming properties, and it is most frequently used as edible wraps for meat or vegetables [66].

In terms of market, soy protein isolates are projected to be the fastest-growing segment of the soy protein ingredients, because their neutral taste and high protein content make them unique as nutritional and functional ingredients in beverages [71]. Soy protein is used as an alternative to meat and dairy in the food industry, especially for vegans; this factor is causing the protein ingredients in the food sector to become more common [71].

3.3.5 Applications in Paper Coatings

The application of soy protein has a long history in papermaking and paper coating industries, for example, as calender sizing agent and as a co-binder to enhance binding strength, water holding, coating immobilization, and rheology. Besides, soy protein isolate can be used alone or in combination with styrene-butadiene rubber latex for paper coating. It has a high affinity for pigments in paper coating compositions and gives paper good ink receptivity and printability [64].

Paper is a material widely used for packaging applications, with good mechanical properties, biodegradable, renewable, and recyclable. However, the paper has poor barrier properties against oxygen, water vapor, grease, and aroma compounds [72].

The paper substrate is rough, porous, and absorbent, whose physical properties change with water. The coating can improve the look and feel, printability, and the functional characteristics of the paper [73]. Coatings consist of pigments, binders, and additives. The most common pigments used are kaolin clay, calcium carbonate, titanium dioxide, and alumina trihydrate. Coating
formulations are based on pigment concentration. Binders are used for linking pigments within the coating layer and the coating layer to the paper fibers. Binders can be naturals, such as starch, soy protein, casein, or synthetic binders, such as latex and polyvinyl alcohol [73].

The pigments have different sizes and shapes, and the synthetics or natural polymers used as binders have considerable effects on the rheological behavior of the coating suspension [74]. These polymers are used as thickeners to adjust the rheological properties and to modify the water retention [75].

Proteins are obtained from renewable and sustainable sources; these features are very much in demand by the industry today. These biopolymers have amphoteric characteristics and can be modified according to a specific application. For paper coating colors, proteins can give several characteristics to the process and final product, such as, rapid coating immobilization, binder migration control, reduced mottle tendency, high porosity (high void volume), better fiber coverage, and coating holdout, reducing the coating weight needed. Besides, proteins improve the uniformity of the layer, providing better ink holdout (printability), ink gloss, and finally, an improved glueability due to the enhanced porosity for packaging applications [76].

Proteins contain a great variety of functional groups, making this biopolymers very versatile and potentially useful for packaging materials. It is possible to change or modify proteins, chemically, enzymatically, or physically, varying the properties of the materials obtained to adjust to the specific needs of a particular application [5].

Among these advantages, proteins present some drawbacks that make the sustainable option a real challenge if a full replacement of synthetic polymers in the industry is wanted. As a biodegradable material, proteins are susceptible to biological degradation, which increases the cost
of operation for storage and handling, using biocides or lower temperatures. Proteins require preparation, such as previous dissolving and/or cooking for the denaturing process. This is a category of requirements that modified proteins can bypass. Due to the strong interaction with other components of the mixture, protein-based formulations require lower solids, bringing higher demand of energy in the drying process to remove the extra amount of water [76], [3], [6].

Proteins modify the behavior of the fluid. A fluid behavior can be categorized into two groups, Newtonian and non-Newtonian. Newtonian fluids are characterized by constant viscosity over a broad range of stress/shear rate; water, alcohol, and most oils are good examples of these fluids. Non-Newtonian materials exhibit changes in the viscosity of suspensions with changes in stress [73]. The coating fluid has a complex rheological behavior by itself, and the application process occurs at high speeds [74]. Thixotropic flow, characterized by hysteresis loops, is a typical behavior in coating colors [75]. The shear-viscosity and viscoelastic properties, along with the time-dependent behavior, need to be measured and correlated in order to ensure a good runnability of the coating process. The coating formulation or coating color is a suspension that requires a high solids content to minimize the drying requirements and to improve the quality of the coating layer [74].

The stability of the coating suspensions relies on the balance of interactions within the fluid [73]. These interactions can be controlled by adjusting the pH, changing the electrolyte content, varying the amount of dispersant and its interaction with the other components, and using different types of dispersants [73]. Water retention and rheology can be modified with different bio-based alternatives, including soy protein, carboxymethyl cellulose (CMC), nanofibrillated cellulose (NFC), and citrus fibers [77].
The purpose of this work is the characterization of tobacco-derived proteins and their
evaluation as binders in a coating formulation. The study compares tobacco and alfalfa proteins
with soy protein. Tobacco protein could be a substitute for soy protein as a component of coating
formulas. This new application could translate into a new market and opportunity, not only for
tobacco, but also for other RuBisCO-containing plants.

3.4 MATERIALS

Tobacco Derived Proteins (TDP) were purified in the Department of Food, Bioprocessing
and Nutrition Sciences at NC State University, and tobacco leaves were provided by Tobacco Rag
Processors. Two samples were provided, called Tob10k and Tob100k. The soy protein isolate
(PROFAM 974) from ADM (Chicago, IL, USA) had a protein concentration of 93% [78]. The
alfalfa protein (AlfaPro) from Swanson (Fargo, ND, USA), had 50% purity [79].

Styrene-butadiene (SB) latex was used as a synthetic binder; it was a water dispersion with
55% of latex (Rovene 4100) from Mallard Creek (Charlotte, NC USA) [80]. The pigments used in
this work were Kaobrite 90 No. 2 coating clay from the Thiele Kaolin Company (Sandersville,
GA, USA), having 95% of particles less than 2 µm and ground calcium carbonate (GCC) (Omyafill
80) from OMYA (Oftringen, Switzerland). SNF Inc. provided the dispersant (Riceboro, GA,
USA), with the brand name Flosperse [81].

Two kinds of paper were used in this project as a base paper for the coating application.
These were brown Linerboard (LB) (unbleached) and White Board Paper #2 (WB) (bleached).
The properties of the base papers are presented in the results section, Table 21. The properties
reported were measured as explained further in the methods section.
3.5 **METHODS**

In this section, the protein characterization is described first, followed by the coating preparation and fluid testing. Lastly, the coating application on the base paper and the paper testing to the uncoated and coated samples are outlined.

3.5.1 **Protein Characterization and Identification**

3.5.1.1 *Protein content*

The purity of the protein was determined by measuring total Kjeldahl Nitrogen (TKN) by Semi-Automated Colorimetry using a factor of 6.25 in the Environmental Analysis Lab of the Department of Biological and Agricultural Engineering of NC State, following the Standard Methods 4500Norg B or EPA Method 351.2 [82].

3.5.1.2 *Isoelectric Point*

The isoelectric point was measured using an Elecrokinetic Charge Analyzer model ECA 2000 P from Chemtrac (North American Filtration, Inc. Company (Norcross, GA, USA). Changing the pH of the protein solution was done by adding NaOH 1N, RICCA Chemical Company (Arlington, TX, USA), or HCl 1N, Fischer Chemical (Hampton, NH, USA). To measure the total charge of the liquid specimen, 5ml of a protein solution at 2.5% (mass/mass) at the adjusted pH was diluted to 200 ml with DI water for the measurement.

3.5.1.3 *Solubility Curve*

The determination of the solubility of proteins was made by preparing the protein solutions at a concentration of 2.5% (m/m) in DI water, adjusting the pH at 2, 4, 6, 7, 8, and 12. The solutions were centrifuged for 5 min at 3000 rpm on a Centrifuge 5702 from Eppendorf (Hamburg, Germany). The supernatant was used for this test. The preparation of the standard curve and the
samples were followed, as described in the Pierce Gold BCA Protein Assay Kit user guide [83],
performing the test tube procedure. The absorbance was measured at 480nm.

Due to interference from the samples during the absorbance measurements, the precipitated solids of the samples were oven-dried and weighed in order to get an estimation of the percentage (%) of total solid solubilized/precipitated.

3.5.1.4 Protein Molecular Weight

The molecular weight of the proteins was determined using the Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique [84]. This test was performed in The Golden LEAF Biomanufacturing Training and Education Center (BTEC). The detailed protocol (SOP) was provided by the institution [85]. For the protein dilution (2-4 mg/ml), Sodium Phosphate Buffer PBS (50 mM) from Corning (New York, USA) was used. As molecular weight standards, the Bio-Rad Precision Plus Protein Dual Xtra Standards, Cat. #161-0377 (store frozen at -20 °C) (Hercules, CA, USA) were used. The denaturing agent was Laemmli Buffer, Bio-Rad Cat. #161-0737 (store at room temperature). For the reduction of proteins, 2-β-mercaptoethanol (BME), Bio-Rad Cat. #161-0710 (store at room temperature) was employed. The proteins were denatured at 95°C for 10 min. The electrophoresis was performed using the Bio-Rad Mini-
PROTEAN Tetra Cell system. The running gels were Bio-Rad Mini-PROTEAN®TGX Long Shelf Life Precast Gels 12% (10-well, 30 μl load, store at 2-8 °C), the staining agent Bio-Safe Coomassie Blue G250 stain (Bio-Rad Cat. #161-0787, store at room temperature), the running buffer 10X Tris/Glycine/SDS (TGS) (Bio-Rad Cat. #161-0772, store at room temperature), and the gel drying solution (Bio-Rad Cat. #161-0752). The gels were immersed in (diluted) 1X running
buffer (TGS), and the system was run at 200V for 30min. The pictures of the gels were taken with the Bio-Rad Gel Doc XR+ system. [86],[87].

3.5.2 Coating Preparation

The performance of the protein as a binder in the paper coating formulations was assessed by preparing a coating fluid and applying it to a paper surface. The tests made, to both the fluids and the coated paper, provided the information needed to compare the performance of the proteins as binders.

Formulations with each protein were prepared using a control latex (synthetic) as a binder. The formulations were prepared using both types of latex and each protein as a binder and co-binder, respectively, as it is usually employed in the paper industry.

The proteins were dissolved at 7.5-10% concentration in water, using a Caframo Stirrer BCD1850 (Wiarton, Ontario, Canada), at 500 rpm, and heating the mixture to 80°C for 30 min [4]. Once the protein solution was at room temperature, the coating fluid was made. The components needed to be added explicitly in the following order: water, clay, calcium carbonate, the dispersant, SB latex, and the protein solution; defoamer, lubricant, and alkali can be added if needed. The slurry was mixed at 500 rpm while every ingredient was slowly added. After all the components were in the mix, the pH was adjusted to 8.5, and the dispersion was mixed for at least 20 min. This procedure was made to avoid the agglomeration of any component and to ensure a correctly dispersed formulation [4].

The coating formulations are typically prepared using a solids target, and every component is calculated based on the pigments (100 parts) on a dry basis. The actual amount needed was calculated using the concentration on a wet basis, of each ingredient, taking into account the parts
needed. Table 15 shows the formulas prepared based on the 100 parts of pigments and the solids target. Entries in white are fixed, whereas entries in grey are based on different amounts of protein or solid targets.

Table 15. Formulation of coating fluids based on 100 parts of pigments. LatexOnly has 10 parts of Latex as binder. The rest of the formulas has either 2.5 or 5 parts of protein, and the rest is latex (10 parts in total). Two solid targets were tested, 40 and 50 %.

<table>
<thead>
<tr>
<th>Dry parts</th>
<th>LatexOnly</th>
<th>Soy-2.5</th>
<th>Soy-5</th>
<th>Tob10-2.5</th>
<th>Tob10-5</th>
<th>Tob100-2.5</th>
<th>Tob100-5</th>
<th>Alf-2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids %</td>
<td>50</td>
<td>40,50</td>
<td>45*</td>
<td>40,50</td>
<td>50</td>
<td>40,50</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>CaCO3</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Clay</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Dispersant</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>SBR-Latex</td>
<td>10</td>
<td>7.5</td>
<td>5</td>
<td>7.5</td>
<td>5</td>
<td>7.5</td>
<td>5</td>
<td>7.5</td>
</tr>
<tr>
<td>Soy</td>
<td>0</td>
<td>2.5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tobacco 10k</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tobacco100k</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Soy 5 was prepared at 45% solids instead of 50% solids due to dispersion stability and mixing problems.

3.5.3 Fluid Coating Properties

Every formula was tested before coating the paper samples. The pH was measured using a Corning pH-meter with a Mettler Toledo (Columbus, OH, USA) Expert Pro pH Electrode. The density was estimated, weighing a fixed volume of the slurry. The solids content was determined using a Sartorius IR moisture analyzer (Göttingen, Germany). The Water Retention Value (WRV) was determined with an AA-GWR Retention meter (Kaltec Scientific Inc, Novi, MI, USA), following the TAPPI T 701 pm-01 [88]. Low shear viscosity was measured using a Brookfield DVIII Ultra viscometer (Brookfield Engineering Company from AMETEK, Berwyn, PA, USA), using the spindle number 1-5 at room temperature between 50 and 250 rpm. The High Shear Viscometer used was the Hercules Viscometer, model DV-10 (Kaltec Scientific Inc, Novi, MI,
USA) at 5000 rpm, ramping the test for 1 min using bob B similarly to the method T 648 om-14
[89].

3.5.4 Coating Application

The application of the coating formula was performed using Mayer rod number 4-16, depending on the viscosity of the mixture targeting a basis weight of 10 to 20 g/m² on two kinds of paper, Brown Linerboard (LB) and White Board Paper #2 (WB). Calendering was done using the NCSU pilot plant calendering machine (two metal rolls), at 100 °C temperature, and 1000 psi pressure.

3.5.5 Coated Paper Properties

The following tests were made to both the base paper and coated paper. The papers were conditioned according to the TAPPI T 402 sp-08 method [30]. All of the subsequent tests were made under these conditions (23 °C and 50% relative humidity, RH). The basis and coating weight was measured according to the TAPPI T 410 om-08 method [90] with a Mettler Toledo analytical balance (PB303-S). The caliper was measured using the method TAPPI T 411 om-15 [91]. The Gurley method (Air Permeability) is an indirect measurement of the porosity; this test was performed following the T 460 om-02 standard [92]. The roughness of the coated paper was quantified following the TAPPI T 538 om-16 standard (Sheffield method) [93]. The wax pick resistance was measured with the TAPPI T 459 om-13 method [94].

For the optical properties, Gloss at 85° was measured following the ASTM D 523 [95] using a BYK micro-tri-gloss, brightness with the standards ISO 2470 or TAPPI T 525 om-18 [96]. For color, the standard ISO 5631-1 (C/2°) [97] was followed using a Technidyne Color Touch X (New Albany, IN, USA).
A surface electro-optics (SEO) contact angle analyzer (Phoenix 300; Surface Electro Optics Co., Ltd., Suwon City, Gyeonggi-do, Korea) was used to determine the water contact angle and the surface tendency of the coated paper to absorb liquid. The angle was measured for six seconds after the drop touched the surface. The equipment software-defined the fast speed at 47 and the slow speed at 32. An industrial needle with a gauge of 27 was used for all tests.

The glueability test was used to measure the strength of the coating layer. The glue was applied to the coated sample and brought into contact with the back of another sample. The Reynolds Company provided a water-based glue; the product name is 157-20. The glue was applied to the coated samples using a Gardco Microm II film applicator, targeting a film thickness of approx. 0.4 mm. Strips of 2.54 cm wide by 10 cm long were prepared for this test in triplicate, and just half of the strips were covered with the glue. A 13 kg steel roller was passed over the sample twice, backward, and forward. The samples were separated after 2 hours of contact with an Instron 4443 tensile testing machine (Norwood, MA, USA), using the portion of the sample with no glue to grip the strips to the machine. The gap was 5 cm, and the speed of the test was 508 mm/min. The test was considered successful if there was more than a 90% fiber tear after a visual inspection of the samples [98] [99].

Field-Emission Scanning Electron Microscope (FESEM) and Time of Flight Secondary-Ion Mass Spectrometry (TOF-SIMS) were performed on the coated samples in the Analytical Instrumentation Facility (AIF) of NC State University, using an FEI Verios 460L (Field Electron and Ion Company, FEI, Hillsboro, OR, USA) and ION-TOF SIMS 5 (ION TOF, Inc. Chestnut Ridge, NY, USA) respectively. The sample preparation for the SEM cross-section images required cutting the samples submerged in liquid nitrogen.
3.6 RESULTS AND DISCUSSIONS

3.6.1 Protein Characterization

3.6.1.1 Protein Content

The results of the Kjeldahl digestion process of organic nitrogen are shown in Table 16. The purity of the soy and alfalfa protein (91.2 and 52.4% respectively) are consistent with the value reported by the manufacturer, 92.7% for soy, and 50% for alfalfa. The Tob10k protein is the most concentrated between the two tobacco samples with a purity of 66.7%; Tob100k has only 19.6% protein. The purity of the proteins will affect the performance of each coating fluid, given that the amounts of pure protein in the mixture are different.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Total Kjeldahl Nitrogen (ppm)</th>
<th>Protein content (N=6.25) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>145870</td>
<td>91.2</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>83780</td>
<td>52.4</td>
</tr>
<tr>
<td>Tobacco 10K</td>
<td>106670</td>
<td>66.7</td>
</tr>
<tr>
<td>Tobacco 100K</td>
<td>31330</td>
<td>19.6</td>
</tr>
</tbody>
</table>

3.6.1.2 Isoelectric Point

The isoelectric point is the point of lower solubility of the protein, where the net charge of the particles in the solution is equal to zero. This point is dependent on the pH and can provide valuable information about the stability of the protein. Figure 12 shows the dependence of the charge of the solution on the pH. The proteins tested had isoelectric points between pH 2 and 4. This is one of the reasons why alkaline pH is used in coating applications: to ensure proper dispersion of the colloidal structure and adequate interaction with the pigments.
Figure 12. Isoelectric Point of protein solution at different pH. Streaming potential vs. pH is plotted. The point of zero potential occurs in the range of pH 2-4, where the proteins are unstable in solution.

3.6.1.3 Solubility curve (Rapid gold BCA protein Assay)

As it was already mentioned, the stability of polypeptides is highly dependent on pH, and the isoelectric point is an important property to know given that the point of no net charge of the molecule dictates the lowest solubility point. Most proteins have a minimum solubility around pH 4, and high solubility in alkaline conditions [100]. The solubility curve for soy, alfalfa, and both tobacco proteins were built using the BCA kit, and the results can be observed in Figure 13.

In Figure 13a, the soy protein exhibits a curve similar to others reported in the literature [101],[102]. Alfalfa protein offers low solubility even at high pH values. This is an indication that the protein did not go through a lysis process during the extraction and purification step, meaning that the protein is most likely trapped in the cell wall or membrane. This will be discussed further in the Molecular Weight section.

Figure 13b shows the curve for the tobacco proteins. Values over 100% are a clear indication of interference in the colorimetric measurement at 480nm. After several trials with different sample
concentrations, in the range of 20-2000 µg/ml for a reliable measurement, and attempting the standard addition method, it was not possible to obtain reliable data for these two proteins.

![Graphs](image)

**Figure 13.** BCA Solubility (%) vs. pH. (a) Soy protein isolate and Alfalfa protein concentrate. (b) Tobacco 10k and Tobacco 100k. Protein initial concentration of 2.5 % by weight.

For the reason mentioned above, the precipitated solids at different pH values were dried and weighed. It was not possible to quantify the proteins and the impurities distribution; hence, this method only provides an estimation. Figure 14 shows the curves of this estimation. For a high purity protein such as the soy isolate, the curve has values similar to the one made with the BCA method. However, as the purity decreases, the distribution of the protein between the solid and the liquid cannot be determined with this method. The values obtained did not show a minimum at pH 4 for the RuBisCO proteins (alfalfa and tobacco) [103]. The solubility for alfalfa was in the same range as the BCA curve, with 5-20 % solubility. The tobacco proteins showed high dissolution even at low pH, another indication of impurities solubilized along with the polypeptides.
Figure 14. Estimated solid solubility (%) vs. pH. Soy protein isolate, alfalfa protein concentrate, Tobacco 10k, and Tobacco 100k. Protein initial concentration of 2.5 % by weight.

### 3.6.1.4 Protein Molecular Weight (SDS-PAGE)

The Molecular Weight (MW) of the protein has a significant impact on the properties and rheological behavior of fluids and the final characteristics of the coated paper [104]. Three gels were run to determine the molecular weight of the polypeptides of each protein. The preparation setup of gel A and B are presented in Table 17 and gel C in Table 18. Gel A (Soy and Tob10k) and gel B (Tob100k and Alf) were used to determine the proper concentration and to study the difference between reduced and non-reduced conditions. All gels were used for the determination of the size (MW) of each band.
Table 17. SDS-PAGE setup. Gel A, reduced, and non-reduced conditions for Soy protein and Tobacco10 protein. Gel B, reduced and non-reduced conditions for Tobacco100 protein and Alfalfa protein.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample (20 µl)</th>
<th>Sample Buffer</th>
<th>Protein (2mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>R</td>
<td>NR</td>
</tr>
<tr>
<td>1</td>
<td>Soy</td>
<td>L</td>
<td>T10</td>
</tr>
</tbody>
</table>

L = Ladder  
R = Reduced  
NR = Non reduced  
E = Empty well  
A = Reducing buffer, Mercaptoethanol (BME) and Laemmli buffer (1:20)  
B = Non-reducing buffer, Laemmli buffer

Table 18. SDS-PAGE setup Gel C. All proteins, reduced conditions. Protein concentration 4 mg/ml.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Sample Buffer</th>
<th>Protein (4mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>10 µl</td>
<td>20 µl</td>
</tr>
<tr>
<td>1</td>
<td>Soy</td>
<td>Alf</td>
<td>T10</td>
</tr>
</tbody>
</table>

L = Ladder  
R = Reduced  
NR = Non reduced  
E = Empty well  
A = Reducing buffer, Mercaptoethanol (BME) and Laemmli buffer (1:20)

Once the gels were run, stained, de-stained, and imaged with the VersaDoc gel imaging system (See Figure 15), the relative migration (Rf) distance of all bands were calculated per the following equation; The distances were measured using the software ImageJ:

\[
Rf = \frac{\text{Migration distance of protein}}{\text{Migration distance of ion front}}
\]

Equation 1
Using the \( R_f \) of all bands and the known molecular weight of the standard bands (ladder), it was possible to make a regression of the log(MW) vs. \( R_f \), and the results of the regression were used to calculate the MW of each band. The regression was limited to the linear part of the resultant curve, and only this section was useful for the MW calculation [87]. Further identification was possible comparing the bands with the results obtained from other authors for the same proteins, using the bands at reducing conditions. The results of the regression for each gel are shown in Table 19. With each linear equation, the MW of each band was then calculated for each gel. The MW of each detected band is presented in Figure 17.

Table 19. Regression results for SDS-PAGE gels A, B, and C. Linear plots can be found in the Appendix.

<table>
<thead>
<tr>
<th></th>
<th>Gel A (Soy and T10)</th>
<th>Gel B (T100 and Alf)</th>
<th>Gel C (All proteins, only reduced conditions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R^2 )</td>
<td>0.9932</td>
<td>0.9932</td>
<td>0.9948</td>
</tr>
<tr>
<td>y-intercept</td>
<td>2.3555</td>
<td>2.3128</td>
<td>2.2841</td>
</tr>
<tr>
<td>Slope</td>
<td>-1.4394</td>
<td>-1.3915</td>
<td>-1.3707</td>
</tr>
</tbody>
</table>

3.6.1.4.1 Soy protein fractions

Soy protein classification is based on the sedimentation constant. The most abundant fractions of soy protein isolates are 11S and 7S (S stands for Svedberg units) [100]. Fraction 2S
and 15S have also been reported in the smaller amount [105], [106]. The fraction 7S is made mainly of β-conglycinin, and the subunits (individual polypeptide chains) that form this structure are α’ (80 KDa), α (70 KDa), β (48 KDa) [105]. The fraction 11S is called Glycinin and is made of the acidic (A) and basic (B) subunits. The acidic subunits are A3 (40 KDa), A1a, A1b, A2, and A4 (34 KDa), A7, and A6 (32 KDa). The basic subunits are B1a, B1b, B2, B4 and B3 (20-22 KDa) [6], [105], [107], [106]. The 2S fraction has the subunit A5, and low MW polypeptides (8-14 KDa) [105] [108]. Another band commonly found in SPI is the enzyme lipoxygenase (LOX) (94KDa), normally reported as part of the 7S fraction [6], [106].

The bands found in this study are shown in Figure 17; it was possible to identify the major fractions from the soy protein β-conglycinin (7S) and glycinin (11S). The differences between the values reported in the literature and those presented in this study are acceptable for this kind of test. The ladder used, or more importantly, the conditions of the isolation of the protein, have a great influence on the final results [100] [105], [106], [107].

3.6.1.4.2 RuBisCO protein fractions

RuBisCO has a molecular mass of 560 kDa and consists of eight small (10-15 kDa each) and eight large (45-55 kDa each) subunits arranged as eight heterodimers [52]. For the Tobacco10k and Tobacco100k proteins studied herein, the results show two major bands at 45 and 12 kDa, being the major differences in the intensity of the bands due to the purity of the proteins. The results obtained are at the lower range of the numbers expected, according to the literature [100] [105], [106], [107].
In the case of the Alfalfa protein, only one band was detected at 66.1 kDa. This can hardly be considered the RuBisCO large subunit. The solids of these proteins are insoluble in the PBS solution. Under the optical microscope (see Figure 16), it is possible to observe the agglomerations of non-disrupted cells forming for this protein concentrate. Most protein powders undergo a purification process to disrupt the cell walls in order to make the RuBisCO protein useful, especially for the purposes intended in this work.

**Figure 16.** Agglomeration of non-disrupted cells of Alfalfa protein. This explains the low solubility and performance of this protein.
Given that the alfalfa protein is trapped in the cells, low concentrations are expected in the protein solution, reducing the binding properties prominently in the coating formulas.

**Figure 17.** SDS-PAGE results. Band identification and corresponding Molecular Weight of Soy protein Isolate (SPI) and RuBisCO enzyme of Alfalfa and Tobacco proteins. Ladder (Protein Standards). Soy Protein: Lipoygenase (LOX). Fraction 7S, β-conglycinin (subunits α’, α, β). Fraction 11S, Glycinin, acidic (A3, A1a, A1b, A2, A4, A7, and A6) and alkaline (B) subunits (B1a, B1b, B2, B4, and B3). 2S fraction 2S, subunit A5. RuBisCO (Tobacco and Alfalfa): large subunits and small subunits.

### 3.6.2 Fluids characterization

The rheology behavior and the water retention capabilities are critical properties during the coating operations; it will determine the stability of the process and the quality of the coated product.

#### 3.6.2.1 Density

The type of binder (synthetic or natural) does not influence the density of the coating fluids, the densities of all formulas are virtually the same at the same solids target. Reducing the solids target also reduces the density of the fluid as shown between Figure 18a, at 50% solids, and Figure 18b at 40% solids.
3.6.2.2 Water Retention Value

In coating applications, the water holding capacity is an important property that affects the runnability of the process. High values (low water retention) relate to fast dewatering of the slurry, changing the stability of the mixture. Low values (high water retention) can affect the drying process and layer consolidation [77]. The values obtained using the conditions of the TAPPI T 701 standard are shown in Figure 19 for the formulas with 40% solids. The soy protein improves the WRV (lower values), due to its water-holding capabilities. The tobacco proteins, with a different structure and functionality in the plant (enzymatic activity), reveal a poor water-holding (high values).
Protein-water interaction depends on the source, processing, molecular weight or polypeptide chain length, swelling capacities, degree of protein denaturation, and amino acid composition, especially the number of polar groups exposed to water binding among other properties [100]. Table 20 shows the amino acid composition according to different authors [78], [109], [110], [111], the most abundant amino acids (around 60%), and the majority fractions are highlighted in bold. The capacity of the amino acid residues to connect with water, forming hydrogen bonds, is mentioned. Soy protein has more aspartic acid and glutamic acid, which can form hydrogen bonds with water. In contrast, RuBisCO has more hydrophobic amino acids such as glycine, alanine, and valine. These differences can explain why RuBisCO protein from alfalfa or tobacco offers lower water holding capabilities than soy protein.

Figure 19. Water Retention Value (WRV) in g/m² of the coating formulas. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. Tob10-5, using 5 parts of Tob10k protein. Tob100-2.5, using 2.5 parts of Tob100k protein. Tob100-5, using 5 parts of Tob10k protein. Solids target 40%.
Table 20. Amino Acid composition reported in the literature of Soy protein, RuBisCO from Tobacco, and RuBisCO from Alfalfa.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>Asp</td>
<td><strong>11.3</strong></td>
<td>6.2</td>
<td>8.7</td>
<td>High</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>Glu</td>
<td><strong>18.7</strong></td>
<td>7.8</td>
<td>9.4</td>
<td>High</td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>4</td>
<td>9.3</td>
<td>4.7</td>
<td>Low</td>
</tr>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>4</td>
<td><strong>8.9</strong></td>
<td>5.6</td>
<td>Low</td>
</tr>
<tr>
<td>*Valine</td>
<td>*Val</td>
<td>4.7</td>
<td><strong>8.1</strong></td>
<td>6.7</td>
<td>Low</td>
</tr>
<tr>
<td>*Leucine</td>
<td>*Leu</td>
<td><strong>7.5</strong></td>
<td><strong>9.9</strong></td>
<td><strong>9.3</strong></td>
<td>Low</td>
</tr>
<tr>
<td>*Phenylalanine</td>
<td>*Phe</td>
<td>5.2</td>
<td>3.3</td>
<td><strong>7.0</strong></td>
<td>Low</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td><strong>7.5</strong></td>
<td>5.4</td>
<td><strong>9.1</strong></td>
<td>High</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>63</td>
<td>59</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

*Essential AA

Most important fractions of AAs in the protein are indicated in **Bold**

The purity of the protein also affects the water-holding capabilities [100]. Tob100k, with the lowest purity, is the protein that offers the highest WRV, and the soy protein is the one with the best results in this matter.

The Alfalfa-based coating reduces the water-holding of the mixture due to the low concentration of protein and due to the hydrophobic groups present in RuBisCO, as explained in the molecular weight section (3.6.1.4). Replacing the synthetic binder (latex) with a low protein concentration in the mixture results in worse (lower) water holding capabilities. This property is also related to the viscosity, especially at high shear, where the stability of the slurry is critical. Poor water retention can generate dewatering at high shear, changing unpredictably the solid content, and the rheological behavior of the coating color. [77] [112]
3.6.2.3 Low shear (Brookfield) Viscosity

All formulas were adjusted to a pH between 8-9 before any measurement of viscosity. A decrease in the viscosity was observed after the pH adjustment. This behavior was expected, as it is reported in the literature [110].

Soy-5 at 50% solids was not possible to prepare due to the lack of initial water to mix the pigments. Most of the water is part of the protein solution. Increasing the concentration of the protein is not possible because of the gelation of the protein. This issue illustrates a general disadvantage of the protein coating. The solid targets are technically difficult to increase, without special modifications to the protein that allows the mixing of the protein as a powder with no further problems.

The viscosity curves are presented in Figure 20. The formulas at 50% solids using 2.5 parts of the protein are in Figure 20a, and the formulas at 50% solid using 5 parts of the protein are in Figure 20b. In addition, the formulas at 40% solid using 2.5 parts of the protein are in Figure 20c.

The latex-only fluid has the lowest viscosity; this value is virtually constant vs. the shear rate (Newtonian fluid). The protein-based fluids are shear thinning, displaying a reduction in viscosity with the increasing shear rate. Among these formulations, soy protein produces the highest viscosity. This is expected given that the soy protein isolate has the highest purity and molecular weight, producing a high viscosity slurry (Figure 20a).

Using proteins as a co-binder not only increases the viscosity of the fluid, but it also changes the rheological behavior of the mixture. More protein further increased the viscosity; however, at 5 parts, Tob100 produces a more viscous fluid (Figure 20b). The solid target is also determinant for the rheology behavior. Reducing the solid target (Figure 20c) for a fixed amount
of protein will change the fluid from shear-thinning to (near) Newtonian type, with a curve similar to the fluid without protein (Latex-only). At 40% solids, only SPI was able to produce a shear-thinning fluid, with a viscosity drop with the increasing shear rate (rpm).
Figure 20. Low shear (Brookfield) Viscosity for coating formulas. (a) Formulas at 50% solids. LatexOnly/50, no protein. SoyADM-2.5/50, using 2.5 parts of soy protein. Tob10-2.5/50, using 2.5 parts of Tob10k protein. (b) Formulas at 45 or 50% solids. LatexOnly/50, no protein. SoyADM-5/45, using 5 parts of soy protein. Tob10-5/50, using 5 parts of Tob10k protein. (c) Formulas at 40% solids. SoyADM-2.5/40, using 2.5 parts of soy protein. Tob10-2.5/40, using 2.5 parts of Tob10k protein. Alf-2.5/40, using 2.5 parts of Alf protein.

(a) Coating colors protein 2.5 parts 50% solids

(b) Coating colors protein 5 parts 50% solids

(c) Coating colors protein 2.5 parts 40% solids
To better understand the behavior of these coating fluids, the high shear viscosity analysis was carried out.

### 3.6.2.4 High Shear (Hercules) Viscosity

The complex structure generated by the interaction protein-latex-pigment-water determines the rheology of the fluid. The performance of the fluid in a coating operation depends on how strong or stable the structure is and how this interaction reacts to the changing shear rate. Therefore, single-point measurements are insufficient to describe the flow characteristics of these mixtures if one wants to understand the rheology of the fluids and correlate them with coating operations.

The rheograms (RPM vs. torque) are positioned to the left (a) of Figure 21, and the viscosity curves to the right (b) of the same figure. Figure 21-1a and 21-1b are the formulas with 2.5 parts of protein with 50% solids. Figure 21-2a and 21-2b are the formulas with 5 parts of protein with 50% solids. Figure 21-3a and 21-3b are the formulas with 2.5 parts of protein at 40% solids.

The proteins change the rheological behavior of the coating fluids dramatically, primarily because of the decrease in the volumetric concentration of solids [113]. The latex-only fluid exhibits a slightly shear thickening behavior [114], where the viscosity increases with the shear rate. At 50% solids, the protein-based fluids exhibit a time-depending or thixotropic behavior.

In the rheograms, the main characteristic of this kind of fluid is the hysteresis in the cycle; the slurry reacts differently, increasing the shear rate than reducing the shear rate, producing a thixotropic loop instead of a single curve. With thixotropic fluids, the internal microstructure breaks during the up curve, and it reforms during the downward curve [113]. Depending on what process happens faster, the breakdown, or the built-up of microstructures, the fluid can suffer shear thinning or shear thickening (dilatancy) [114].
With the degree of thixotropy change between the fluids, at 50% solids, and using 2.5 parts of the protein (Figure 21-1a and 21-1b), the soy isolate produces the highest viscosity and a greater area on the hysteresis loop. This indicates a stronger entanglement of the pigments and proteins that takes more energy to break under shear action.

At 50% solids and 5 parts of the protein (Figure 21-2a and 21-1b), the fluids behave as plastic-dilatant thixotropic, and a certain amount of stress is needed before developing flow. At stresses smaller than that, the systems behave like elastic solids. Plastic flow behavior (Bingham) is due to strong particle-particle interaction and association due to secondary forces that must be destroyed before flow can begin (yield stress) [113], [114].

Another characteristic is the dilatancy, increasing the viscosity at high shear rates instead of reaching a minimum value. In the rheogram (Figure 21- a), the thixotropy area decreases, and the fluid behaves the same no matter whether the shear rate increases or decreases, similar to the shear-thickening behavior. The change of rheological regime at high shear has been reported in cement mixtures, aqueous bentonite gels, bulged complex hysteresis loops, and heavy crude oil with nanoparticles [115], [116], [117]. This information indicates that 5 parts of protein increase the thixotropic response noticeably, and without a substantial improvement on the paper properties of the coated paper, this formulation should be avoided.

Dilatancy could be observed to some degree in all the samples; in terms of the coating operation, this should be avoided because it may cause several problems on the runnability, including blade scratching, lines running along the machine direction, or deposits of coating material on the coating equipment. Also, they require greater pumping capabilities and transportation [113],[76]. Dilatant fluids are not always a problem; they could be advantageous in the sense that they form more
stable laminar layers before metering. Nonetheless, the issue can be solved by changing the formula, varying the ratio of the pigments or ratio binder and co-binder, as well as adjusting the solid target.

Some of the samples coated with the 50% solids formulas had lines or “rod marks.” The fluid properties explained above are related to this issue, and it was decided to change the solids target. The coating colors with 2.5 parts were also prepared at 40% solids (Figure 21-3a and 21-3b). These conditions allowed study of the impact on the rheology, particularly to remove the line formation on some of the coating samples.

At lower concentrations, the rheology of the suspensions is primarily dictated by the particle-liquid interfacial interactions. The macromolecules are solubilized, and the formation of gel-like networks is less pronounced. This allows more mobility to the particles; therefore, the thixotropy loops are significantly mitigated. With these formulas, no rod marks were observed. However, some degree of dilatancy was still present (Figure 21-3b).

So far, two key features of coating fluids have been discussed, the WRV and the rheological behavior of the fluid. Adding protein as co-binder to the formula greatly altered both. Soy and RuBisCO gave rise to a shear-thinning fluid (with soy protein producing higher viscosities), which is often an advantage during the metering process. However, adding protein as a co-binder requires formulas with lower solids contents, particularly due to the water needed for the protein dissolution.

Tobacco proteins negatively affected the water retention, a disadvantage comparing with soy protein that could affect the stability of the fluids, and the final layer consolidation process.
Figure 21. High shear (Hercules) viscosity. (a) RPM vs. Torque. (b) Apparent viscosity vs. Shear rate. (1) Formulas at 50% solids. LatexOnly, no protein. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. (2) Formulas at 45 or 50% solids. LatexOnly, no protein. SoyADM-5/45, using 5 parts of soy protein. Tob10-5, using 5 parts of Tob10k protein. (3) Formulas at 40% solids. SoyADM-2.5, using 2.5 parts of soy protein. Alf-2.5/40, using 2.5 parts of Alfalfa protein. Tob10-2.5, using 2.5 parts of Tob10k protein.
3.6.3 Paper Testing

The coating application using a Mayer rod showed consistency in the coating weight regardless of the formula used. The differences in density, viscosity, and rheological behavior showed little to no impact on the coating weight, compared to the rod number used and the base paper used. For the study herein, a coating weight range between 10 and 20 gsm was reached by adjusting the rod number.

All the properties of interest for this study were measured first on the uncoated base-paper. The properties are summarized in Table 21. The supplemental information contains color images of the coated and uncoated board.

Table 21. Base paper Properties. All properties were measured at standard conditions of 23 °C and 50% relative humidity (RH), according to TAPPI T 402. Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Brown LinerBoard (LB)</th>
<th>WhiteBoard #2 (WB#2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basis Weight (g/m^2)</strong></td>
<td>130.9±1.7</td>
<td>271.7±2.5</td>
</tr>
<tr>
<td><strong>Caliper (µm)</strong></td>
<td>178.8±3</td>
<td>330.5±2</td>
</tr>
<tr>
<td><strong>Apparent Density (kg/m^3)</strong></td>
<td>732.2±14.3</td>
<td>822.1±8.9</td>
</tr>
<tr>
<td><strong>Roughness (Sheffield units)</strong></td>
<td>405.3±6.5</td>
<td>164.8±9.3</td>
</tr>
<tr>
<td><strong>Porosity (Gurley) (sec/100ml)</strong></td>
<td>32.7±2.3</td>
<td>31.6±3.3</td>
</tr>
<tr>
<td><strong>Gloss (85°)</strong></td>
<td>2.3±0</td>
<td>10.7±0</td>
</tr>
<tr>
<td><strong>Color (L, a, b)</strong></td>
<td>61.6±0.08,4.52±0.03,20.13±0.08</td>
<td>94.4±0.13,-1.05±0.03,4.24±0.24</td>
</tr>
<tr>
<td><strong>Brightness</strong></td>
<td>17±0.1</td>
<td>82.5±0.3</td>
</tr>
<tr>
<td><strong>WaxPick</strong></td>
<td>23.0</td>
<td>23.0</td>
</tr>
</tbody>
</table>

3.6.3.1 Coating Properties

The graphs in Figure 22 and Figure 23 show the caliper, apparent density, roughness, and porosity of the coated paper. In Figure 22, the paper coated with the 50% solids formulas for un-calendered and calendered samples are presented. Similarly, Figure 23 shows the paper coated
with 40% solids formulas, for un-calendered and calendered samples. In each graph, the apparent density scale is to the right side; the rest of the values can be read on the left scale, using the corresponding units. The graphics to the left (a) present the values for the linerboard paper; similarly, the graphics to the right (b) show the characteristics for the whiteboard.

The caliper was practically independent of the formulation, given that the coated weight target was fixed. For a similar coated weight, there was a difference in the caliper of the coating layer between base-papers. In linerboard was around 9 µm and in whiteboard around 15 µm as an average. This can be explained due to the roughness of the linerboard, since more coating material is needed to cover the irregularities of the base-paper.

There is not a clear trend on the influence of the proteins in the roughness measurements. This property remains similar in linerboard (50% solids) with Latex-Only (LO) and Soy-5 formulation (Figure 22). However, it is reduced by 37% with the Soy-2.5 formulation. The tobacco formulation also decreases this property by a maximum of 16.5%. In comparison, the roughness of the whiteboard is lower, and among the coated samples, the roughness has similar values. Soy-2.5 and Tob100-5 achieved the lowest values, 50 and 21% lower, respectively.

As expected, the values of Gurley porosity (s/100ml) increase significantly (less porosity) in coated samples. A fair comparison can be made between the coating formulas with and without proteins. Adding protein to the coating formula produces a more porous consolidated layer [76]. For linerboard, there was a porosity increase (lower times) for all the formulations except Tob100-2.5. In the case of the whiteboard, the porosity varied between 168 and 245 s/100ml, with Tob100-5, being the more porous and Tob10-2.5 the less. The properties of the base paper had important
influence on the final porosity of the coated paper. In general, coated linerboard samples had higher porosity (Gurley seconds < 150 s/100ml) than the coated whiteboard samples.

The results in Figure 23 show that reducing the solid target from 50 to 40% also had a big impact on the coated paper. For both linerboard and whiteboard, the porosity increased (lower times) up to 60 and 30%, respectively. An increase in the void volume, as consequence of the higher porosity, it was also reflected with a thicker coating layer and lower apparent density.

![Graph](image-url)

(1a) Coated Linerboard 50% solids formulas

(1b) Coated Whiteboard 50% solids formulas
Figure 22. Paper properties of coated samples with 50% solids formulas. Apparent density (kg/m^3), Caliper (µm), Porosity (sec/100ml). Roughness (Sheffield units). Apparent density is read on the right scale. Each property should be expressed with the corresponding unit in the legend.
Calendering compresses the coated paper, increasing the apparent density and reducing the caliper. This process reduces the irregularities of the surface, making it smoother (lower roughness). At the same time, the porosity significantly decreases, increasing the time needed to complete the test. This effect is greater on linerboard than on whiteboard. The reduction of porosity is more intense on the linerboard coated only with latex.

The results indicate that tobacco proteins can offer similar features as soy proteins in terms of density, bulkiness, porosity, and roughness without sacrificing significantly the characteristic properties offered by latex formulations. The use of proteins increased the porosity of the coated layer compared with the synthetic binder. This can be especially useful for packaging applications, facilitating the gluing of the paper surface.
3.6.3.2 Wax Pick

The wax pick test is used to measure the surface strength of paper, where a higher number of wax pick means stronger surface. When the wax is stronger (higher wax number), it will disturb the surface of the sample, picking fibers or coating, or the occurrence of blisters, breaks or lifts, Figure 24. According to this test, the linerboard and the white board had the same surface strength; both failed at wax number 23 (see Table 22). The values with a dash in Table 22 mean that surface of the sample failed with the weakest wax (number 2). The coated layer was not as strong as the paper in those cases. This was demonstrated with the reduction of the wax number in all the coated samples. Whiteboard offers better bonding capacities than the linerboard, meaning that the coating layer binds stronger to the white paper. One reason could be the amount of lignin in the unbleached paper (LB), reducing the amount of hydrogen bonding that can be formed with the coating layer. In summary, there was a reduction in strength in the Z direction of all coated LB samples.
Comparing the synthetic formula with the protein formulas, the formulas composed entirely with latex, made a stronger coating layer. This was consistent with expectations. The presence of protein increases the disorder of the matrix, leading to an increase in the void spaces between the particles, making less intimate contact among the particles. The result is a weaker, more porous consolidated coating layer, incapable of withstanding the forces of the stronger wax.

When comparing the different protein formula ions, the soy formulations yielded a stronger paper, using 5 parts for both papers. The opposite occurred with the tobacco coatings, where the layers with lower protein content yielded a stronger surface. This could be due to the presence of impurities, which disrupt the formation of a continuous and strong layer. Increasing the amount of impure protein also could enhance this effect. Another explanation could be that soy, with a higher affinity for water, gives rise to greater moisture content of the layer, losing mechanical strength. This result has been observed in other work using citrus fiber as water retention and rheology modifier [77].
Table 22. Wax pick number. Higher the number, stronger the wax needed to break the surface of the sample. The coating is not as strong as the base paper. Dash indicates that paper fails with wax 2.

<table>
<thead>
<tr>
<th>Coating ID</th>
<th>LB</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>LatexOnly</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>SoyADM-2.5</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>SoyADM-5</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Tob10-2.5</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Tob10-5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tob100-2.5</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Tob100-5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3.6.3.3 Glueability

This test is intended to demonstrate that proteins improve the glueability of the coated paper. The glued system fails as a function of the weakest link. If the glued system fails by the glue seam or in the pigment coating, the tested is considered failed. For this reason, the fiber tear percentage is measured; the paper should be the weakest point of the system. In that case, the glue and the coating layer are strong enough for the intended application. A visual inspection is the most practical way to determine the success of the test. The results are summarized in Table 23.
Table 23. Glueability test for coated Linerboard (LB) and Whiteboard (WB). Glue, coating, and fiber fail percentage (%) after 3 tests. Visual inspection. Highest fiber failure is intended for a glue/coating/paper system.

<table>
<thead>
<tr>
<th>Coating ID</th>
<th>Linerboard (LB)</th>
<th>Whiteboard (WB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROD/Coating Weight (gsm)</td>
<td>Tear/Fail %</td>
</tr>
<tr>
<td></td>
<td>Glue</td>
<td>Coating</td>
</tr>
<tr>
<td>LatexOnly</td>
<td>8/16.3</td>
<td>13</td>
</tr>
<tr>
<td>SoyADM-2.5</td>
<td>8/16.8</td>
<td>27</td>
</tr>
<tr>
<td>SoyADM-5</td>
<td>8/15.1</td>
<td>48</td>
</tr>
<tr>
<td>Tob10-2.5</td>
<td>12/20.3</td>
<td>62</td>
</tr>
<tr>
<td>Tob10-5</td>
<td>8/19.5</td>
<td>10</td>
</tr>
<tr>
<td>Tob100-2.5</td>
<td>8/19.6</td>
<td>3</td>
</tr>
<tr>
<td>Tob100-5</td>
<td>8/20.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Once again, the base paper makes a difference in the behavior of the system. The same formulations performed better in the WB, resulting in a lower percentage of failure of the coating layer and higher fiber tear percentage. Comparing the fiber tear percentage of the same paper, the protein-based formulations offer an improvement. The coating formula without protein had lower performance in terms of glueability, with low fiber tear and glue failing in the WB, and coating failing in the LB.

Better glueability is an important characteristic of protein coatings, due to the increased porosity and the affinity of protein to water. The glue can penetrate properly through the coating layer and bind the entire system together. The objective of the glued system is to force the failing of the fibers first when separating the glued layers [118]. Table 23 shows an increasing fiber tear percentage with the protein formulas in comparison with the formula without proteins, meaning that the glueability has been enhanced.

In the wax pick section it was demonstrated that the soy-containing surfaces were stronger than RuBisCO coated surfaces. Results of the glueability test support the wax pick results; more failures were observed on samples coated with RuBisCO than soy.
The Tob100-2.5 formula achieved a 100% fiber tear, meaning a satisfactory performance of the system. It was surprisingly better than the other combinations of tobacco proteins and concentrations. The low concentration of protein exposes more pigments to the surface, as shown in the images of ToF-SIMS (shown later in section 3.5.3.6) (Figure 35). In addition, a weaker matrix, as explained on the wax pick section, and more exposed surface could be causing this behavior.

Good glueability requires a strong paperboard, with good surface strength. Coating composition and glue selections are key factors to perform well. This test is semi-quantitative at best and exhibits great variability; however, it offers a good indication of the differences of the coating system using different proteins [99].

3.6.3.4 Water Contact Angle

The water contact angle is an indication of the relative hydrophobicity of the surface, and it is related to the porosity and the roughness of the coated layer. Figure 25 displays the water contact angle as a function of time for the coated LB and WB with the formulas of 40% solids after calendaring, compared with calendered uncoated paper.

The water contact angle decreases in comparison to the calendered uncoated paper, angles greater than 90° are generally related to hydrophobic surfaces. The chemistry, roughness, and porosity of the material have big impacts on the solid-liquid interaction [42]. The calender process compresses the paper and drastically reduces the porosity, partially sealing the surface. Calendering produces a smoother surface and increasing the smoothness. Thus, the difference between uncoated LB and WB is due to the presence of lignin on the unbleached paper (LB)
(Figure 25). The lower affinity of LB with water generated larger water contact angles; in this case, there was about 10° difference.

For the coated samples (calendered), LatexOnly and Soy formulation offered similar water contact angles, between 60-70° for both papers. On the other hand, RuBisCO-based formulas, Alf-2.5/40 and Tob100k-2.5/40, produced the lowest water contact angles (more hydrophilicity).

In general, the coating layer fills the surface with the hydrophilic pigments and biopolymers, resulting in an increased affinity of the paper to water, decreasing the water contact angle [119]. For the coated samples (calendered), LatexOnly and Soy formulation offered similar water contact angles between 60-70° for both papers; and RuBisCO-based formulas, Alf-2.5/40 and Tob100k-2.5/40, produced the lowest water contact angles.

**Figure 25.** Water Contact angle for the calendered coated paper, using 40% solids formulas in comparison with calendered base paper (uncoated). Soy and latex are overlapping in Linerboard

Soy protein, as a non-thermoplastic material, forms a sealing layer that does not allow the pigments to be present on the surface (see ToF SIMS Section, Figure 35) [76]. On the other hand,
it seems the RuBisCO protein does not limit the migration of the hydrophilic pigments to the surface, increasing the hydrophilicity of the surface (see ToF Section, Figure 35). The calendering process compresses the paper, and drastically reduce the porosity, partially sealing the surface and increasing the smoothness. Soy protein, as a non-thermoplastic material, controls the migration of latex to the coated surface [76]. The migration process can be altered with the presence of a natural binder, and it can have significant effects on the final layer characteristics [120] [121].

To summarize, soy protein did not affect the water contact angle in comparison to latex; on the other hand, RuBisCO protein decreased the water contact angle. Overall, the coating layers provided more hydrophilicity to the surface, in a combined effect of each component chemical affinity and the overall surface characteristics [42].

3.6.3.5 Optical properties

3.6.3.5.1 Color

The color of the samples can be expressed in the CIELAB color space with the ($L^*$, $a^*$, $b^*$) values. All the formulas offered a slightly different color. The latex only (LO) and soy formulas were the closest to the white color (100,0,0). The Tob10k formulations generated a yellow/brown paper, and the Tob100k formulations produced a red/brown paper. The lightness in all the samples was higher in the whiteboard than the linerboard.

Table 24 and 25 show the ($L^*$, $a^*$, $b^*$) values of linerboard and whiteboard with the formulations at 50% solids before and after calendering. The calendering process did not affect the overall color of the samples.
Table 24. CIELAB color space. (L, a, b) values of coated paper, Linerboard (LB), and Whitepaper (WB) as base paper. All formulas. Formulas at 50% solids: LatexOnly, no protein. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. SoyADM-5, using 5 parts of soy protein. Tob10-5, using 5 parts of Tob10k protein. Formulas at 40% solids. SoyADM-2.5/40, using 2.5 parts of soy protein. Alf-2.5/40, using 2.5 parts of Alfalfa protein. Tob10-2.5/40, using 2.5 parts of Tob10k protein. Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Linerboard (LB)</th>
<th>Whiteboard (WB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>Uncoated</td>
<td>61.6±0.08</td>
<td>4.52±0.03</td>
</tr>
<tr>
<td>LatexOnly</td>
<td>85.8±0.15</td>
<td>0.2±0.03</td>
</tr>
<tr>
<td>SoyADM-2.5</td>
<td>87.26±0.14</td>
<td>0.03±0.02</td>
</tr>
<tr>
<td>SoyADM-5</td>
<td>83.22±0.28</td>
<td>0.78±0.04</td>
</tr>
<tr>
<td>Tob10-2.5</td>
<td>82.59±0.78</td>
<td>-0.78±0.53</td>
</tr>
<tr>
<td>Tob10-5</td>
<td>81.05±0.18</td>
<td>-0.8±0.13</td>
</tr>
<tr>
<td>Tob100-2.5</td>
<td>84.85±1.33</td>
<td>1.74±0.16</td>
</tr>
<tr>
<td>Tob100-5</td>
<td>83.86±0.18</td>
<td>1.35±0.25</td>
</tr>
<tr>
<td>SoyADM-2.5/40</td>
<td>84.45±0.79</td>
<td>0.75±0.33</td>
</tr>
<tr>
<td>Alf-2.5/40</td>
<td>81.8±1.5</td>
<td>0.9±0.31</td>
</tr>
<tr>
<td>Tob10-2.5/40</td>
<td>78.01±0.68</td>
<td>0.79±0.36</td>
</tr>
<tr>
<td>Tob100-2.5/40</td>
<td>90.82±0.31</td>
<td>-0.25±0.07</td>
</tr>
</tbody>
</table>
Table 25. CIELAB color space. (L, a, b) values of coated paper after calendered, Linerboard (LB), and Whitepaper (WB) as base paper. All formulas. Formulas at 50% solids: LatexOnly, no protein. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. SoyADM-5, using 5 parts of soy protein. Tob10-5, using 5 parts of Tob10k protein. Formulas at 40% solids. SoyADM-2.5, using 2.5 parts of soy protein. Alf-2.5/40, using 2.5 parts of Alfalfa protein. Tob10-2.5, using 2.5 parts of Tob10k protein. Mean value ± Standard deviation.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Calendered Linerboard</th>
<th>Calendered Whiteboard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>Uncoated</td>
<td>61.6±0.08</td>
<td>4.52±0.03</td>
</tr>
<tr>
<td>LatexOnly</td>
<td>82.3±0</td>
<td>0.42±0</td>
</tr>
<tr>
<td>SoyADM-2.5</td>
<td>85.51±0</td>
<td>-0.05±0</td>
</tr>
<tr>
<td>SoyADM-5</td>
<td>77.89±0</td>
<td>1.36±0</td>
</tr>
<tr>
<td>Tob10-2.5</td>
<td>79.4±1.5</td>
<td>0.9±0.31</td>
</tr>
<tr>
<td>Tob100-2.5</td>
<td>80.01±0.68</td>
<td>0.79±0.36</td>
</tr>
<tr>
<td>Tob100-5</td>
<td>81.88±1.76</td>
<td>0.81±0.3</td>
</tr>
<tr>
<td>SoyADM-2.5/40</td>
<td>79.4±1.5</td>
<td>0.9±0.31</td>
</tr>
<tr>
<td>Alf-2.5/40</td>
<td>78.01±0.68</td>
<td>0.79±0.36</td>
</tr>
<tr>
<td>Tob10-2.5/40</td>
<td>80.82±0.31</td>
<td>-0.25±0.07</td>
</tr>
</tbody>
</table>

Figure 26 shows the (L, a, b) values of linerboard and whiteboard with the formulations at 50% solids. The values are expressed in a two-dimensional space to provide a better idea of the differences in color of the coating layer of the color obtained. Plots corresponding to other formulations can be found in the Appendix.
Figure 26. CIELAB color space. (L, a, b) values of coated paper with 50% solids formulas. LatexOnly, no protein. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. Tob10-5, using 5 parts of Tob10k protein. Tob100-2.5, using 2.5 parts of Tob100k protein. Tob100-5, using 5 parts of Tob10k protein.
To determine how noticeable the difference in colors can be, the Delta E values were calculated using the LatexOnly formula as a reference; using the simplest version of the Delta E formula (Equation 2). The results are plotted in Figure 27. Values greater than 2 indicate that the difference in color is perceptible at a glance [122]. The calendaring process seems to reduce the difference in the whiteboard of the formulas at 50% solids. Values between 1 and 2 mean that the difference is perceptible through close observation. Only Soy-2.5 and Alf-2.5 yielded values under 2, and in some cases, under 1, where there is no perceptible difference in color for human eyes.

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Equation 2

Figure 27. Delta E values of coated paper, Linerboard (LB), and Whitepaper (WB) as base paper (a) Formulas at 50% solids formulas. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. Tob10-5, using 5 parts of Tob10k protein. Tob100-2.5, using 2.5 parts of Tob100k protein. Tob100-5, using 5 parts of Tob10k protein. (b) Formulas at 40% solids. SoyADM-2.5/40, using 2.5 parts of soy protein. Alf-2.5/40, using 2.5 parts of Alfalfa protein. Tob10-2.5/40, using 2.5 parts of Tob10k protein.
3.6.3.5.2 Brightness and Gloss

Figure 28 summarizes the results for Brightness and Gloss before and after calendaring for all the formulas at 40 and 50% solids. Each value must be read in brightness or gloss units corresponding to the same scale. The gloss of the coated sheet exhibited greater increase after calendering. The brightness, on the other hand, did not change much with calendaring.

The samples coated with Tob10k had the lowest brightness. LatexOnly and the soy-containing formulas produced similar numbers, and the tobacco formulations offered a more opaque and dark appearance.
Figure 28. Brightness and Gloss of coated paper samples. Linerboard (LB) and Whitepaper (WB) as base paper. (1a, 1b) Formulas at 50% solids formulas. SoyADM-2.5, using 2.5 parts of soy protein. Tob10-2.5, using 2.5 parts of Tob10k protein. Tob10-5, using 5 parts of Tob10k protein. Tob100-2.5, using 2.5 parts of Tob100k protein. Tob100-5, using 5 parts of Tob10k protein. (2a, 2b) Formulas at 40% solids. SoyADM-2.5/40, using 2.5 parts of soy protein. Alf-2.5/40, using 2.5 parts of Alfalfa protein. Tob10-2.5/40, using 2.5 parts of Tob10k protein.

The coating solids showed no direct effect on these two properties. In all cases, the values remained between 45 and 85 brightness units.
To summarize, the protein used affected the optical properties of the coated paper, particularly the color of the samples. The tobacco-based coatings were obviously (following the Delta E criteria) more yellow and brown. Calendering had a big impact on gloss, with little to no impact on color and brightness.

3.6.3.6 Coating components distribution and coverage

3.6.3.6.1 Scanning Electron Microscope (SEM)

SEM and TOF SIMS were used to understand the distribution of the components of the coating layer. The micrographs of the coated layer with each formulation at low (1000x) and high magnification (10000-15000x) are presented in this section. Figure 29 shows the influence of calendering on the surface, and Figure 30 and Figure 31 illustrate the effect of the proteins and their content on the surface morphology. Also, Figure 32 and Figure 33 display the cross-section (500x) of the coated papers.

The SEM images show how the pigments were distributed across the substrate evenly, fully covering the fibers. The influence of calendaring in the decrease of porosity and roughness, it is clearly supported by the SEM micrographs shown in Figure 29 for the latex formulations. The compression of the surface by the calendaring rolls created a smooth and packed layer that makes difficult the diffusion of air through the paper. This behavior was similar in the other formulations.

Overall, the SEM micrographs support the previous observations about the increase in porosity due to the presence of the proteins in comparison to the papers coated only with latex (surface: Figure 29 vs. Figure 30 (soy) and Figure 31 (tobacco), and cross-section: (Figure 32 and Figure 33). Nevertheless, the roughness did not seem to be affected. As previously mentioned, there was no specific trend in terms of rugosity due to the addition of proteins and, despite the
increase in porosity, the values were similar among samples coated with fluids containing proteins. These images demonstrate that the tobacco protein can bind pigments between the coating formula and the paper in the same way that the soy protein can.

In terms of protein content (Figure 30), no noticeable differences were observed when increasing the protein content from 2.5% to 5%, supporting the preceding findings in porosity.
Figure 29. Verios SEM micrographs of the coated samples for LatexOnly formulas: uncalendered (left) and calendered (right).
Figure 30. Verios SEM micrographs of the coated samples (un-calendered) for SoyADM – 2.5 (left) and SoyADM – 5 (right).
Figure 31. Verios SEM micrographs of the coated samples (un-calendered) for Tob10k (left) and Tob100k (right).
Figure 32. Verios SEM cross-section micrographs of the coated samples with the different formulas (un-calendered). Linerboard
Figure 33. Verios SEM cross-section micrographs of the coated samples with the different formulas (un-calendered). Whiteboard
3.6.3.6.2 Time-of-Flight Secondary Ion Mass Spectrometry (ToF SIMS)

ToF SIMS spectra are shown in Figure 34; it was possible to identify peaks corresponding to the polypeptide chains of the proteins; these peaks were not present in the latex control. The pigments showed characteristic negative (SiO$_3^-$, SiO$_3$H$^-$, Si$_3$CH$_3$O$_5^-$, Al$_2$O$_4$H$^-$) and positive (C$_8$H$_9^+$, Ca$_3$O$_3$H$_{10}^+$, Ca$_4$O$_4$H$_{15}^+$) ions on the spectra. The latex in the formulations was observed as C$_7$H$_7^+$ and C$_8$H$_9^+$, hydrocarbon fragments. Finally, ion fractions of the amino acids on the proteins were also present: negative ions (CNO$^-$, CN$^-$, C$_3$N$^-$) and positive ions (CH$_4$N$^+$, C$_4$H$_8$N$^+$).

Figure 35 shows the distribution of the components on the surface of the coated paper. The pigments were distributed evenly throughout the surface, covered with the latex (control). In the high-resolution images of the samples coated with proteins, the CN groups can be observed on top of the rest of the components, where the green color in the spectra represents the protein chains. Among the proteins, Tob100k was the the least detected on the surface; nonetheless, it provided full coverage and even distribution along with pigments and the synthetic latex. As was already mentioned, the coverage of the surface by the different components of the coating formulation had an effect on the water contact angle (hydrophilicity).

As discussed previously, tobacco proteins have lower purity, which might affect the coverage of the protein, and it might explain why the tobacco-coated papers had a more brittle and hydrophilic surface. The lower MW of the chains and smaller structural matrix of the RuBisCO protein may also affect these results.
Figure 34. ToF SIMS Spectra of coated paper. (a) Negative spectra (top) and (b) Positive Spectra (bottom).
**Negative Ions** Red - SiO₃⁻, SiO₃H⁻, Si₃CH₃O⁻, Al₃O₄H⁻ Green - CNO⁻, CN⁻, C₃N⁻

**Positive Ions** Red - C₇H₇⁺, C₈H₉⁺, Ca₃O₃H⁺, Ca₄O₄H⁺ Green - CH₄N⁺, C₄H₈N⁺

**Figure 35.** ToF SIMS High Resolution Images. Negative (top) and positive (bottom) ions spectra.
3.7 CONCLUSIONS

This work shows evidence that the RuBisCO enzyme, present in all photosynthetic leaves of plants, can have a potential application in the coating industry as a co-binder, allowing the reduction of synthetic polymers as part of the efforts to improve the sustainability of the coating industry. The natural difference between the soy protein, a storage protein, and the RuBisCO protein, an enzyme (bio-catalytic action), had a major impact on critical properties such as water retention.

The soy protein has more hydrophilic amino acids than RuBisCO, which was found to provide a coating prepared with the tobacco protein less water-binding. However, other properties are dependent on the quality of the concentrated proteins (purity). An example is the color given to the coating paper by the tobacco proteins. Impurities in the extract can alter the color of the fluids and the stability of the slurry. In the case of the RuBisCO from alfalfa, impurities as part of the cell walls of the plants may discourage the usage of the protein in the intended application.

It is essential to mention that the soy protein employed was a highly purified product, intended to be used in the food industry. On the other hand, the tobacco protein samples tested in this project did not have high purity. Nonetheless, most of the tests done demonstrated that the tobacco protein could offer the critical properties of a pigment co-binder, changing the rheology of the slurry, providing better metering and application, a porous consolidated layer for improved gluing and printability, and even distribution of pigments and adequate structural strength. In some cases, the coating layer offered virtually the same properties (roughness and porosity) using soy or tobacco.
An optimization of the RuBisCO isolation process from tobacco leaves as feedstock could provide a high purity and high-quality protein isolate that would offer properties similar to the current technologies, allowing the introduction of such products to the packaging industry.
CHAPTER 4. SUMMARY AND FUTURE DIRECTIONS

4.1 SUMMARY OF PAPER STRAWS CHAPTER 2

Plastic waste reduction is a need that the society is demanding. In particular, plastic straws are at the center of discussion, given the large amount of straws used globally and how harmful to the environment they can be. The paper-based version of this common item has returned to the market. However, in terms of durability, paper straws have a lack of performance, given that once in contact with a beverage, they rapidly lose physical integrity and mechanical strength.

The objective of this chapter was to cover the lack of a formal procedure to test paper straws in comparison with plastic straws. In this way, a methodology was developed to test paper straws under controlled conditions, simulating typical use conditions. With this new methodology, the loss of mechanical strength was determined. Tracking the time-dependent weight gain and compressive strength of the straws under immersion in liquids, the type of fibers of the straws were analyzed to characterize the material. To determine the interaction with liquids, the surface characteristics, and the release of the solid during long immersion times were also calculated. This was done by measuring the water contact angle and turbidity, respectively.

Results of the study indicated that the wetted paper straws gained weight, absorbing water along with a rapid loss in mechanical strength by up to 90% of the dry values. The characterization of the material showed that surface treatment increased the hydrophobicity. In addition, the paper straws did not release observable amounts of particulates to the liquids after long periods. In contrast, plastic straws do not suffer any mechanical strength loss or water absorption.
4.2 Future Work for Paper Straws Chapter 2

The next step of this research will be to determine whether the paper straws release any kind of soluble substances into the liquids. The components of the paper straws could be dissolved into the liquids, bringing unwanted characteristics to the beverages (color or flavor) or affecting the users.

It is important to test different coating materials to reduce the water uptake and mechanical strength, keeping the biodegradable characteristics of the straws. This can be applied as a coating, enhancing the properties of the paper straws, making it more durable and water permeable without losing their bio-friendly characteristics.

It is needed to explore different approaches for the development of an improved version of paper straws, addressing the issues detected with this work.

4.3 Summary for Tobacco Protein-Containing Coating Chapter 3

The current problem with synthetic plastics has brought the necessity of more sustainable options in the coating and packaging applications. The approach with this research was to address this problem using a new alternative to current synthetic plastics as a binder in coating colors. Using RuBisCO isolated proteins as new co-binders in coating applications. These biopolymers are present in the leaves of plants, meaning that they are the most abundant proteins on Earth, and easy to obtain. Potentially they can be obtained from Agro-industrial waste.

Two RubisCO-loaded extracts from tobacco and alfalfa leaves were used, and the properties of pigment coating colors using the proteins as co-binders, were compared. The work includes the characterization of the proteins, the rheological and water retention analysis of different coating fluids, and the measurement and comparison of the coated paper properties. Comparing the
proposed option with the current natural alternative, the soy protein, and the synthetic option, SBR Latex.

The main findings show that the tobacco protein offers a suitable option as co-binders, offering rheology modification, enhanced glueability, and comparable coating properties. However, water retention of the coating color and the final color of the samples are aspects that need improvements.

4.4 Future work for tobacco protein-containing coating chapter 3

Improving the isolation process to obtain better quality and higher purities. A better isolation process will potentially reduce the interferences in critical aspects such as the color of the coating layer and solution stability.

Higher protein purity is needed because it will also allow the development of a full procedure to characterize and determine all the fundamental properties of the protein, such as amino acid composition and sequence. With these properties, protein modifications could be used to improve critical properties such as water retention or rheology behavior.

Thus, it is important to study the economic aspects to determine the cost-effectiveness of RuBisCO-based protein and its potential in the industry as a sustainable alternative.
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45740772/most-paper-straws-are-made-in-asia-but-now-uk-firms-are-starting-to-produce-them-again.


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APPENDIX
Figure 36. Linear regression plots for Molecular Weight determination using SDS-PAGE.
Figure 37. Pictures of paper samples. Comparison between un-coated samples and coated samples using all formulas for Linerboard. From b-h, 50% solids formulas, from i-l, 40% solids formulas. (a) Base paper. (b) LatexOnly, no protein. (c) SoyADM-2.5, using 2.5 parts of soy protein. (d) SoyADM-5, using 5 parts of soy protein. (e) Tob10-2.5, using 2.5 parts of Tob10k protein. (f) Tob10-5, using 5 parts of Tob10k protein. (g) Tob100-2.5, using 2.5 parts of Tob100k protein. (h) Tob100-5, using 5 parts of Tob100k protein. (i) SoyADM-2.5/40, using 2.5 parts of soy protein. (j) Alf-2.5/40, using 2.5 parts of alfalfa protein. (k) Tob10-2.5/40, using 2.5 parts of Tob10k protein. (l) Tob100-2.5/40, using 2.5 parts of Tob10k protein.
Figure 38. Pictures of paper samples. Comparison between un-coated samples and coated samples using all formulas for Whiteboard. From b-h, 50% solids formulas, from i-l, 40% solids formulas. (a) Base paper. (b) LatexOnly, no protein. (c) SoyADM-2.5, using 2.5 parts of soy protein. (d) SoyADM-5, using 5 parts of soy protein. (e) Tob10-2.5, using 2.5 parts of Tob10k protein. (f) Tob10-5, using 5 parts of Tob10k protein. (g) Tob100-2.5, using 2.5 parts of Tob100k protein. (h) Tob100-5, using 5 parts of Tob10k protein. (i) SoyADM-2.5/40, using 2.5 parts of soy protein. (j) Alf-2.5/40, using 2.5 parts of alfalfa protein. (k) Tob10-2.5/40, using 2.5 parts of Tob10k protein. (l) Tob100-2.5/40, using 2.5 parts of Tob10k protein.
Figure 39. CIELAB color space. (L,a,b) values of calendered coated paper with 50% solids formulas
Figure 40. CIELAB color space. (L,a,b) values of coated paper with 40% solids formulas
Figure 41. CIELAB color space. (L,a,b) values of calendered coated paper with 40% solids formulas