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DEVELOPMENT OF A COLORIMETRIC INDICATOR, USING A RESIDUE FROM GRAPE JUICE PROCESSING, FOR FOOD FRESHNESS MONITORING

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Advisor: Prof. Caciano Pelayo Zapata Noreña, Ph.D.

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ABSTRACT

Colorimetric indicator films were developed for food freshness assessment through the solid residue from the centrifugal separation process of grape juice upcycling, a non-pomace residue (NPG) currently unexplored in the development of intelligent systems for food packaging. The film-forming matrix was obtained by polyelectrolyte complexation of alginate and gelatin and alginate and chitosan, with and without NPG addition, with the following composition (w/w): alginate and gelatin (75 % + 25 %, AG); alginate, gelatin and NPG (75% + 25% + 0.5 g 100 g $_{complex}^{-1}$, AG0.5); alginate, gelatin and NPG (75% + 25% + 1.0 g 100 g $_{complex}^{-1}$, AG1.0); alginate and chitosan (75% + 25%, ACH); alginate, chitosan and NPG (75% + 25% + 0.5 g 100 g $_{complex}^{-1}$, ACH0.5); alginate, chitosan and NPG (75% + 25% + 1.0 g 100 g $_{complex}^{-1}$, ACH1.0). The NPG addition affected the stability and the rheological behavior of the AG and ACH complexes, which presented predominantly viscous and viscoelastic characteristics, respectively, evidenced by their creep-recovery behavior. The NPG affected the water-related properties of the indicator films. It decreased their moisture by up to 35 %, increased the solubility of the AG films, and had no significant effect on their water vapor permeability, except for the ACH1.0 sample, in which microstructure changes promoted by the drying process may have increased the diffusion through the film. The mechanical and optical properties were also affected, as well as the morphology with the formation of films with lower light transmission, and more heterogeneous and rigid structures. This behavior was corroborated by the films' surface topography evaluation, whose average roughness values for the AG and ACH films increased by about 91 % and 52 %, respectively, after the highest content of NPG addition. The samples' diffractograms and FTIR spectra demonstrated that NPG interacted differently with the two complexes, and the NPG phenolic compounds may have acted as a crosslinking agent for the ACH complex, which enhanced intermolecular and intramolecular interactions between alginate and chitosan. This behavior was evidenced in the wettability evaluation, which was increased by the NPG addition, as well as in the evaluation of thermodynamic properties. The films' water adsorption isotherms were determined at 20, 30, and 40 °C, from which the thermodynamic properties of sorption were obtained. The GAB model presented the best fit for the adsorption data, which was an enthalpy-controlled process. The lowest mass losses up until 100 °C were found for the ACH films, which showed the highest storage and loss modules in dynamic mechanical analysis. A new method was proposed to evaluate the biodegradability of films, by area loss assessment through digital image analysis. NPG addition decreased the films' biodegradability, whose area losses ranged

from 90.02 % to 35.90 % after 30 days under indoor soil conditions. Colorimetric efficiency tests of the films were performed in acid lactic / lactate buffer solutions and in the gaseous phase with ammonia, as model systems for the deterioration of dairy and meat products, respectively. They were also applied as freshness indicators in whole milk samples. In general, lower NPG contents were more efficient for color differentiation by the viewer. The red chromatic shift quantification as a function of pH was promising to evaluate the visual color change by digital image analysis for the films' application on food packaging.

Keywords: Food packaging, pH indicator, upcycling, anthocyanins, biopolymer films, digital image analysis.

RESUMO

Filmes indicadores colorimétricos foram desenvolvidos para avaliação da qualidade de alimentos através do reaproveitamento do resíduo sólido, do tipo não-bagaço (NPG), da centrifugação do suco de uva, atualmente inexplorado em sistemas inteligentes para embalagens de alimentos. As dispersões foram obtidas por complexação polieletrolítica de alginato e gelatina e alginato e quitosana, com e sem adição de NPG, nas seguintes composições (m/m): alginato e gelatina (75 % + 25 %, AG); alginato, gelatina e NPG (75% + 25% + 0.5 g 100 g $_{complexo}^{-1}$, AG0.5); alginato, gelatina e NPG (75% + 25% + 1.0 g 100 g $_{complexo}^{-1}$, AG1.0); alginato e quitosana (75% + 25%, ACH); alginato, quitosana e NPG (75% + 25% + 0.5 g 100 g $_{complexo}^{-1}$, ACH0.5); alginato, quitosana e NPG (75% + 25% + 1.0 g 100 g $_{complexo}^{-1}$, ACH1.0). A adição de NPG afetou a estabilidade e o comportamento reológico dos complexos AG e ACH, que apresentaram características predominantemente viscosas e viscoelásticas, respectivamente, evidenciadas pelo comportamento de *creep-recovery*. O NPG afetou as propriedades hidrofílicas dos filmes indicadores. A umidade diminuiu em até 35 %, a solubilidade dos filmes AG aumentou, e não houve efeito significativo na permeabilidade ao vapor de água, exceto para a amostra ACH1.0, na qual alterações microestruturais promovidas pelo processo de secagem podem ter aumentado a difusão através do filme. As propriedades mecânicas e ópticas também foram alteradas, assim como a morfologia, formando-se filmes de menor transmissão de luz com estruturas mais heterogêneas e rígidas. Esse comportamento corroborou-se pela avaliação da topografia superficial dos filmes, cujos valores de rugosidade média para os filmes AG e ACH aumentaram cerca de 91 % e 52 %, respectivamente, após a adição do maior teor de NPG. Os difratogramas das amostras e os espectros de FTIR demonstraram que o NPG interagiu de maneira diferente com os dois complexos, e os compostos fenólicos do NPG podem ter atuado como um agente de reticulação para o complexo ACH, o que aumentou as interações intermoleculares e intramoleculares entre alginato e quitosana. Isto evidenciou-se na molhabilidade, que aumentou após adição de NPG, bem como na avaliação das propriedades termodinâmicas. As isotermas de adsorção de água dos filmes foram determinadas a 20, 30 e 40 °C, a partir das quais foram obtidas as propriedades termodinâmicas de sorção. O modelo GAB apresentou o melhor ajuste para os dados de adsorção, que foi um processo controlado por entalpia. As menores perdas de massa até 100 °C foram encontradas para os filmes ACH, que apresentaram os maiores módulos de armazenamento e perda na análise dinâmico-mecânica. Um novo método foi proposto para avaliar a biodegradabilidade dos filmes, por meio da avaliação da perda de área por análise

digital de imagens. A adição de NPG diminuiu a biodegradabilidade, com perdas de área de 90,02 % a 35,90 % após 30 dias em ambiente fechado. Testes de eficiência colorimétrica dos filmes foram realizados em soluções tampão ácido lático / lactato e na fase gasosa com amônia, como sistemas modelo para a deterioração de produtos lácteos e cárneos, respectivamente. Eles também foram aplicados como indicadores de frescor em amostras de leite integral. Em geral, menores teores de NPG foram mais eficientes para diferenciação de cores pelo visualizador. A quantificação do desvio cromático vermelho em função do pH foi promissora para avaliar a mudança visual de cor por análise digital de imagens para aplicação dos filmes em embalagens de alimentos.

Palavras-chave: Embalagem de alimentos, indicador de pH, reaproveitamento, antocianinas, filmes biopoliméricos, análise digital de imagens.

SUMMARY

INTRODUCTION

Packaging is fundamental for the food industry, being responsible for protecting food from storage to distribution to the consumer. However, they do not provide a record of the environmental conditions in which the food was during storage. For this reason, the intelligent packaging concept has been explored, whose composition allows to check internal changes in the food as well as in the storage environment, and in this way, it communicates to the consumer whether the consumption of a product is safe (Restuccia, Spizzirri, Parisi, Cirillo, & Curcio, 2010). Different technologies have been used to produce intelligent packaging, the main one being the development of indicators, with emphasis on indicators sensitive to pH changes caused by the degradation of food products (Dalmoro et al., 2017).

pH-sensitive colorimetric indicators can be used to assess the freshness and estimate the shelf life of foods from animal or plant sources, based on changes in the pH of the food that leads to changes in the indicator color. These pH changes are caused both by chemical and enzymatic degradation as well as by the production of metabolites coming from microbial metabolism (Balbinot-Alfaro et al., 2019). For the elaboration of colorimetric indicators, it is necessary to prepare a solid support, usually a hydrophilic film, which can store and transport the indicator compound. This support allows colorimetric indicators to be incorporated into food packaging, attached inside or outside it, helping companies and consumers in detecting and monitoring changes in the conditions of packaged products according to variations in the color of the indicator (Maciel, Yoshida, & Franco, 2012).

In this context, residues and by-products from vegetable processing have been widely explored in the development of colorimetric indicators of filmogenic matrix (Crizel, Costa, Rios, & Flôres, 2016; Luchese, Sperotto, Spada, & Tessaro, 2017; Travalini, Lamsal, Magalhães, & Demiate, 2019). These materials can be used as a source of pigmented compounds sensitive to pH changes, as well as due to their high fiber content, to reinforce and fill the polymeric matrix of the support film (Hussain, Jõudu, & Bhat, 2020).

Among agro-industrial residues and by-products, those generated by viticulture stand out, which comprise an important portion of agriculture in Brazil, with an estimated annual production of 1.7 million tons of grapes (IBGE, 2022). Concentrated grape juice and table grapes account for the main commodities of the sector (Camargo, Tonietto, & Hoffmann, 2011). During the grape juice processing, the grape pomace consisting of skins and seeds is separated by pressing, with the juice removal. Then, clarification processes are applied that include unit operations of centrifugation and stabilization of the grape juice. During

centrifugation, the suspended solids are removed giving rise to a wet and solid non-pomace residue (Haas, Toaldo, Müller, & Bordignon-Luiz, 2017; Rizzon & Meneguzzo, 2007). This material is rich in anthocyanins, which change color when exposed to pH changes. These compounds may be used as colorimetric indicators when the pH changes are associated with the food deterioration processes.

This study aims to develop and characterize colorimetric pH indicators for monitoring food freshness. The filmogenic support was elaborated by polyelectrolyte complexation of sodium alginate and gelatin, and sodium alginate and chitosan, with the addition of the non-pomace residue of the grape juice centrifugal separation process, which is currently unexplored in the development of intelligent systems for food packaging.

It is organized as follows: Chapter 1 constitutes the theoretical foundation, which addresses the main topics of the research; Chapters 2 and 3 present the development of the study in the format of scientific papers, presenting abstract, introduction, methodologies, results and discussions, and conclusions; Chapter 4 presents the general discussion of the study and, in the end, the final considerations and the research perspectives.

OBJECTIVES

GENERAL OBJECTIVE

Development and characterization of colorimetric indicators using a non-pomace residue of grape juice (NPG) processing as a source of anthocyanins, for application as indicator compounds, and of fibers, as reinforcement material in the support film-forming matrix, produced from alginate, gelatin, and chitosan biopolymers, by polyelectrolyte complexation.

SPECIFIC OBJECTIVES

- Characterize the NPG in terms of centesimal composition and anthocyanin content;
- Produce film-forming dispersions through the polyelectrolyte complexation of alginate-gelatin and alginate-chitosan with and without the addition of NPG;
- Study the rheological behavior of the film-forming dispersions;
- Produce indicator films employing the casting method;
- Characterize the indicator films produced for their moisture, water solubility, water vapor permeability, and contact angle;
- Characterize the structure of indicator films by scanning electron microscopy (SEM), mechanical profilometry, and X-ray diffraction (DRX);
- Evaluate the interactions between biopolymers and NPG in the formation of the indicator's film-forming matrix by Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR);
- Evaluate the thermal properties of the indicator films by thermogravimetric analysis (TGA) and dynamic mechanical analysis (DMA), as well as the mechanical and optical properties of the indicator films;
- Obtain the water adsorption isotherms of the indicator films at 20, 30, and 40 °C;
- Assess the biodegradability of indicator films;
- Evaluate the efficiency of the colorimetric response to pH changes, using lactic acid and ammonia as model systems for the deterioration of dairy products and meat products, respectively;
- Apply the films as freshness indicators in whole milk samples.

CHAPTER 1

THEORETICAL FOUNDATION

1 Colorimetric indicators

The growing consumer demand for fresh and safe food has driven the development of tools that can access information about product quality. In this trend, there are sensors or indicators that, when incorporated into the packaging, can provide information about the integrity of the food, guaranteeing its quality and safety for consumption (Kuswandi, 2017). Thus, besides the protection and preservation of food are the main functions of packaging (Robertson, 2013), they can also provide information to the consumer about the quality, shelf life or expiration date, traceability, and/or indication of a violation of food safety.

Sensors are devices that detect, record, and transmit quantifiable information about the presence of a specific analyte. They are divided into chemical sensors, biosensors, and gas sensors, which provide the content of chemical compounds of the environment, the metabolites produced during the food deterioration process, and the change of packaging atmosphere, respectively (Dalmoro et al., 2017). Although the terms “sensors” and “indicators” are often used interchangeably, unlike sensors, indicators do not include signal reception and transduction components, communicating only information through a direct visual change (Butler & Kerry, 2008; Kuswandi, 2017). Indicator systems are widely used due to the simplicity and rapid transmission of information or response, which is usually communicated through the visual perception of colorimetric changes (Ghaani, Cozzolino, Castelli, & Farris, 2016). The type of response involved can be of a qualitative or semi-quantitative nature, and the indicators can be classified into three categories: package integrity, time-temperature, and freshness indicators (Butler & Kerry, 2008).

Integrity indicators are used mainly in modified atmosphere packages and allow for assessing whether there was damage and violation of the packaging during food storage, as well as whether there was adulteration in the gaseous composition inside the package. Among the integrity indicators, the gas indicators stand out, which monitor the atmosphere inside the packaging, whose response is revealed by the change of color of the indicator due to the presence of gas, such as O_2, CO_2, ethylene, and H_2S (Dalmoro et al., 2017; Vu & Won, 2013).

Time-temperature indicators (TTIs) provide visual information on the temperature history of perishable food from storage to the time of product use. In general, TTIs are small

self-adhesive labels that, through a change in the color of the indicator, inform when the product is stored at inappropriate temperatures. These indicators are especially useful for chilled or frozen products, given that temperature variations have a great influence on microbial growth and the kinetics of physical and chemical deterioration, as they allow monitoring of storage conditions and indicate whether the appropriate temperatures have been applied correctly (Kerry, O'Grady, & Hogam, 2006). Therefore, TTIs can be used as shelf life indicators, as well as information providers about a possible break in the cold chain (Dalmoro et al., 2017).

Freshness indicators act by monitoring the presence and/or products of reactions formed during ripening or post-harvest or post-slaughter metabolism of plant or animal foods (organic acids, CO_2, volatile nitrogen compounds, sulfur derivatives, etc.). Predominantly, the presence of these compounds is a consequence of alterations in the chemical composition of the products, from reactions such as oxidation and hydrolysis, as well as products from microbial metabolism (Butler & Kerry, 2008; Dalmoro et al., 2017).

The indicator response consists of a visible color change, which allows the assessment of the quality of the food product inside the package (Poyatos-Racionero, Ros-Lis, Vivancos, & Martínez-Máñez, 2018). This color change is possible due to the use of dyes in the production of indicators, mainly the ones sensitive to pH changes, such as bromothymol blue (Chen, Zhang, Bhandari, & Guo, 2018; Lyu et al., 2019), methyl red (Rukchon, Nopwinyuwong, Trevanich, Jinkarn, & Suppakul, 2014), or anthocyanins (Sanches et al., 2021; Tirtashi et al., 2019). For the transport of the dye, a film with hydrophilic characteristics is generally used. This greater polarity of the polymeric matrix surrounding the dye is necessary due to the colorimetric response mechanism, as the indicators normally respond to pH changes due to the dissolution of compounds released by food (CO_2, volatile nitrogen compounds, etc.) in water (Mills & Skinner, 2010).

1.1 Colorimetric pH indicators

Changes in food pH during storage are generally related to chemical, enzymatic or microbial degradation, such as the production of free fatty acids by lipid oxidation, as well as the increase in microorganisms whose metabolites (organic acids, sulfur compounds, etc.) cause changes in the pH of the medium. These events can be detected by the consumer through pH-sensitive colorimetric indicators when they change their color (Balbinot-Alfaro et al., 2019). Usually, colorimetric pH indicators are composed of two parts: the indicator

compound, which must be sensitive to pH change, and the solid support for it (Pourjavaher, Almasi, Meshkini, Pirsa, & Parandi, 2017). The indicator compound can interact with the solid support in three ways: being immobilized by physical adsorption on the solid support, usually an ion exchanger; covalently attached to hydrophilic support, such as cellulose or silica; or being physically encapsulated in polymer matrices (Abolghasemi, Sobhi, & Piryaei, 2016). In this way, the solid support allows colorimetric indicators to be incorporated into food packaging, attached inside or outside it, helping to detect and monitor changes in the conditions of packaged products due to variations in the indicator color (Maciel et al., 2012).

Several studies have been carried out on the use of indicators in dairy and meat products. Weston et al. (2020) developed a shelf life indicator for milk, using anthocyanins extracted from red cabbage as a dye and a mixture of polyvinyl acetate (PVA) and agarose as a solid support, based on the change in color of the indicator that identify the increase of lactic acid content promoted by microbial growth during storage. This indicator was successful in distinguishing fresh milk, the beginning of degradation, and spoiled milk. For ground beef degradation, Sanches et al. (2021) developed an indicator with anthocyanins extracted from red cabbage as indicator compounds and a mixture of starch and whey as a solid support. In this case, the degradation of proteins provoke the production of free amino acids which induces the formation of amines (histamine, putrescine, tyramine, etc.) and ammonia, alkaline products that increase the pH of meat foods during their deterioration (Butler & Kerry, 2008).

Another of the main applications of colorimetric pH indicators is their use in the development of intelligent packaging, which transmits information related to food freshness, such as pH and temperature, to stakeholders in the production and supply chains, such as manufacturers, retailers, and consumers (Restuccia et al., 2010). For this purpose, the indicator can be produced with a film as a solid support, where the indicator compounds will be added to a polymeric dispersion that will be the film-forming matrix (Balbinot-Alfaro et al., 2019; Kuswandi, 2017).

1.2 Elaboration of the indicator support

Biopolymers, such as polysaccharides and proteins, are used to produce the film-forming matrix supporting the indicators due to the intrinsic hydrophilic properties of most of these compounds, as well as their biodegradability (Guilbert, Gontard, & Gorris, 1996; Krochta, 2002).

For the formation of these films, there must be interaction between the structures of the compounds used, through the formation of covalent bonds, the establishment of hydrophobic (Van der Waals) or hydrophilic (hydrogen bonds) intermolecular interactions, or electrostatic interactions (polyelectrolyte complexation). To ensure that the formed film-forming matrix is malleable and flexible, the addition of a plasticizer, such as glycerol, is necessary (García, Martino, & Zaritzky, 2000; Han, 2014), which can lead to a decrease in cohesion between the biopolymer chains (Guilbert et al., 1996; Krochta, 2002).

However, in order to compensate this negative effect of the plasticizer, fibers can be used as reinforcing material in the film-forming matrix interacting with the biopolymers. As a source of fibers, as well as dyes for the development of indicators, the use of residues from the food industry has been highlighted, such as pomace from fruits such as grapes (Abolghasemi et al., 2016), cranberry (Park & Zhao, 2006) and blueberry (Tagliani, Perez, Curutchet, Arcia, & Cozzano, 2019), rich in pigmented compounds that have potential application as indicator dyes, since the color of these compounds is modified by pH and temperature.

1.3 Application as intelligent films

In line with the trend towards healthier and safer food, active and intelligent packaging development has stood out in packaging production. Active materials are so called because they interact with food, while intelligent ones monitor the state of the product based on the product condition or the storage medium (European Commission, 2004). The intelligent system can be the packaging or an external component incorporated into the final packaging, as is the case with colorimetric indicators, which aim to provide more convenience and inform consumers about the quality of food (Ghaani et al., 2016; Kerry et al., 2006).

2 Biopolymers used in the preparation of the biodegradable film-forming matrix

Biopolymers consist of polymers produced from natural sources, chemically synthesized from a biological material, or fully biosynthesized by living organisms. They are biodegradable, whose decomposition occurs predominantly by the enzymatic action of microorganisms resulting in CO_2, CH_4, H_2O, and residual organic matter (biomass)(Peelman et al., 2013). Thus, when this type of material decomposes in the soil, the residual biomass from the decomposition can be used as fertilizer (Peelman et al., 2013). Due to the hydrophilicity necessary for the indicator's colorimetric response mechanism, biopolymers are

widely explored in the development of indicators for the construction of the solid support, such as films based on sodium alginate (Fabra, Falcó, Randazzo, Sánchez, & López-Rubio, 2018), gelatin (Rawdkuen, Faseha, Benjakul, & Kaewprachu, 2020) and chitosan (Yoshida, Maciel, Mendonça, & Franco, 2014). There is also the possibility of using a combination of these materials, which have excellent film-forming properties. In addition to the ability of complexation by electrostatic interaction between the negatively charged carboxyl group of alginate and the positively charged amino groups of gelatin and chitosan under appropriate pH conditions, there is the formation of hydrogen bonds between the polar groups present in these molecules (Bonilla & Sobral, 2016; Haghighi et al., 2019).

2.1 Sodium alginate

Sodium alginate (Figure 1a) is a naturally non-toxic anionic polymer, typically obtained from brown seaweed, widely used in the food, biomedical, and pharmaceutical industries (Lee & Mooney, 2012). It is preferably used in the form of sodium alginate, due to its higher solubility in water (Funami et al., 2009). Each block that constitutes the polymeric structure of alginate has different properties, such as solubility and structural rigidity, due to the different conformations adopted. The G blocks (α-L-guluronic acid) represent the alginate fraction insoluble in water, whose structure presents greater rigidity when compared to the M blocks (β-D-mannuronic acid. They represent the soluble fraction, which has a higher steric hindrance around glycosidic bonds (Funami et al., 2009; Larsen, Salem, Sallam, Mishrikey, & Beltagy, 2003).

When dispersed in a liquid medium, the alginate structure is also influenced by the pH of the medium, due to the presence of carboxylic groups (Yang, Chen, & Fang, 2009). They are protonated at a pH between 3.0 and 4.5, which decreases their interaction with water molecules and the alginate chains solubility. At a pH lower than 3.0, alginic acid precipitation occurs (Damodaran & Parkin, 2017). Because of this, dispersions whose pH values are between 5.0 and 10.0 are usually used, the range in which the alginate remains soluble (Yang et al., 2009). Being a material generally considered safe by the U.S. Food and Drug Administration (FDA, 2019), alginate is widely used in various industries such as food, beverage, textile, printing, and pharmaceutical as a thickening agent, stabilizer, emulsifier, chelating agent, encapsulating wall material, and film-forming polymer (Parreidt, Müller, & Schmid, 2018).

2.2 Gelatin

Gelatin (Figure 1b) consists of a protein with a high content of proline, glycine, and hydroxyproline prepared by the thermal denaturation of collagen, available in the skin and bones of animals (Figueroa-Lopez, Andrade-Mahecha, & Torres-Vargas, 2018). It is found commercially in 2 forms, type A and type B. Type A gelatin is obtained by acid pretreatment, and type B is obtained by basic pretreatment. They have an isoelectric point between 8.0 and 9.0, and 4.8 and 5.4, respectively (Aramwit, Jaichawa, Ratanavaraporn, & Srichana, 2015). Their use is mainly as a gelling agent in the food, pharmaceutical, and cosmetic industries, due to their ability to form transparent, elastic, and thermoreversible gels.

At the molecular level, the gelation process involves the renaturation of the structure, starting from the disordered state until the formation of triple helix structures (characteristics of collagen in the native state). The renaturation of the triple helix acts in the connection of the three-dimensional network, and the organization of the network and the physical properties of the gelatin gel are mainly maintained by the formation of the triple helix structures, a behavior that can be evaluated by the mechanical properties of the gel (Deshmukh et al., 2017). The physicochemical properties of gelatin films, such as the mechanical properties, are influenced by the drying temperature used in their production process. Then, it is important that drying occurs slowly so that intramolecular interactions can be established between the polymeric chains responsible for a suitable microstructure formation to the filmogenic support development (Suhag, Kumar, Petkoska, & Upadhyay, 2020; Velaga, Nikjoo, & Vuddanda, 2018).

2.3 Chitosan

Chitosan (Figure 1c) is a polysaccharide derived from chitin, a nitrogenous polysaccharide from the exoskeleton and internal structure of invertebrates. It can be obtained by deacetylation and depolymerization of native chitin, (partial) deacetylation of chitin in the solid state under alkaline conditions (concentrated NaOH), or enzymatic hydrolysis in the presence of a chitin deacetylase (Zargar, Asghari, & Dashti, 2015). Among the most important physicochemical parameters of this polymer, the degree of deacetylation (GDA) stands out. It is defined as the ratio glucosamine / N-acetyl glucosamine, which increases as chitin is converted into chitosan. GDA influences all physicochemical properties, such as molecular weight, viscosity, solubility, etc. This parameter can also affect the solubility of the

polymer in organic or aqueous solvents, where increasing the GDA increases the solubility in water (Zargar, Asghari, & Dashti, 2015).

In general, this polymer has been reported as non-toxic, biodegradable, and biocompatible, in addition to having antimicrobial and antioxidant potential, being widely used in the food industry, in the biomedical and pharmaceutical areas, and for the controlled release of drugs and bioactive substances. Due to its chemical structure, chitosan becomes an excellent alternative to be employed as a base in the production of nanoparticles, microparticles, hydrogels, and films (Abdel-Aleem & El-Aidie, 2018; Fráguas, Simão, Faria, & Queiroz, 2015).

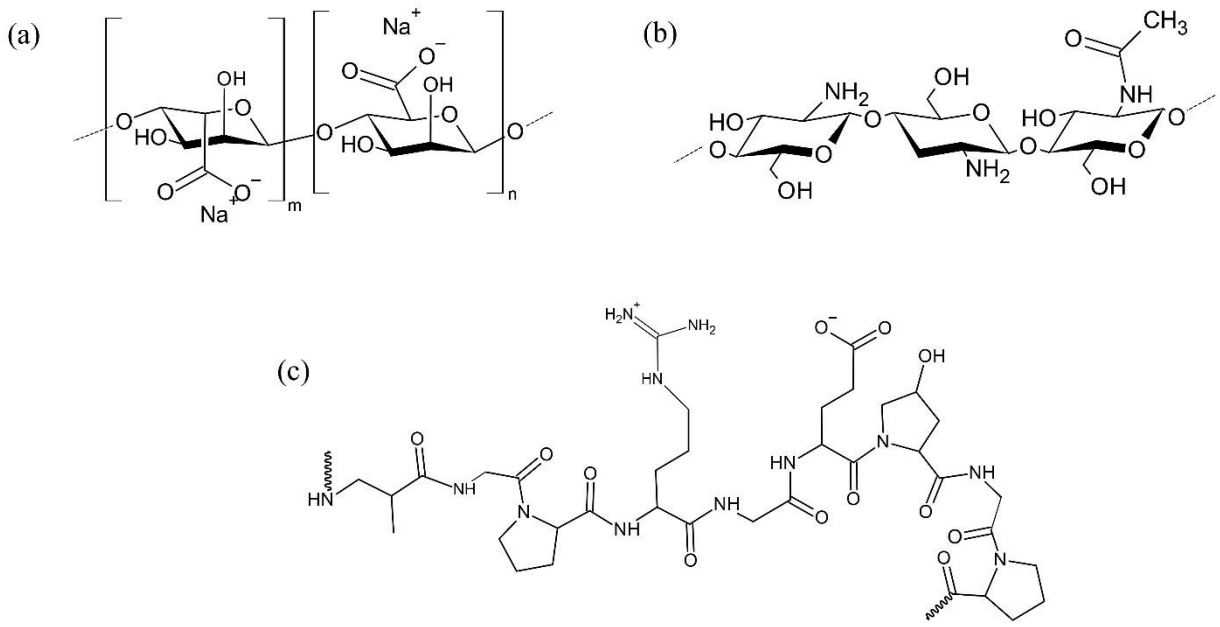

Figure 1 – Basic chemical structures of sodium alginate (a), chitosan (b), and gelatin (c).

Source: Adapted from Mahadevappa, Arjumand e Ravindra (2014); Noor et al. (2021); Zargar, Asghari e Dashti (2015).

3 Food industry residues in the production of colorimetric indicators

The increase of agricultural production, combined with inadequate handling and post-harvest technologies, as well as the generation of by-products during the various stages of food processing, has led to the generation of high amounts of residues and by-products in the food industries, mainly in the processing of fruits and vegetables (Hussain et al., 2020). According to the Food and Agriculture Organization of the United Nations (FAO), annually about 15 % of global food production is wasted, with 60 % of this portion corresponding to the production of horticulture (fruits, vegetables, and tubers) (FAO, 2019).

In the composition of these residues and by-products of the industry, several compounds applicable to the formation of colorimetric indicators can be found. For example, pigmented phenolic compounds, which can signal changes in the characteristics of the food by changing the color, and fibers, which can act in reinforcing the polymeric network of the supporting film (Hussain, Jõudu, & Bhat, 2020). Juice production, in particular, generates a significant amount of residue. Among worldwide fruit production, almost 50% of the crops are being processed as juice, which may account for 20−80 % of the whole fruit being generated as residues or byproducts (Kandemir, Piskin, Xiao, Tomas, & Capanoglu, 2022). In this sense, the grape juice and winemaking industry generates solid residues rich in fibers and phenolic compounds, such as anthocyanins. For example, there are the pomace and the non-pomace residues, such as plant remains, lees, and sediments from the clarification and centrifugation process (Beres et al., 2017).

3.1 Non-pomace residues of grape juice

Grape juice is obtained in several stages. The process can be summarized in the following unit operations: reception of the raw material, separation of the stems, crushing, heat treatment, enzymatic treatment, draining, pressing, centrifugation, cooling, vacuum filtration, heat treatment, and packaging (Rizzon & Meneguzzo, 2007). The world's production of juices generates approximately 5.5 million metric tons of residues and byproducts per year, which are rich in bioactive compounds (Hussain et al., 2020).

Usually, their destination is animal feed and fertilization, but sometimes they are discarded without any treatment, potentially causing environmental damage (Rockenbach et al., 2011). While pomace is widely studied (Sousa et al., 2014; Yeler & Nas, 2021; L. Zhang et al., 2017), the non-pomace residues are less explored, especially the solid residues from grape juice centrifugation. They are constituted by the suspended solids of the juice and represent approximately 4 to 8% of the initial volume (Rizzon & Meneguzzo, 2007). This material is rich in anthocyanins (Haas, Toaldo, Burin, & Bordignon-Luiz, 2018), which could be employed to produce colorimetric indicator films, and also contains fibers to fill and reinforce the film support matrix.

3.2 Anthocyanins

Some synthetic pigments such as bromocresol purple, bromocresol green, bromophenol blue, chlorophenol red, and cresol red have been used as pH-sensitive dyes.

However, when it comes to food safety, natural dyes are more favorable for application in food packaging (Balbinot-Alfaro et al., 2019). Natural colorants such as curcumin, betalains, carminic acid, carotenoids, chlorophylls, and especially anthocyanins have shown potential as pH, gas, or temperature-responsive dyes (Becerril, Nerín, & Silva, 2021).

Anthocyanins are soluble phenolic compounds, ranging in color from red to blue, belonging to the class of flavonoids (Delgado-Vargas, Jiménez, & Paredes-López, 2000). They are found in fruits in the form of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, and are naturally glycosylated, usually linked to monosaccharides such as glucose, fructose, and galactose (Fernandes et al., 2014; Zhang & Hamauzu, 2004). In grapes, anthocyanins are mainly responsible for color and are found in higher concentration in the skins as monoglycosides of malvidin, cyanidin, petunidin, peonidin and delphinidin (Monagas, Bartolomé, & Gómez-Cordovés, 2005; Obreque-Slier et al., 2017).

These compounds have stood out in the food industry as an alternative to synthetic dyes, considering that they can be obtained in different shades and their consumption can bring health benefits, due to the intrinsic antioxidant activity of these molecules (He & Giusti, 2010; Sigurdson, Robbins, Collins, & Giusti, 2019). However, anthocyanins are unstable after their extraction from the plant matrix, showing sensitivity to O_2, temperature, light, and especially pH changes, thus affecting the structure of the molecules and, consequently, their color (Fernandes et al., 2014).

Depending on the pH value to which they are exposed, anthocyanins present different colors, and the color change is related to structural transformations, most of which are reversible (Figure 2) (Sigurdson et al., 2019; Tang et al., 2019)). These reactions can be applied to the development of food freshness indicators.

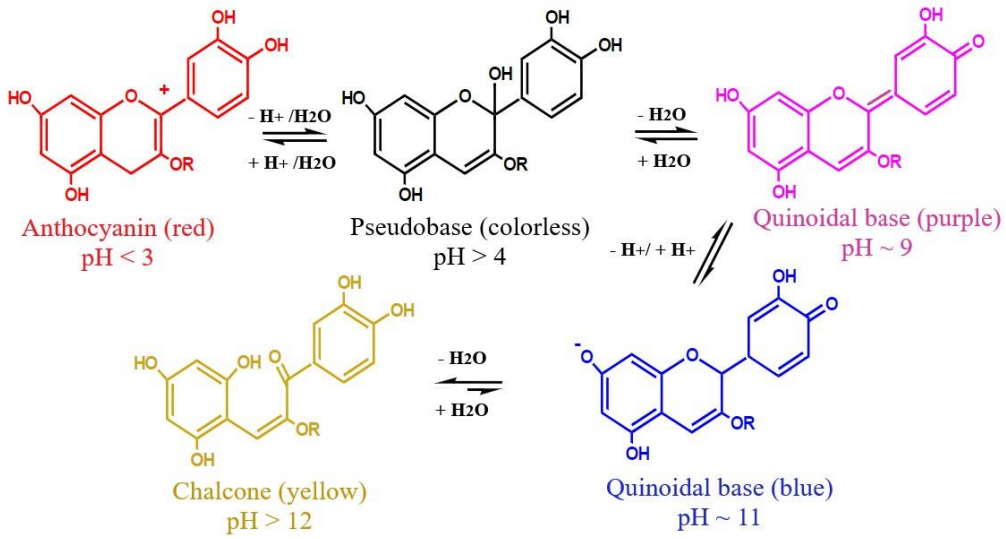

Figure 2 – Anthocyanin structures associated with changes in pH, where R = glycoside.

Source: Adapted from Ananga et al. (2013).

3.3 Fibers

Several studies have emerged focusing on the use of natural fibers as reinforcement in composites with polymeric matrices for the production of biodegradable films (Crizel et al., 2016; Moro, Ascheri, Ortiz, Carvalho, & Meléndez-Arévalo, 2017; Pereira et al., 2015). The fibers are employed to compensate for the negative effects of the addition of plasticizers, such as glycerol and sorbitol, which are necessary for the formed structure to be malleable and flexible. Depending on the content of the plasticizer, despite increasing the flexibility of the film, reducing its fragility to breakage and impacts, they can lead to an excessive decrease in the intermolecular interactions between the polymeric chains of the biopolymers that form the films, which modifies their mechanical and barrier properties of the films (Moro et al., 2017).

In higher content in red grapes (Deng, Penner, & Zhao, 2011), both soluble and insoluble fibers can be found in grape juice processing residues. The major contribution comes from the presence of the skins, where mainly insoluble fibers are found, such as pectin (Figure 3a), cellulose (Figure 3b), and lignin (Figure 3c) (Deng et al., 2011; L. Zhang et al., 2017).

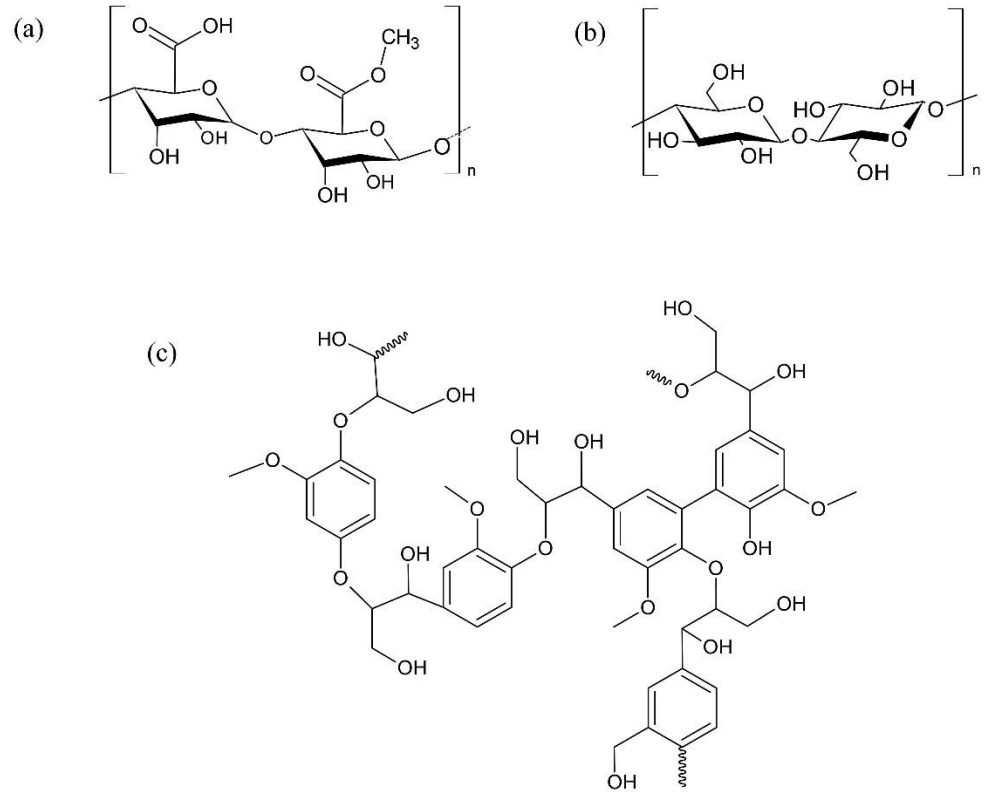

Figure 3 – Basic chemical structures of pectin (a), cellulose (b), and lignin (c).

Source: Adapted from (Ali, El-Regal, & Saeed, 2015; Damodaran & Parkin, 2017; Mahmood et al., 2018).

CHAPTER 4

GENERAL DISCUSSION

This study aimed to develop colorimetric indicators for monitoring food freshness, using the solid residue from the centrifugation of grape juice as a source of anthocyanins, to be used as indicator compounds, and fibers to reinforce the support of biopolymeric films. The film-forming dispersions were obtained by polyelectrolyte complexation. Sodium alginate was used as an anionic polyelectrolyte, while gelatin and chitosan were used as cationic polyelectrolytes. The indicator films were obtained by the casting method followed by drying and characterized in terms of their physicochemical properties. Its colorimetric response performance was also evaluated at different pH's, in solution, in the gas phase, and in a food model. The results found were presented in the form of two scientific papers. Article 1 (Chapter 2) focuses on obtaining the indicator films, evaluating their main physicochemical and structural properties, as well as their efficiency of colorimetric response to pH changes, using lactic acid and ammonia as model systems for the deterioration of dairy products and meat products, respectively. Article 2 (Chapter 3) presents the evaluation of the thermodynamic properties, as well as the performance of the films in whole milk samples, which were acidified with lactic acid to simulate its spoilage.

In Chapter 2, the solid residue from grape juice centrifugation, the non-pomace residue (NPG), was freeze-dried, fragmented, and sieved to obtain a finely divided powder. This powder was then characterized in terms of moisture content, proteins, lipids, ash, carbohydrates, total monomeric anthocyanins (TMA), and its behavior in UV-Vis when exposed to different pH's. The centesimal composition of NPG, as well as the TMA of 11.07 ± 0.47 mg $_{\text{malvidin-3,5-diglycoside}}$ $g^{-1}_{\text{d.b.}}$, were similar to the data reported for grape pomace (Gorrasi, Viscusi, Gerardi, Lamberti, & Giovinazzo, 2022; Kalli, Lappa, Bouchagier, Tarantilis, & Skotti, 2018). Its UV-Vis absorption spectrum (Figure 1; Chapter 2) demonstrated the typical behavior of anthocyanins in the pH range (2 – 13) studied.

After its characterization, NPG was added to the dispersions of polyelectrolyte complexes at the following concentrations: alginate and gelatin, without NPG (75 % + 25 %, AG); alginate, gelatin and NPG (75% + 25% + 0.5 g 100 g $_{\text{complex}}^{-1}$, AG0.5); alginate, gelatin and NPG (75% + 25% + 1.0 g 100 g $_{\text{complex}}^{-1}$, AG1.0); alginate and chitosan, without NPG (75% + 25%, ACH); alginate, chitosan and NPG (75% + 25% + 0.5 g 100 g $_{\text{complex}}^{-1}$, ACH0.5); alginate, chitosan and NPG (75% + 25% + 1.0 g 100 g $_{\text{complex}}^{-1}$, ACH1.0). These

six complex dispersions were characterized according to their ζ-potential and rheological behavior. The results showed that while the addition of NPG decreased the stability of colloidal systems of the AG complex, there was an increase in the ACH complex stability. It may have occurred due to the adsorption of the polymers present in the NPG to the surface of the ACH complex, which would increase the repulsion between particles by steric hindrance, increasing the system stability (Chhabra & Basavaraj, 2019).

The flow curves of the biopolymer dispersions and the complexes (with and without NPG addition) were obtained (Figure 2; Chapter 2), and the power law was used to describe the data (Table 3; Chapter 2). The polyelectrolyte complexation, as well as the addition of NPG, increased the viscosity of the systems. The power law fitted the data well ($R^2 \geq 0.92$), corroborating the behavior of non-Newtonian fluids with pseudoplastic properties observed in the flow curves. The ACH complex formed the dispersions with the highest viscosity, with the ACH0.5 dispersion having the highest K (consistency index) equal to 2.427 ± 0.059 Pa s^n.

The viscoelastic behavior of the complexes was evaluated by means of frequency sweeps and creep and recovery tests, through which it was observed that the behavior of the dispersions of the AG complex was predominantly viscous, while for ACH was viscoelastic (Figure 4; Chapter 2). Burger's model was used to fit the creep data of the ACH complexes, while its recovery was fitted by an exponential decay function. Both functions fitted the data well, with $R^2 \geq 0.99$ and $R^2 \geq 0.9$, respectively (Tables 4 and 5, Chapter 2). It was observed that an increase in NPG content decreased the strength of the network structure of the complexes.

The casting method was used to obtain the indicator films, where the dispersions of the complexes were placed on silicone molds and dried with forced air circulation at 30 ± 5 °C for 36 h. The water-related and mechanical properties were influenced by both the complex composition and the NPG content (Tables 6 and 7, Chapter 2). In general, the ACH0.5 film was less soluble and permeable to water vapor, showing greater structural rigidity, and desired characteristics in the development of the indicators. Both complexes formed heterogeneous films after the addition of NPG, which was not uniformly distributed in the filmogenic matrix (Figures 5 and 6; Chapter 2). The ACH complex films showed lower mass loss up to 100°C, whose values ranged from 22.2 to 26.2 % (Figure 8; Chapter 2). However, to state that these samples have higher thermal stability, additional studies are needed about the state of anthocyanins in the supports, bearing in mind that from 45 °C the anthocyanins are more susceptible to decomposition (Oancea & Drăghici, 2013).

To evaluate the biodegradability of the films, a new methodology was proposed to reduce the interferences inherent to the usually employed gravimetric method (Martucci & Ruseckaite, 2009). The quantification of sample area loss after 30 days of incubation in natural organic soil through digital image analysis proved to be a promising method for assessing the biodegradability of solid samples of known areas. The area losses ranged from 90.02 % to 35.90 % after 30 days under indoor soil conditions and were obtained after processing the images of the samples in the ImageJ software (Figure 10; Chapter 2). These results were consistent with the macroscopic observation of the films (Figure 9; Chapter 2), demonstrating the potential application of the proposed method.

The efficiency of the colorimetric response of the films was evaluated through colorimetric tests in buffer solutions of lactic acid and lactate, and with ammonia (gaseous), as model systems for the deterioration of dairy products and meat products, respectively (Figures 11 and 12; Chapter 2). Except for the ACH1.0 sample, the color difference presented by the indicator films in the pH ranges 2 – 3, 3 – 4, 4 – 5, and 5 – 6 was differentiable by the viewer (Table 9; Chapter 2). As for ammonia response, the following exposure intervals were evaluated: 10 – 30, 30 – 60, and 10 – 60 min (Table 10; Chapter 2). The difference in the color of samples with higher concentrations of NPG, AG1.0, and ACH1.0, in the evaluated time intervals, is not differentiable by the viewer. Based on the results obtained in Chapter 2, it can be seen that while a higher concentration of NPG forms films with more favorable mechanical properties for application as indicators, the colorimetric response performance was better for films with a lower NPG content.

The objective of article 2 (Chapter 3) was to understand the polymeric support structure formation, analyzing changes in crystallinity by X-ray diffraction (XRD) and the chemical bonds present by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Surface topography, as well as wettability and adsorption phenomena, were also evaluated. Finally, a colorimetric response test was carried out in samples of whole milk acidified with lactic acid as a food model. It allowed assessing whether the behavior presented in the buffer solution of acid lactic/lactate presented in Article 1 is conserved, considering the interferences in a food matrix.

The topography of the films was evaluated quantitatively by mechanical profilometry to obtain roughness parameters, and qualitatively by converting scanning electron microscopy (SEM) images of the films in the ImageJ software to 3D plot images. The surface heterogeneity of the films was greater for the samples with higher NPG content (Figure 1;

Chapter 3), which can be explained by the difficulty of distributing the NPG uniformly in the dispersions of the complexes. Corroborating the qualitative evaluation, the films with the highest NPG content, especially the ACH complex films, presented the highest roughness parameters (Table 1; Chapter 3). This may explain the colorimetric response performance of the ACH1.0 film in the solution and gas phase tests presented in Chapter 2, whose high roughness, such as an average roughness (*Ra*) of 0.450 ± 0.006, may have modified the contact area between the film and the H^+ ions.

The evaluation of the interaction between the polyelectrolytes for the formation of each complex by XRD (Figure 2; Chapter 3) and ATR-FTIR (Figure 3; Chapter 3) demonstrated that the alginate interacted with gelatin and chitosan in different ways. Because of this, NPG was incorporated in different ways into the filmogenic matrix. An increase in the crystallinity of the ACH complex was observed after the addition of NPG, as well as the emergence of a sharp peak around 1607 cm^{-1}, the region of C=C stretching of the NPG aromatics. It suggests that some compounds present in NPG, such as phenolic acids, may have mediated a layered organization of the polyelectrolytes (Li, Zhu, Guan, & Wu, 2019). A factor that was not possible in the AG complex, probably due to the steric impediment of the protein structure.

The wettability of the films was evaluated by determining the contact angle by the tangent method. It was noticed that there was a significant difference ($p < 0.05$) between the right and left contact angles of the samples with the highest NPG content, which can be explained by the heterogeneity of their surfaces. Corroborating this hypothesis, it could be stated that roughness correlated with wettability ($R_{Pearson's} \geq 0.8$).

To know under what storage conditions can act the indicator films, as well as to know the thermodynamic properties of sorption, the adsorption isotherms at 20, 30, and 40 °C were elaborated. Different adjustment models were used for data sorption, being that the GAB model presented the best fit for the adsorption data, with $R^2 \geq 0.99$. The results showed that the drying processes applied to the ACH complex films altered their polar binding sites, a behavior not observed for the AG complex samples, reinforcing that there are differences in the bonds forming the structure of each complex. For all samples, the adsorption process was enthalpy-controlled and not spontaneous, with Gibbs free energy (ΔG) > 0, indicative of higher storage stability (Carvalho & Noreña, 2015).

Finally, the colorimetric response of the films in samples of whole milk at pH 4.0 (spoiled), 5.5 (drink soon), and 6.8 (fresh) was tested. Samples AG0.5, AG1.0 and ACH0.5

showed differences in color that could be identified by the viewer and, therefore, were chosen for the construction of red chromatic shift (RCS) curves. The RCS curves as a function of pH facilitate the interpretation of the result by the viewer. With the aid of an image analyzer software capable of extracting the red, green, and blue (RGB) values of the indicator, it is possible to obtain the pH of the sample. The RCS curves showed a good data fit, with $R^2 \geq 0.94$, demonstrating a potential way of interpreting the data by the viewer, who with a smartphone camera and access to an image analysis application, could assess the pH of the product during its distribution, minimizing the possibility of food waste.

FINAL CONSIDERATIONS

The presented results demonstrate the potential of upcycling underexplored vegetal residues, such as non-pomace residues from grape juice production. It encourages the full use of the fruit, as well as contributes to the reduction of waste and better management of residues and by-products in the grape processing industry with the development of products that can be reinserted into the food market. The indicator films may minimize food waste during the distribution chain and after purchase by the consumer, contributing to establishing the shelf life of the food on the market and providing a chemically and microbiologically safe product for the consumer. In addition, they are biodegradable, helping to encourage the production of environmentally friendly packaging.

To define which indicator is most suitable for each food, the composition of the food matrix must be considered, as well as the pH range of interest for monitoring by the indicator. With the characterization presented throughout the work, highlighting the colorimetric efficiency tests, it can be stated that the lowest concentration of NPG was more efficient for the desired colorimetric response, and the film composed of alginate, chitosan and lower NPG content (ACH0.5) showed the best physicochemical characteristics for incorporation in food packaging.

PERSPECTIVES

To optimize the developed films, as well as to evaluate their commercial suitability, the following steps are suggested:

- Characterization of the fibers present in the non-pomace residue, as well as their isolation for use as support biopolymers;
- Extraction of anthocyanins, adding the extract to the support, aiming at the formation of more homogeneous films;
- Evaluate variations in physicochemical properties, such as fiber and anthocyanin content, of different batches of NPG to standardize the performance of the indicator to be produced.
- Study the stability of anthocyanins in the filmogenic support under different conditions of temperature and humidity;
- Evaluate the performance of easily accessible image analyzer applications on smartphones, such as *PhotoMetrix*, in processing RCS curves, as well as in correlating the concentration of anthocyanins with the food pH.

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