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EFFECT OF CHITOSAN INCLUSION ON THE PROPERTIES OF XANTHAN GUM-BASED EDIBLE FILMS

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ABSTRACT

Researches in food preservation have evolved into use of edible polysaccharide products and the most utilized polysaccharide is xanthan gum. In this study, xanthan gum and chitosan were used in composite coating material. The composite films were developed by casting using xanthan gum and chitosan. The films were prepared in varying proportion of xanthan gum and chitosan thus; 90:10, 92.5:7.5, 95:5, 97.5:2.5, and 100:0. The films were analyzed for mechanical properties (Young's modulus, elongation at break, strain at break, load at break, stress at break and energy at break). Using the elongation at break and Young's modulus as reference variables, films with the following proportion of xanthan-chitosan; 90:10, 95.5:5, 97.5:2.5 were further evaluated for barrier properties (thickness, solubility in water, oxygen permeability and water vapour permeability) and optical properties (opacity and colour parameters). The elongation at break of each composite film increased with decreased young modulus. The xanthan gum-based films brought about changes in thickness, water vapor permeability and solubility of films but did not change significantly ($p \leq 0.05$) the oxygen permeability. Furthermore, the produced films had low opacity values and the luminosity values of the films were directly linked to the transparency of the films. The films with the highest L* value had the least ΔE value and highest WI value. The findings showed that the developed composite polysaccharide films could be useful in reducing environmental problems associated with synthetic packaging.

Keywords: Barrier properties, Chitosan, Colour, Films, Mechanical properties, Xanthan gum

INTRODUCTION

Edible films and coatings have been used as an alternative to synthetic plastic for food applications because of their advantages over synthetic films, the main advantage of edible films over traditional synthetics is that they can be consumed with food products; they have also received considerable attention in recent years (Dhanapal *et al*., 2012). There is also no package to be disposed of and it could still contribute to the reduction of environmental pollution even if the films are not consumed. Edible films are produced exclusively from edible ingredients which are renewable and can therefore degrade more readily compared to synthetic materials (Enujiugha and Oyinloye, 2019).

The organoleptic properties of packaged foods can be enhanced by edible films provided they contain various components such as flavorings, sweeteners, and colorings. Edible films and coatings can be produced with a variety of natural products such as proteins, polysaccharides, and lipids; also with the addition of surfactants and plasticizers (Dhanapal *et al.*, 2012). Edible film's performance and functionality mainly depend on its color, and mechanical and barrier properties which depend on the composition and the formation process of the film while for edible coatings; the most important parameters are the capacity of the coating to adhere to the surface and the method of application on the product. Coating of food products is carried out by dipping or spraying which forms a thin film on the food surface and also acts as a semi-permeable membrane thereby helping to control moisture loss and/or suppress gas transfer (Lin and Zhao, 2007). Edible coating materials for fruits and vegetables are driven by anti-fungal activities, biodegradability, and eco-friendliness (Martinez-Romero *et al.*, 2006).

The application of protein and lipids in film formation has some limitations (Enujiugha *et al.*, 2013), especially the denaturation of protein and the enzymatic degradation of lipids (Oguntoyinbo *et al.*, 2023). Carbohydrate-based films are therefore most preferred as coating materials.

Xanthan gum is a high molecular weight extracellular polysaccharide produced by *Xanthomonas campestris* using glucose and sucrose as sole carbon sources (Freitas *et al.*, 2011; Palaniraj and Jayaraman, 2011). It is water-soluble and non-toxic and imparts a high viscosity in aqueous media at low concentrations with a strong shear-thinning behavior. Its solutions have rheological properties that are quite stable in a wide range of pH, temperature values, and ionic strength (Faria *et al.*, 2011; Freitas *et al.*, 2013).

Chitosan is a high molecular weight cationic polysaccharide with reported antibacterial and antifungal activities obtained from the deacetylation of chitin. Different factors such as alkali concentration, incubation time, temperature, ratio of chitin to alkali, and chitin source can affect its properties. It is insoluble in water but soluble in acidic solvents, non-toxic, biodegradable, and has great film-forming capacities (Ferreira *et al.*, 2009; Campos *et al.*, 2011).

Different plasticizers are essentially added to starch-based film because films prepared with starch alone are difficult to handle due to their brittleness and poor mechanical as well as barrier properties (Anjum *et al.*, 2017). Plasticizers are composed of small molecules of low volatility that interact with the starch chains, reduce internal hydrogen bonding, and improve the flexibility of the resultant films. They confer plastic-like properties and also allow easier removal of film from the forming support. They also affect the barrier and sorption characteristics of film and produce better packaging attributes. This study seeks to evaluate the use of xanthan gum and chitosan in the formation of edible coating material.

MATERIALS AND METHODS

Sample Collection

Chitosan and Xanthan gum powders and glycerol were obtained from Delson Pascals Ventures, Akure, Nigeria. The biochemical reagents used in the study were manufactured by Sigma-Aldrich, and supplied by Bristol Scientific, Lagos, Nigeria. All the reagents and chemicals used were of analytical grade and deionized distilled water used was obtained via distillation and deionization.

Preparation of films

The preparation of the films was carried out using the casting method described by Araujo-Farro *et al.* (2010) with slight modifications. Xanthan gum solution was prepared by a slow addition of the powder in 50 mL of distilled water under constant stirring at room temperature for 30 min (Arismendi *et al.,* 2013). The chitosan was dispersed in 50 mL of acetic acid solution (1% v/v) with a gentle stirring at room temperature until complete dissolution.

Five formulations of films were studied by varying the proportion of xanthan gum to chitosan thus; 100:0; 97.5:2.50; 95:5; 92.5:7.50 and 90:10. Glycerol at a concentration of 0.30 mL glycerol/g dry biopolymer was added to the dispersions of xanthan gum and chitosan for each treatment, as a plasticizer. The dispersions were later subjected to stirring using a homogenizer at 2000 rpm for 10 min to achieve complete homogenization (Lima *et al.,* 2017) and then subjected to centrifugation at 1000 rpm for 10 min to remove the bubbles present in the mixture.

Film casting

The film mixture was weighed (15 g) into petri dishes (9 cm diameter) and dried in a chamber (oven) under a controlled temperature at 38 ^{o}C for 18 h (Lima *et al.,* 2017). The dried films were peeled from the petri dishes and kept in a desiccator at 25 ^{o}C before analysis (Arismendi *et al.,* 2013).

Determination of film barrier properties

Film thickness measurement

Film thickness was measured by digital micrometer (Vernier caliper). The measurements (n=5) were taken for each film: one at the sample center and four more at varied perimeter sites. An average value was therefore calculated for each sample and used in Water Vapour Permeability and Oxygen Permeability determinations (Jangchud and Chinnan, 1997).

Solubility of the Film in Water

Solubility is the percentage of dry matter that is been solubilized after 24 h of film immersion in distilled water with respect to the initial dry matter (Gontard *et al.,* 1992). 2 cm diameter disks of the dried film were further dried in a vacuum oven at 100 ^{o}C for 24 h to determine the initial dry matter of the film. Other discs were cut, weighed and immersed in 50 ml of distilled water with stirring for 24 h at 25 ^{o}C. The films that did not solubilize were taken out and dried at 100 ^{o}C for 24 h; this is to determine the final weight of dry matter. Three replicates were taken for each sample (Arismendi *et al.,* 2013). The solubility was therefore calculated as follows:

$$MS\ (\%) = 100\ \text{X}\ \frac{\text{Mi}-\text{Mf}}{\text{Mi}} \quad (1)$$

Where, Mi is the initial mass of the dried film; Mf is the mass of the dried film insoluble in water after 24 h.

Oxygen Permeability (OP)

OP was determined with a MOCON unit (Ox-Tran 100A, Modern Control, Inc., Minneapolis, MN) according to Standard Method D3985-81 (ASTM, 1993c). Film samples were double masked by aluminum foil with an effective film test area of 50 cm^2. Testing was performed at 38 ^{o}C and 0% RH. OP was calculated by multiplying oxygen gas transmission rate (OGTR) by the thickness and dividing by partial pressure difference of oxygen across the film surface (Jangchud and Chinnan, 1999).

$$OP = \frac{\text{Oxygen gas transmission rate x thickness}}{Partial\ pressure\ difference\ of\ oxygen\ across\ the\ film\ surface} \quad (2)$$

Water Vapour Permeability (WVP)

WVP was measured using a modified ASTM method which was reported by Avena- Bustillos and Krochta (1993). Films were sealed in a glass permeation cup and kept in a cabinet desiccator with silica gel (0% RH); silica gel was heated at 180 °C for at least 3 hours, then cooled prior to use. The weight of the edible film was periodically observed until a constant weight is reached and WVP ($gm^{-1}s^{-1}Pa^{-1}$) of the film was calculated as follows:

$$WVP = \frac{\text{Weight of film (g)}\ x\ thickness\ of\ film\ (mm)}{Area\ of\ film\ (m2)\ x\ time\ (h)\ x\ P2{-}P1\ (kPa)} \quad (3)$$

Where, P1 is the Initial pressure; P2 is the Final pressure

Opacity of the Film

The film samples were cut into rectangles and placed on the internal side of a spectrophotometer cell according to a modified standard procedure of the British Standards Institution (Zuo *et al.,* 2019). Film opacity was calculated with the area under the absorbance curve with respect to wavelength from (400 to 600 nm) and was taken as the opacity of the film.

The following equation according to the method described by Gontard and Guilbert (1994) was used to calculate opacity of the films.

$$Opacity = absorbance\ at\ 500nm\ x\ film\ thickness \quad (4)$$

Determination of Colour

The colour of the films was determined by the method of Zhang *et al.* (2016) using a HunterLab ColourFlex EZ 45 colourimeter (HunterLab Associates Laboratory, Colourflex, USA). A white standard plate was used to calibrate the instrument. Lightness (L) and chromaticity parameters a (red-green) and b (yellow-blue) were measured by placing the film samples over the standard white plate. Three readings from random sites of each film were recorded. The total colour difference (ΔE) whiteness index (WI) and Yellowness Index (YI) were calculated using the following formulas:

$$\Delta E = (\text{L}* - \text{L}) + (\text{a}* - \text{a}) + (\text{b}* - \text{b}) \quad (5)$$

$$WI = 100 - (100 - L*) + a* + b* \quad (6)$$

$$YI = 142.86\ x\ \frac{L*}{b*} \quad (7)$$

Where L, a and b are the colour parameter values of the white standard (L is 91.83, a is −0.73, b is 1.52), and L*, a*, and b* are the colour parameter values of the films.

Determination of Mechanical Properties

The mechanical properties such as stress and strain at break, load at break, elongation at break and the modulus of elasticity of the produced film were determined according to ASTM standard method (ASTM D1037-99) using a Universal testing machine at Engineering Materials Development Institute (EMDI), Ondo State, Nigeria. The samples were placed in the testing area and held firmly in place by grips. The testing machine then applied force to the sample until it deformed. The results of the test were displayed on the screen

of the machine. The test was repeated for each sample and the readings were averaged.

RESULTS AND DISCUSSION

Mechanical Properties of Films

Films were analyzed in terms of their compressive modulus, compressive stress at yield and at break as well as elongation at yield and at break. Generally, the mechanical properties of edible films and coatings are determined by the film constituents, relative proportions and preparation conditions (Antares and Chiralt, 2016). Specific interactions between the film constituents such as crosslinks, different structural arrangements of the components or the formation of heterogeneous biphasic structures, must be taken into consideration. The mechanical properties of the films in current study are presented in Table 1. Elongation at break is a measure of the flexibility of the film and defines the ability of the film to deform in place before it collapses while the modulus is a recognized measure of mechanical strength in any material. It measures the stiffness of a solid material and gives a straightforward overview of the ability of the material to withstand stress without deformation. For all the films produced, it was observed that the higher the elongation at break, the lower the Young's modulus. Samples A, D and E had longer elongations at break ranging from 83.92 mm to 174.83 mm than samples B and C with values ranging from 42.68 mm to 44.65 mm. Samples B and C had increased Young's modulus with values ranging from 24040 KPa to 27250 KPa when compared to samples A, D and E which had values ranging from 111.38 KPa to 305.68 KPa. Since all the films were of uniform area and diameter before testing, the percent (%) deformation (strain) is directly proportional to the elongation. The load at break describes the maximum load which the film was able to withstand before deformation. It is a measure of the structural integrity of the film under stress. The stress at break can also be said to be the force at break. It is greatly affected by the load at break. Sample D (92.5:7.5 xanthan: chitosan) had the highest stress at break with value 1679 KPa as a direct result of being able to withstand more load while sample E (97.5:2.5 xanthan: chitosan) had the least with value 17.58 KPa. Energy at break represents the amount of energy in joules required to deform the film. The energy at break values affirm samples A and E having the highest value ranging from 0.08 J to 0.09 J when compared with samples B, C and D having the least values ranging from 0.01 J to 0.04 J. Therefore, using the Young's modulus, elongation at break and energy at break as reference, samples B, C and E were then used for further studies.

Film Barrier Properties

Edible films have some physicochemical properties which are required to keep integrity through production, transportation and storage. These properties are affected by the type of film, the quality and structure of the components and the production technology and conditions (García *et al.,* 2009). The barrier properties of the composite carbohydrate films produced in this study are presented in Table 2.

The film thickness is a vital parameter for the calculation of mechanical and barrier properties. The thickness of the films ranged from 0.003 mm to 0.004 mm. Film thicknesses observed were comparable to those obtained by Ekrami and Emam-Djomeh (2014) on salep-based films.

Film opacity is an important parameter of the packaging film as consumers prefer to be able to see the packed food items (Kanatt and Makwana, 2019). Opacity is a recognized measurement of the transparency of a film. A higher value of opacity means lesser transparency (Pereda *et al.,* 2011). Transparency is a relevant property of film since it has a direct impact on the appearance of the packaged product. Transparency is important to the function of edible film and can be expressed as a variable (Fang *et al*., 2006). The produced films had low opacity values ranging from 0.86 to 0.99. The inclusion of chitosan in xanthan gum films did not affect the oxygen vapor permeability of the films. However, solubility, opacity, water vapour permeability and thickness were changed significantly.

Table 1: Mechanical properties of composite carbohydrate films from Xanthan gum and Chitosan

Sample	Elongation at Break (mm)	Strain at Break (mm/mm)	Load at Break (N)	Stress at Break (KPa)	Energy at Break (J)	Young Modulus (KPa)
A	174.83±0.03^{a}	4.60±0.01^{a}	0.21±0.01^{b}	51.83±0.40^{d}	0.09±0.03^{a}	228.34±0.35^{e}
B	42.68±0.01^{e}	0.69±0.03^{d}	0.05±0.02^{e}	129.00±0.58^{c}	0.04±0.01^{c}	27250.00±0.42^{b}
C	44.65±0.02^{d}	0.74±0.05^{c}	0.06±0.01^{d}	211.80±0.10^{b}	0.01±0.05^{e}	24040.00±0.21^{c}
D	83.92±0.03^{c}	0.20±0.01^{e}	0.47±0.02^{a}	1679.00±0.21^{a}	0.03±0.03^{d}	111.38±0.32^{a}
E	103.08±0.01^{b}	3.82±0.02^{b}	0.07±0.03^{c}	17.58±0.58^{e}	0.08±0.02^{b}	305.68±0.58^{d}

*Values expressed as means ± standard deviation

*Values with the same superscript on the same column indicate no significant difference at p ≤0.05

A = 100% xanthan: 0% chitosan; B = 90% xanthan: 10% chitosan; C = 95% xanthan: 5% chitosan; D = 92.5% xanthan: 7.5% chitosan; E = 97.5% xanthan: 2.5% chitosan

Table 2: Barrier properties of composite carbohydrate films from Xanthan gum and Chitosan

Sample	Solubility in water (%)	Opacity	Oxygen Permeability ($gm^{-1}s^{-1}Pa^{-1}$)	Water Vapour Permeability ($gm^{-1}s^{-1}Pa^{-1}$)	Thickness (mm)
B	43.44±0.01^{c}	0.92±0.06^{b}	0.01±0.02^{a}	0.76±0.04^{b}	0.004±0.003^{a}
C	43.61±0.01^{b}	0.86±0.02^{c}	0.01±0.06^{a}	0.73±0.02^{a}	0.003±0.001^{b}
E	44.12±0.02^{a}	0.99±0.04^{a}	0.01±0.05^{a}	0.89±0.05^{c}	0.004±0.003^{a}

*Values expressed as means ± standard deviation

*Values with the same superscript on the same column indicate no significant difference at p ≤0.05

B = 90% xanthan: 10% chitosan, C = 95%xanthan: 5%chitosan, E = 97.5%xanthan: 2.5%chitosan

Solubility can be defined as the maximum quantity of a substance that can be completely dissolved in a given amount of solvent and represents a primary concept in research fields such as chemistry, physics, food science, pharmaceutical, and biological sciences. Film solubility may be regarded as the degree of water resistance and the integrity of a film (Rhim, *et al.,* 1999). The produced films had high solubility values ranging from 43.44% to 44.12%. This increased solubility is because polysaccharide edible films are often hydrophilic and so, the majority of these films have higher solubility in water (Ekrami and Emam-Djomeh, 2014).

Oxygen in food causes oxidation which in turn affects several food properties like colour, flavour and sometimes the nutrient content (Munoz-Bonilla and Fernandez-Garcia, 2012). The ability of films to retard oxidation or degradation of the product is an important characteristic that affects the final product quality and food shelf-life. Films made from carbohydrates are excellent barriers to oxygen, because of their tightly packed, ordered hydrogen-bonded network structure (Yang and Paulson, 2000). Oxygen permeability of the films showed that they had good oxygen impedance with values ranging from 0.0072 $gm^{-1}s^{-1}Pa^{-1}$ to 0.0089 $gm^{-1}s^{-1}Pa^{-1}$. This is in agreement with the work of Gaudin et al. (2000) where the antiplasticisation and oxygen permeability of starch-sorbitol films were carried out.

The water vapour permeability (WVP) is an important indicator of the ability of a film to retard food spoilage and increase the shelf-life of the product to which it is applied. WVP is affected by film composition, chemical structure, degree of cross linking and use of plasticizers. Other factors which affect the WVP are temperature and relative humidity (Olivas *et al.,* 2009). The films had low water vapour permeability values ranged from 0.727-0.891 $gm^{-1}s^{-1}Pa^{-1}$. This result is similar to the results of other studies on edible film (Nazan-Turhan and S¸ahbaz 2004; Amadi *et al*. 2012). Plasticizers may aid in the formation of rigid structures in the film matrix.

Colour Parameters of the Produced Films

Colour is an essential feature for the acceptability of biopolymer packaging film; it also reflects consumer's willingness and purchasing behavior for a food product (Bharti *et al.,* 2020). Consumer acceptability of the product may be affected by the film colour. Generally, the desirable characteristics in edible film packaging and coating are high transparency and lightness (Razavi *et al.,* 2015; Ren, 2008). The measured colour parameters including Lightness/darkness (L*), red/green index (a*), yellow/blue index (b*), total colour difference (ΔE), whiteness index (WI) and yellowness index (YI) of composite carbohydrate films and the CIE L*a*b* coordinates are presented in Table 3. It can be seen in all the films that luminosity values ranged from 92.70 to 94.20 (indicator of lightness) while a* values ranged from 3.27 to 3.65 (indicator of the tendency towards redness) and b* values ranged from 11.97 to 12.76 (indicator of the tendency towards yellowness) and were directly linked to the transparency of the films. This is in agreement with the findings of Ghasemlou et al. (2011) where the development and characterization of a new biodegrable edible film made from kefiran, an exopolysaccharide was obtained from kefir grains.

Other colour functions such as ΔE, WI and YI can be used to interpret the colour changes of films. The ΔE shows the degree of total colour difference of the films and it was observed that the film with the highest L* value had the least ΔE value. The results are in agreement with those of Siracusa et al. (2018) where it was observed that films from Citral Essential Oil, Alginate and Pectin with higher ΔE values were less transparent. Opposite trends were observed for WI as the film with the lowest L* value had the lowest WI value. The differences might be owing to the chemical compositions of film-forming solutions. YI values were also significantly different for all the films produced. Colour is of crucial importance for biopolymer films and is greatly influenced by factors such as substrate type, manufacturing parameters and storage conditions (Zhang *et al.,* 2016).

Table 3: Colour parameters of composite carbohydrate films from Xanthan gum and Chitosan

Sample	L*	a*	b*	ΔE	WI	YI
B	92.70 ± 0.58^{c}	3.65 ± 0.01^{a}	12.76 ± 0.02^{a}	12.06 ± 0.010^{a}	84.84 ± 0.02^{c}	19.66 ± 0.01^{a}
C	94.20 ± 0.10^{a}	3.27 ± 0.02^{c}	11.97 ± 0.04^{c}	11.44 ± 0.01^{c}	86.30 ± 0.02^{a}	18.15 ± 0.01^{c}
E	93.40 ± 0.10^{b}	3.51 ± 0.01^{b}	12.50 ± 0.03^{b}	11.87 ± 0.03^{b}	85.43 ± 0.01^{b}	19.12 ± 0.02^{b}

*Values expressed as means ± standard deviation

*Values with the same superscript on the same column indicate no significant difference at $p \leq 0.05$

B = 90% xanthan: 10% chitosan, **C =** 95% xanthan: 5% chitosan, **E =** 97.5% xanthan: 2.5% chitosan **L* -** luminosity, **a* -** red/green index, **b* -** yellow/blue index, **ΔE –** total colour difference, **WI –** whiteness index, **YI –** yellowness index

CONCLUSION

Composite carbohydrate films with good mechanical properties and barrier properties were produced from Xanthan gum and Chitosan in this study. The increased addition of chitosan improved the mechanical and barrier properties. This work has shown the potential of Xanthan gum and Chitosan mix in the formation of edible film.

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