

Functional Properties of Edible Agar-Based and Starch-Based Films for Food Quality Preservation

THE D. PHAN,[†] F. DEBEAUFORT,^{*,†,§} D. LUU,[#] AND A. VOILLEY[†]

ENSBANA-IMSAPS, Université de Bourgogne, 1 esplanade Erasme, F-21000 Dijon, France;
 IUT-Génie Biologique, Boulevard Dr. Petitjean, B.P. 17867, F-21078 Dijon Cedex, France; and
 University of Technology, 268 Ly Thuong Kiet, HoChiMinh City, Vietnam

Edible films made of agar (AG), cassava starch (CAS), normal rice starch (NRS), and waxy (glutinous) rice starch (WRS) were elaborated and tested for a potential use as edible packaging or coating. Their water vapor permeabilities (WVP) were comparable with those of most of the polysaccharide-based films and with some protein-based films. Depending on the environmental moisture pressure, the WVP of the films varies and remains constant when the relative humidity (RH) is > 84%. Equilibrium sorption isotherms of these films have been measured; the Guggenheim–Anderson–de Boer (GAB) model was used to describe the sorption isotherm and contributed to a better knowledge of hydration properties. Surface hydrophobicity and wettability of these films were also investigated using the sessile drop contact angle method. The results obtained suggested the migration of the lipid fraction toward evaporation surface during film drying. Among these polysaccharide-based films, AG-based film and CAS-based film displayed more interesting mechanical properties: they are transparent, clear, homogeneous, flexible, and easily handled. NRS- and WRS-based films were relatively brittle and have a low tension resistance. Microstructure of film cross section was observed by environmental scanning electron microscopy to better understand the effect of the structure on the functional properties. The results suggest that AG-based film and CAS-based films, which show better functional properties, are promising systems to be used as food packaging or coating instead of NRS- and WRS-based films.

KEYWORDS: Water vapor permeability; water absorption rate; surface hydrophobicity; sorption isotherm; agar; rice starch; cassava starch

INTRODUCTION

Since the end of the 1980s, edible films and coatings have been used as innovative food packagings that have been defined as “a type of packaging that like (is) a film, a sheet, a thin layer or coating and it is the integral part of (the) food and can be eaten together with (it)” (1). It has been introduced as a barrier layer to improve the consumer-acceptable shelf life of food products. The protective function of edible film and coating is to prevent the transfer of moisture, oxygen, flavor, and/or oil content between food and the surrounding medium and/or between different compartments in a heterogeneous food. Usually, the film-forming substances are based on proteins, polysaccharides, lipids, and resins or on a combination of these (2).

With regard to the polysaccharides, because of their wide variety of structures, various film-forming behaviors may occur

during the manufacture. Consequently, films made from different types of polysaccharides are possible to display a wide range of properties (3). The polysaccharides may be classified on the basis of both their physical properties and their composition in monosaccharides. Starch and agar are obviously different in their component units, but they can be also distinguished by their chemical structure, starch being granular and agar being fibrous (4). For the film formation and properties, these structure differences are important: starch forms a thick gel after gelatinization, whereas, even at high concentration (4–5% w/w), agar forms a slightly viscous solution after solubilization in hot water and then becomes a thermoreversible gel when the temperature is brought down.

Starch is mainly composed of two macromolecular polymers of α -D-glucopyranosyl, amylose, and amylopectin. Amylose is a linear glucan with α ,1–4 glycosidic linkages and has a molecular mass between 10^5 and 10^6 g·mol⁻¹ (5). Conversely, amylopectin is a highly branched polymer composed of short linear chains branched on longer chain by α ,1–6 linkages. Its molecular chain is > 10^8 g·mol⁻¹ (6). Depending on botanical origin and environmental conditions during growth and at harvest, the amylose content in cassava starch ranges from 17

* Address correspondence to this author at Lab. Imsaps, Ensbanana, 1 Esplanade Erasme, F-21000 Dijon Cedex, France [telephone +33 (0) 3 80 39 68 43; fax +33 (0) 3 80 39 66 11; e-mail debeaufort@u-bourgogne.fr].

[†] ENSBANA-IMSAPS.

[§] IUT-Génie Biologique.

[#] University of Technology.

to 24%, and a formation of a translucent gel with high viscosity and a tendency to retrograde was observed after a heat–moisture treatment (7). On the other hand, the amylose content in rice has been reported to vary from 0 to 33%. Rice varieties may be classified as waxy (or glutinous), very low, low, intermediate, or high with 0–2, 5–12, 12–20, 20–25, and >25% of apparent amylose, respectively (8). Starches separated from various rice cultivars showed significant differences in physicochemical, thermal, and rheological properties (9).

Agar, which exists in algae as a gel at the temperature of the natural environment, is a gelatinous product from the red algae class (Rhodophyceae). Agar is a heterogeneous complex mixture of related polysaccharides having the same backbone chain structure. The main components of the chain are D-galactopyranose and 3,6-anhydro-L-galactopyranose, which alternate through α -(1,4) and β -(1,3) linkages. Agar is lightly sulfated; the main fractions are agarose, a neutral polymer, and agaropectin, a sulfated polymer. In addition, the charged chains have pyruvic acid bound in ketal form. Depending on the source of the agar, the molecular weight of the chains varies from 80 000 to 140 000. Agar is insoluble in cold water and slightly soluble in ethanolamine, whereas in the dried state, it is soluble in hot water (10, 11).

Polysaccharide film-forming materials such as starch and starch derivatives, cellulose derivatives, alginate, carrageenan, chitosan, pectinate, and various gums have been studied extensively for the development of edible packaging. However, few studies have been carried out on edible films made from rice starch, cassava starch, and agar. On the other hand, these materials are produced in Vietnam for use in the manufacture of foodstuffs. Indeed, although rice starch and cassava starch are efficient thickeners and binding agents, they are used mainly and extensively in the production of soups, sauces, pastry filling, etc., whereas agar is usually used as a gel builder in candy and desserts. The low cost and the difference in their gel-forming behaviors are the main driving forces inducing investigations on new applications of these polysaccharides. The objective of this work is to study the potential use of these materials as edible packaging and their functional properties.

MATERIALS AND METHODS

Materials. Agar (AG) (food quality, Ha Long Co.), cassava starch (CAS) (99.8% of starch), waxy rice starch (WRS) (91.4% of starch), and normal rice starch (NRS) (99.1% of starch) (food grade, Tai Ky Food Enterprise) were used as the film-forming component to provide a continuous matrix of edible film. Anhydrous glycerol (98% purity, Fluka Chemical) was added to improve their mechanical properties. Two homogeneous edible films were also composed of pure hydroxypropylmethylcellulose (HPMC; E15 LV Premium, Dow Chemical) and arabinoxylan (AX, ULICE, D quality). They are used as a reference for comparison of the film properties. Two synthetic films were also used as standards: a cellophane film (300P, Courtauld's), hydrophilic but not soluble in water, and a low-density polyethylene film (LDPE, Riblène FF30, EnChem). All of the test films are considered to be dense and nonporous.

Preparation of Homogeneous Edible Films. Agar films were obtained after solubilization of 3 g of AG in 100 mL of osmosed water at 95 °C for 30 min under a 600 rpm magnetic stirring. Then, glycerol was added at a concentration of 15% of the total dry basis. The film-forming solution was kept for 10 min under the same conditions of temperature and stirring prior to being spread onto a glass plate that had been previously covered with a polyvinyl chloride adhesive sheet to prevent the sticking of dried films. A continuous thickness of 1000 μ m was applied for the cast solution using a thin-layer chromatography spreader. All of the film was dried for 5 h in a ventilated cupboard (KBF 240 Binder, ODIL) with temperature and relative humidity (RH)

fixed at 40 °C and 30% RH, respectively. The CAS, NRS, WRS, and AX films were prepared according to the same procedure except that the film-forming solutions was composed of 5 g for the CAS and NRS films, 4 g for the WRS film, and 16 g for the AX film in 100 mL of water. In the case of AG and AX, the choice of concentration for film-forming solutions was based mainly on their solubility in hot water and solution viscosity, allowing its spreading for the film formation. For films of CAS, NRS, and WRS, the dry matter was chosen to obtain an appropriate viscosity that facilitates the air bubbles' removal by vacuum. For the HPMC film, a solution of 9 g of HPMC dissolved in 100 mL of water/ethyl alcohol mixture (75:25, w/w) was prepared; casting and drying remained the same procedure as that previously described.

Chemical Analysis. Nitrogen content N was determined according to the micro-Kjeldahl method (Büchi AG B-339), and then protein content was calculated as $N \times 6.25$. The lipid content was determined using the Soxhlet method with diethyl ether as solvent. Total starch, amylose, and amylopectin contents were determined according to the method of Jarvis and Walker (12). Defatted starch (20 mg) was dissolved in 5 mL of 1 M KOH solution, and then 5 mL of distilled water was added. Next, 1 mL of this solution was neutralized with 5 mL of 0.1 M HCl, iodine reagent (0.5 mL) was added, and the volume was made up to 50 mL with distilled water. All measurements were carried out at room temperature (18 °C). Absorbances were read, using a Varian DMS80 spectrophotometer, at the following wavelengths: 504, 525, 548, 580, 630, 700, and 800 nm. Pairs of simultaneous equations based on Beer's law were used.

$$\text{Abs}_1 = E_{0,\text{am}1} \times C_{\text{am}} + E_{0,\text{ap}1} \times C_{\text{ap}}$$

$$\text{Abs}_2 = E_{0,\text{am}2} \times C_{\text{am}} + E_{0,\text{ap}2} \times C_{\text{ap}}$$

Abs₁ and Abs₂ are the sample absorbance at the first and second wavelengths; $E_{0,\text{am}1}$ and $E_{0,\text{am}2}$ are the absorptivities of amylose at the first and second wavelengths; $E_{0,\text{ap}1}$ and $E_{0,\text{ap}2}$ are the absorptivities of amylopectin at the first and second wavelengths; C_{am} is the amylose concentration ($\mu\text{g}\cdot\text{mL}^{-1}$); and C_{ap} is the amylopectin concentration ($\mu\text{g}\cdot\text{mL}^{-1}$). The following equations were derived from the above two simultaneous equations and used to estimate C_{am} and C_{ap} :

$$C_{\text{am}} = \frac{(\text{Abs}_2 \times E_{0,\text{ap}1}/E_{0,\text{ap}2}) - \text{Abs}_1}{(E_{0,\text{am}2} \times E_{0,\text{ap}1}/E_{0,\text{ap}2}) - E_{0,\text{am}1}}$$

$$C_{\text{ap}} = \frac{\text{Abs}_1 - (E_{0,\text{am}1} \times C_{\text{am}})}{E_{0,\text{ap}1}}$$

A Microsoft Excel spreadsheet was used to solve these simultaneous equations for C_{am} and C_{ap} for each of 21 combinations of the seven wavelengths and calculate the mean of C_{am} and C_{ap} estimations.

Characterization of the Films. *Film Thickness.* The thickness of films was determined by using an electronic gauge (Multicheck FE, SODEXIM) with a precision range between 0.1 and 1% as a function of thickness value (0–100 or 0–1000 μ m). Ten replicates were done on each film-making. *Film microstructure* was also observed by environmental scanning electron microscopy (ESEM, Phillips XL 30 ESEM). A 5 \times 10 mm² film was fixed on the support using double-side adhesive tape, with an angle of 90° to the surface, which allowed observation of the film cross section.

Water Vapor Permeability (WVP). The WVP at a relative humidity differential of 57–22, 84–22, or 99–22% was measured using a modified French standard method (13), homologous to the ASTM E96-80 method (14) and adapted to edible materials by Debeaufort et al. (15) and McHugh et al. (16). Prior to measurement of WVP, all film samples were equilibrated at 25 °C for 48 h in a desiccator over potassium acetate saturated solution (CH₃COOH, Merck), which fixed the RH at 22%. Film samples were then placed between two Teflon rings on the top of the glass cell containing a saturated salt solution of sodium bromide, potassium chloride (NaBr; KCl, Merck), or distilled water, of which the water activities were 0.57, 0.84, and 0.99, respectively, at 25 °C. The test cell was introduced into a climate-

Table 1. Main Composition of Film-Forming Materials

	film-forming material			
	agar	cassava starch	normal rice starch	waxy rice starch
carbohydrates	93.78	99.80	99.11	91.44
starch				
amylose		26.78	19.50	3.64
amylopectin		73.02	79.61	87.80
protein	6.17	0.15	0.73	8.26
lipid	0.05	0.04	0.13	0.28

controlled chamber (KBF 240 Binder, ODIL) regulated at 22% RH and 25 °C. The test cell was periodically weighted to a constant weight variation rate. WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was calculated using the equation

$$\text{WVP} = \frac{\Delta m \times x}{A \times \Delta t \times \Delta p}$$

where Δm is the weight loss (g) of the test cell, x is the film thickness (m), and A is exposed area ($8.11 \times 10^{-4} \text{m}^2$) during Δt duration (s) under Δp partial water vapor pressure (Pa).

Moisture Sorption Isotherm. The moisture sorption isotherm was determined at 25 °C. Film samples were conditioned in a desiccator for 7 days at $\approx 0\%$ RH to reduce the initial moisture content. Samples were then placed over saturated salt solutions having the desired 22, 32, 57, 84, and 90% RH conditions. The equilibrium conditions were reached when the two consecutive weighings were equal. Equilibrium moisture content was calculated from the increase in mass of the sample after equilibration reported to the dried mass obtained after drying in an oven at 103 °C for 10 h. The Guggenheim–Anderson–de Boer (GAB) model was used to fit film sorption isotherm data

$$M = \frac{M_0 C k a_w}{(1 - k a_w)(1 - k a_w + C k a_w)}$$

where M is the equilibrium moisture content at the water activity a_w , M_0 is the monolayer moisture content, C is the Guggenheim constant, and k is the corrective constant taking into account properties of multilayer molecules with respect to the bulk liquid. GAB equation parameters were calculated from the Water Analysis software (ver. 97.4) performed by Labuza (2000).

Film Solubility. The film solubility was determined at 25 °C; for this purpose, the film samples at first were kept in a desiccator containing silica gel (SiO_2 , Prolabo) until they reached a constant weight, and then ~ 500 mg of each film was immersed in a beaker containing 50 mL of distilled water under 500 rpm magnetic stirring for 24 h. The content in the beaker was filtered using a glass microfiber filter (catalog no. 1822 090, Whatman). The insoluble matter was determined according to the oven method at 105 °C. The percentage of soluble matter (%SM) of the films was calculated and referred to the initial dry sample weight.

Table 2. Water Vapor Permeability, Contact Angle, and Water Adsorption Rate of Agar (AG), Cassava Starch (CAS), Normal Rice Starch (NRS), and Waxy Rice Starch (WRS) Based Films and of Hydroxypropylmethylcellulose (HPMC), Arabinoxylan (AX), Cellophane, and Low-Density Polyethylene (LDPE) Films at 25 °C^a

composition of film	lipid content in raw material (g/100 g)	thickness (μm)	water vapor permeability ($10^{-11} \text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$)			contact angle (deg)	water adsorption rate ($10^{-3} \mu\text{L s}^{-1}$)
			$\Delta\text{RH} = 22\text{--}57\%$	$\Delta\text{RH} = 22\text{--}84\%$	$\Delta\text{RH} = 22\text{--}99\%$		
AG + 15% glycerin	0.05	37.38 ± 1.17	$7.21 \pm 0.320\text{b}$	$12.52 \pm 0.295\text{cd}$	$13.70 \pm 0.935\text{b}$	$92.58 \pm 3.48\text{c}$	$143 \pm 27\text{bc}$
CAS + 15% glycerin	0.04	47.77 ± 5.81	$5.58 \pm 0.226\text{a}$	$11.19 \pm 1.003\text{c}$	$11.22 \pm 0.696\text{ab}$	$50.44 \pm 4.42\text{a}$	$478 \pm 54\text{d}$
NRS + 15% glycerin	0.13	49.12 ± 9.73	$7.23 \pm 1.087\text{b}$	$16.59 \pm 2.604\text{e}$	$16.75 \pm 1.811\text{c}$	$90.13 \pm 4.68\text{c}$	$88 \pm 24\text{b}$
WRS + 15% glycerin	0.28	34.96 ± 7.47	$12.26 \pm 1.650\text{c}$	$21.49 \pm 3.450\text{f}$	$21.03 \pm 3.941\text{d}$	$92.57 \pm 2.12\text{c}$	$829 \pm 69\text{e}$
HPMC + 15% glycerin	0.00	35.33 ± 2.58	nd ^b	$19.73 \pm 0.556\text{f}$	nd	$64.59 \pm 1.26\text{b}$	$444 \pm 25\text{d}$
AX + 15% glycerin	nd	57.82 ± 2.16	nd	$13.70 \pm 0.720\text{d}$	nd	$65.55 \pm 3.77\text{b}$	$193 \pm 27\text{c}$
cellophane	0	22.00 ± 0.41	$4.54 \pm 0.134\text{a}$	$7.94 \pm 0.253\text{b}$	$8.37 \pm 0.072\text{a}$	nd	nd
LDPE	0	30.00	nd	$0.19 \pm 0.010\text{a}$	nd	$105.45 \pm 0.52\text{d}$	$20 \pm 09\text{a}$

^a Values in the same column followed by the same letter are not significantly different at the $p < 0.05$ level. ^b Not determined.

Surface Hydrophobicity and Wettability. The surface hydrophobicity and wettability of the film were evaluated from the contact angle measurement of water droplet deposited on the film surface using a G1 Krüss goniometer (KRÜSS GmbH) equipped with image analysis software (Drop Shape Analysis, KRÜSS). Kinetics of contact angle and liquid moisture adsorption rate were determined. Prior to measurement, all film samples were conditioned at 57% RH and 25 °C.

Mechanical Properties. Tensile strength at breaking (TS) and percentage of elongation (%E) were measured using a Universal Testing Instrument (Instron UTTI 1122, Instron Ltd.) with a 5 kN load cell. Samples were stored at 57% RH over the sodium bromide saturated solution for 10 days at 25 °C prior to measurement. A total of 20 samples for each type of film were stretched at a constant rate of 100 mm min^{-1} . The effective dimension of the film before the stretching was $20 \times 60 \text{mm}^2$.

Significance. Each film attribute was measured at least in triplicate, and the differences between means were tested at the $p < 0.05$ level using Statgraph software.

RESULTS AND DISCUSSION

The main composition of materials used in film formulation is presented in **Table 1**. The principal difference among the three starches is the amylose content. In addition, the lipid content of these three types of starch seems to be inversely proportional to the amylose content. Cassava starch (CAS) contains the highest amylose fraction but the lowest lipid content. On the contrary, waxy rice starch (WRS) has the lowest amylose fraction but the highest lipid content. It is important to note that the protein fraction of WRS is relatively high in comparison with other starches.

The film obtained from an aqueous solution of 3% AG and plasticized with glycerin was transparent, clear, homogeneous, flexible, and easily handled. A similar appearance was observed for the film made of CAS, except it is less flexible than the AG-based film. However, films obtained from the rice starches (NRS and WRS) were slightly opaque and brittle, especially the WRS film. The average thickness of the films is given in **Table 2**. AG- and WRS-based films have the same range of thickness, which is from 35 to 38 μm , whereas that of CAS and NRS is slightly higher, varying between 48 and 50 μm . These differences could be due to the differences in the viscosity and the matter concentration of the film-forming solutions. The AG solution is slightly viscous, whereas CAS and NRS form a thick gel after gelatinization; their thickness is thus higher than that of AG-based film. Moreover, the lower thickness of WRS-based film could be explained by its lower film-forming gel concentration. It could be noted that the film thickness, to some extent, can affect their functional properties such as WVP and tensile strength. The reason should be the over-plasticizing effect

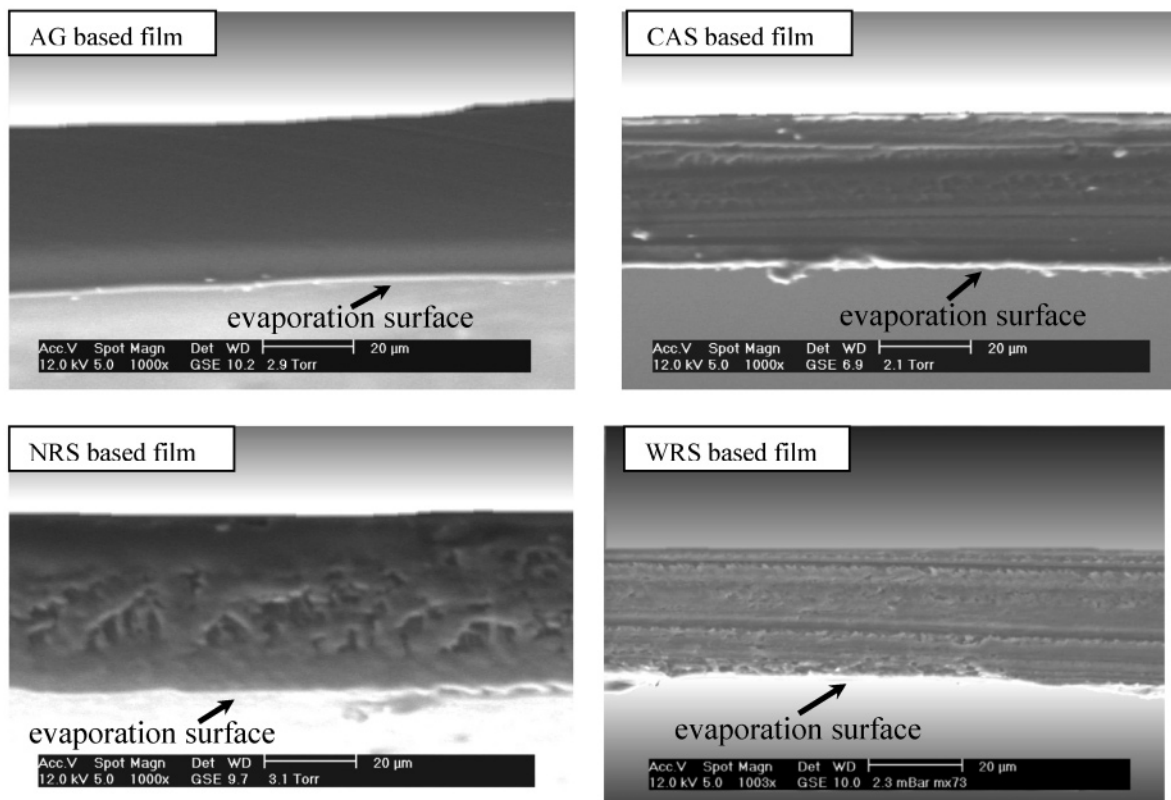


Figure 1. ESEM micrographs ($\times 1000$) of the cross section of AG-, CAS-, NRS-, and WRS-based films.

of water when the film is exposed to the high relative humidity. However, several authors observed that film WVP is not significantly affected because the thickness is over a range of 30–60 μm (2, 17–19). Moreover, as the barrier thickness is taken into account with the WVP and mechanical properties, they could be compared and discussed even if the film thickness is slightly different.

Film Microstructure. Environmental scanning electron microscopy (ESEM) observations of cross sections of films are reported in Figure 1. The films were cut with a razor blade prior to ESEM observation; therefore, some defects could be observed on some film cross sections. An AG-based film micrograph shows a relatively smooth and continuous cross section without pores or cracks, which confirms a dense and homogeneous structure. This may result from a formation of strong and homogeneous AG gel prior to setting of the film. A micrograph cross section of starch-based films displayed an irregular and rough structure. This suggests a heterogeneous structure due to the retrogradation and partial crystallization of gelatinized starch before the formation of the film. However, at this magnification (1000 \times), the micrograph does not allow one to detect the network structure of the films to ensure their structural homogeneity and structural integrity.

WVP of Films. WVP of films for the relative humidity differentials of 57–22, 84–22, and 99–22% was measured at 25 $^{\circ}\text{C}$. WVP values are presented in Table 2. It is well-known that thickness influences the transfer rate and thus the permeability of moisture through edible films (20, 21). Fick's law requires that permeance, under a given vapor pressure gradient, should vary inversely with film thickness. However, many authors observed that WVP is not significantly affected because the thickness is over a range of 30–60 μm (2, 17–19). Therefore, if the permeability is a property of the film and is homogeneous throughout the film, the value will be unaffected by film thickness and becomes the intrinsic permeability (22).

Consequently, in our case, the WVP of the films can be compared and discussed for the reason that the film thickness is in a range of 35–50 μm .

From Table 2 it is evident that all of the edible films obtained exhibited poor moisture barrier properties compared to LDPE because of the inherent hydrophilicity of the polysaccharides and the plasticizers used in the formulations. They have WVP nearly 2–3 times greater than that of cellophane (hydrophilic synthetic film) and nearly 2 orders of magnitude greater than that of LDPE film. However, they are of the same order of magnitude as that of gluten films (23) or whey protein films (24). For the same reasons, environmental RH and moisture pressure gradient significantly affect the WVP of the films studied. Indeed, our results show that WVP increases with RH gradient. Nevertheless, the WVP measured at 84–22% and those at 99–22% RH differential were not significantly different. WVP of films tends to reach a plateau at high moisture environments. In this case, the moisture tends to plasticize the biopolymer network, which probably swells at RH up to 80%. Therefore, the “swollen network of starch or agar facilitate(s) the diffusion of water molecules”. On the other hand, for edible films, water is a common plasticizer, and the adsorption of water can convert the film glassy state into an amorphous rubbery state. In this case, the internal viscosity decreases and internal mobility increases; the water molecule diffusion into and through rubbery material is thus more rapid than through the glassy material. Consequently, the exposure of one of the film sides to high environmental humidity could induce this transition, which may affect their WVP. Moreover, from mechanical measurement (discussed later), the shape of stress–strain curves (Figure 6) gives an idea of the state of the material at 53% RH and 25 $^{\circ}\text{C}$ according to the works of Gibson and Ashby (25). Indeed, AG-based film exhibits a typical rubbery behavior with a large extension, whereas NRS and WRS are in a glassy state characterized by a linear-elasticity to fracture. For CAS-based

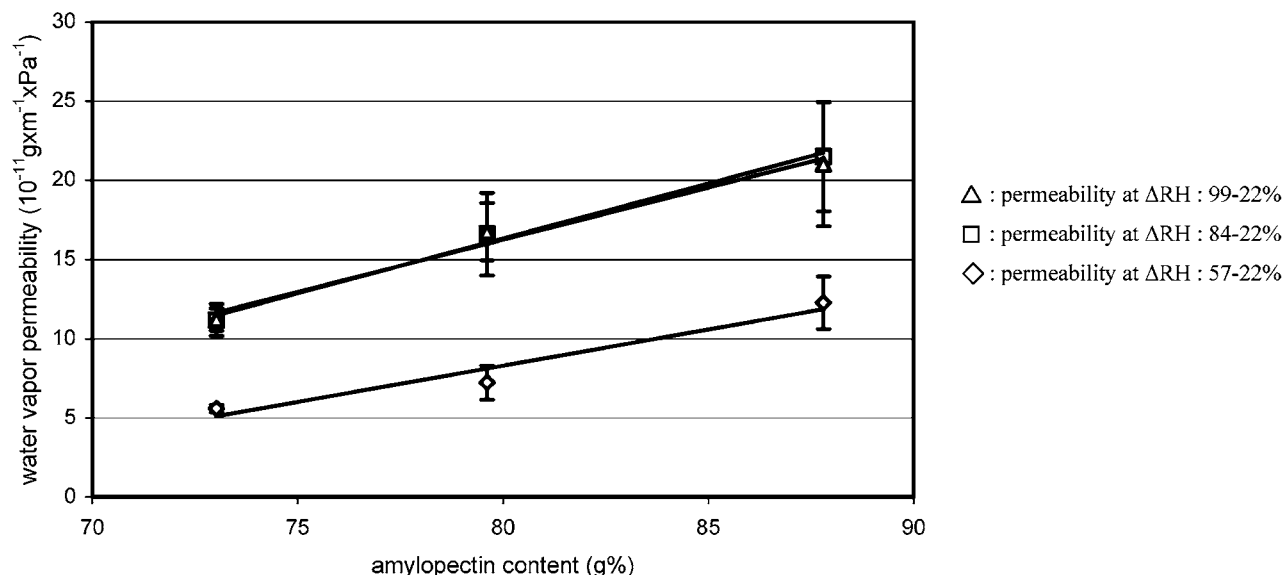


Figure 2. Water vapor permeability as a function of amylopectin content in starch-based films.

film, a yield point followed by a small extension shows that this film is near the glass transition state.

Compared to starch films, at 57–22% RH differential AG-based film has a WVP higher than that of CAS film, but it is nearly the same value as that of rice starch films. However, in the case of high water vapor pressure, the WVP of AG-based film was comparable with that of CAS film and significantly lower than that of rice starch films. Consequently, the moisture barrier of films seems to depend not only on the RH environment but also on the nature and chemical structure of the film-forming substance (17). The differences in apparent cross-section micrographs may contribute to some extent to the differences in WVP; the more compact and homogeneous structure of AG-based film and CAS starch film leads to a lower WVP.

Among starch-based films, it could be also noticed that CAS film seems to have a better moisture barrier efficiency than WRS-based film. As all starch films were prepared according to the same process, this difference can be due to the variation of amylose content in film-forming starches (26, 27). The percentage of amylose and amylopectin in the film-forming raw materials was thus determined. As shown in Figure 2, the WVP of the films is directly proportional to the amylopectin content. Similar results on the better barrier property of amylose films compared with that of amylopectin films were reported by Rindlaw-Westling et al. (28). On the other hand, the effect of amylose content on the WVP of the starch-based films could be suggested by the crystallization of amylose chains in the dried films as demonstrated Myllärinen et al. (29); amylose films showed B-type crystalline structures, whereas amylopectin films were completely amorphous. Indeed, diffusion of moisture is easier in amorphous systems than in crystalline one.

Moisture Isotherm Sorption. The GAB equation was applied to sorption isotherms of the films studied. The GAB equation parameters have been determined and are presented in Table 3. The values of k (< 1) and the correlation coefficient ($R^2 > 0.98$) show that the GAB equation gives a good fit to experimental values. Figure 3 represents a GAB moisture sorption isotherm curve of the films at 25 °C. In general, the moisture sorption isotherm of all of the films studied displays sigmoidal shape and indicates that the equilibrium moisture content increases slowly with increasing environmental a_w up to 0.7, beyond which a steep rise in moisture content in film samples was observed. The sigmoidal shape of the sorption

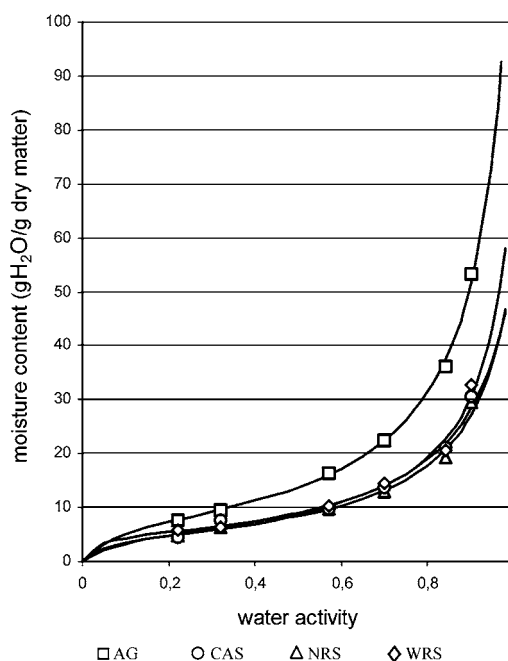


Figure 3. Isotherm sorption of AG-, CAS-, NRS-, and WRS-based films: (□, ○, △, ◇) experimental points; (—) GAB model.

Table 3. GAB Equation Parameters for AG, CAS, NRS, and WRS Films at 25 °C

composition of film	M_0 (g of H ₂ O/g of solids)	C	K	R^2
AG + 15% glycerin	0.0793	12.697	0.9432	0.996
CAS + 15% glycerin	0.0546	11.897	0.9014	0.982
NRS + 15% glycerin	0.0487	17.051	0.9148	0.982
WRS + 15% glycerin	0.0491	38.457	0.9342	0.983

isotherm is well cited in the literature for most food material, including edible films (30, 31).

Gennadios et al. (32) reported that the hydrophilic characteristic (evaluated from moisture sorption behavior) of film polymers was the most important factor in determining the RH sensitivity of films. Indeed, AG-based film shows higher monolayer moisture content compared to all starch films, which

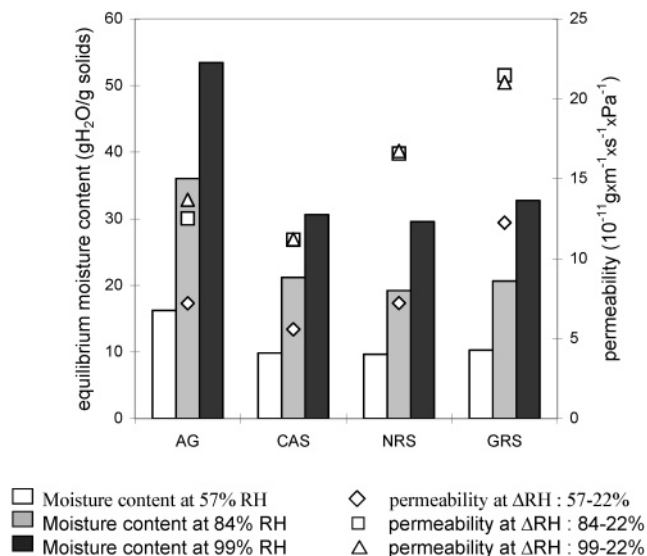


Figure 4. Relationship between equilibrium moisture content and permeability of AG-, CAS-, NRS-, and WRS-based films.

indicated that the monolayer moisture content and moisture sorption were attributable to the nature of the film-forming substance. Combined with sorption isotherm curves in **Figure 3**, it could be deduced that the AG-based film is more hygroscopic than starch-based films, whereas no significant difference is observed between starch films in terms of moisture sorption. Thus, the AG-based film absorbs higher amounts of moisture than starch films in the same RH conditions. This trend is due to the more hydrophilic characteristics of AG compared to starch.

Related to the WVP, **Figure 4** gives the equilibrium moisture contents of films at 57, 84, and 99% RH and their WVP of films studied at 57–22, 84–22, and 99–22% RH conditions. It could be assumed that although the WVP depends on the RH gradient, adsorbed moisture has a plasticizing effect on films, thereby WVP tends to steady when one side of the film is exposed to high environmental RH. This sensitivity to the environmental humidity confirms the hydrophilic nature of film-forming polymers. However, AG-based film was recognized as the most hydrophilic film, but its WVP was comparable with that of CAS film and significantly lower than that of NRS and WRS films. The hydrophilic nature of the film-forming substance limits their ability to form films with good moisture barrier ability; however, the results obtained do not confirm that the more hydrophilic the film is, the poorer its water vapor barrier is.

It can be also observed that the C value of WRS is clearly higher than that of CAS- and NRS-based films. According to Timmermann et al. (33), C is the energy constant, related to the difference of chemical potential of the adsorbed molecules in the monolayer (first sorbed state) and in the upper sorption layers. Moreover, water molecules are associated with each type of biopolymer by different mechanisms (20). Thus, the value of C constant for WRS film may be attributed to the high amylopectin and protein fractions (**Table 1**), for which water attraction is more important than amylose chains in the low range of a_w . Moreover, Al-Muhtaseb et al. (34) show that in the case of sorption, C constant for high-amylopectin starch is always higher than that of high-amylose starch in the temperature range from 30 to 60 °C.

Solubility in Water. Solubility in water of the films was determined at 25 °C. AG-based film shows a solubility of

0.06%; this value allows us to suggest that AG-based film is insoluble in water. In reality, the film was swollen but remains intact even under moderate stirring during 24 h. Conversely, the solubility of CAS-based film was 79.33%; this film was considered to be partially soluble and is completely disintegrated. No particle of the film could be observed in water. For NRS-based film and WRS-based film, the solubilities are 0.84 and 17.99%, respectively; they were also partially soluble but much less than that of CAS-based film. NRS-based film was disintegrated, but numerous big particles were observed, whereas very small particles were shown in the case of WRS.

Surface Hydrophobicity and Wettability. Contact angle (surface hydrophobicity) and water adsorption rate (wettability) of the films were used to estimate the resistance of the films to liquid moisture transfer. The values are given in **Table 2**, and **Figure 5** shows the initial shape of the deposited water droplet on which the contact angle was measured. Except the LDPE, a hydrophobic film used as reference, which displays the highest value of contact angle, WRS- and NRS-based films exhibit a higher value compared to CAS-based film. This difference may be attributed to the fact that rice starch contains more lipid than cassava starch (0.28, 0.13, and 0.04% in WRS, NRS, and CAS, respectively) and eventually the migration of lipid to the evaporation surface as observed Debeaufort et al. (35) for emulsified films when the drying temperature is higher than the melting point of lipid component. Besides this, the initial contact angle of AG-based film was found to be higher than that of HPMC film and AX film (the two other films based on fibrous film-forming substances). In this case, the contact angle could be affected by the solubility in cold water of HPMC film and AX film. Moreover, the sensitivity of films to the liquid moisture transfer was evaluated by the determination of the adsorption rate of the water droplet. However, this characteristic might be affected when the excess water evaporates during the measurement; thus, the “adsorption” rate of LDPE was determined to estimate the evaporation rate of the measurement, considering that LDPE does not significantly absorb water during the measurement. The results show the liquid water adsorption rate varies inversely with contact angle except for the case of WRS film. The higher the contact angle is, the lower the adsorption rate is. The WRS film does not obey this trend, probably due to its disintegrative property in cold water and its partial solubilization in the droplet. The contact angle and water adsorption rate of cellophane film were not determined because the surface of the film is so hydrophilic that the water droplet spreads out as soon as it is deposited. **Figure 5** displays the changes in volume of the water droplet deposited on the films as a function of time after the deposition. Differences in the overall wetting behaviors of the films could be observed. In the case of AG-based film, the film is flexible and hygroscopic but insoluble in cold water, which displays a hydration at the first time followed by a regular adsorption rate. Inversely, CAS-based film exhibits at first time a very short steady step, then a promptly declining step, probably due to the solubility of the film in cold water and a stability in final step. The liquid water adsorption rate of the NRS-based film was nearly linear, whereas WRS displays a high rate the first time and becomes constant in the following step. The low rate observed in the final step of liquid water adsorption of some films could suggest that after the liquid water adsorbed, the physical state of the film surface is drastically changed, especially when the film was dissolved or completely hydrated. Indeed, when a polymer matrix in a glassy state is transformed into a rubber state by adsorbing water, the loss of molecular arrangements occurs spontaneously. This

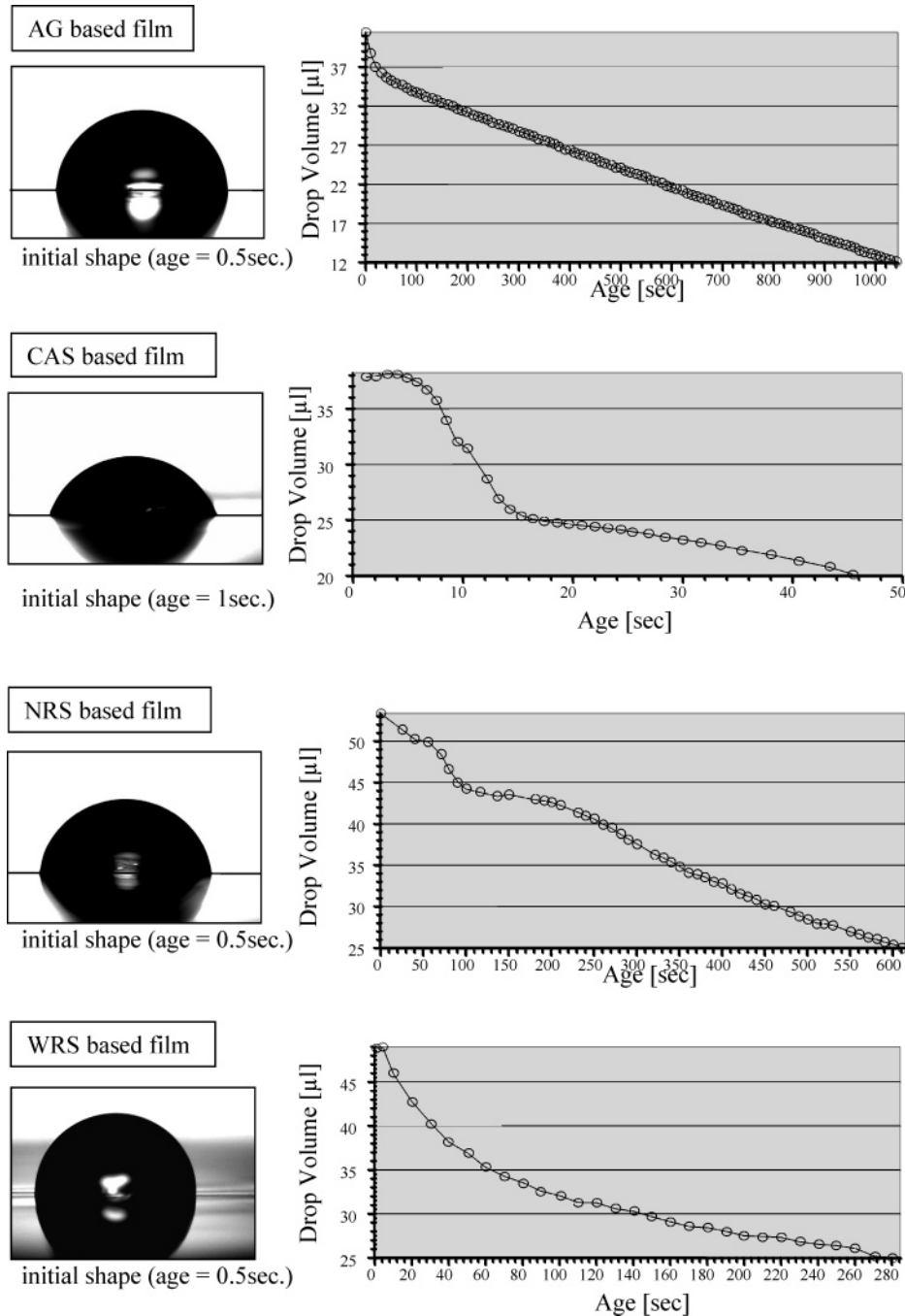


Figure 5. Water drop shape and changes in volume of water drop deposit on AG-, CAS-, NRS-, and WRS-based films as a function of time after the drop deposition.

phenomenon for glassy polymers is generally referred to as network relaxation and directly affects physical attributes of the materials (36). Then, the disposal water for the sorption was restricted, and this phenomenon can only occur at the periphery of the droplet area. Thereby, the volume of deposited droplet becomes finite, and the adsorption rate reaches a plateau value corresponding to the evaporation rate.

Mechanical Properties. Tensile strength (TS) and percentage of elongation (%*E*) at the break point of the films studied are presented in **Table 4**, and **Figure 6** displays their typical force–deformation curves. It could be noted that when the effective dimension of the film sample before the stretching is constant, it is evident that the load of break is directly proportional to initial film thickness. For this reason, TS values are normalized by dividing the strength at breaking by the initial cross-section

Table 4. Tensile Strength and Percentage of Elongation of AG-, CAS-, NRS-, and WRS-Based Films and of HPMC, AX, Cellophane, and LDPE Films^a

film	tensile strength (MPa)	elongation (%)
AG + 15% glycerin	42.11 ± 3.27d	6.51 ± 0.96c
CAS + 15% glycerin	35.17 ± 4.60c	2.64 ± 0.73ab
NRS + 15% glycerin	28.85 ± 3.53bc	2.07 ± 0.49a
WRS + 15% glycerin	8.51 ± 3.12a	0.72 ± 0.16a
HPMC + 15% glycerin	31.89 ± 4.39c	11.08 ± 3.70d
AX + 15% glycerin	22.30 ± 2.97b	5.46 ± 1.89bc
cellophane ^b	55–124	16–60
LDPE ^c	9–17	500

^a Values in the same column followed by the same letter are not significantly different at $p < 0.05$ level. ^b Taylor 1986. ^c Briston 1986.

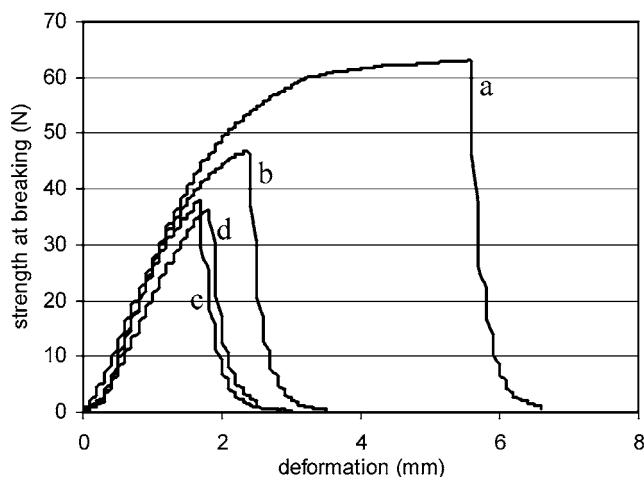


Figure 6. Typical force–deformation curves for AG-based (a), CAS-based (b), NRS-based (c), and WRS-based (d) films.

area of film samples. Therefore, the value should not be affected by film thickness. Starch and agar films exhibit a stronger tensile resistance compared to most protein films (37, 38). Except for WRS film, all of the films tested have higher TS than the LDPE film. Especially, AG-based film appears to have TS as high as cellophane and significantly higher than that of starch-based films. On the other hand, the TS of CAS-based film and NRS-based films was comparable to that of HPMC film. Only the WRS film displays a weak mechanical resistance. Values obtained for starch films allow us to note that TS of the films tends to increase with the amylose content. CAS-based film with 27% amylose has an average TS of 35.17 MPa, NRS-based film containing 19.5% amylose displays a TS close to 28.85 MPa, and the TS of WRS-based film is 8.51 MPa with 3.6% amylose. Published mechanical results give similar values: Rindlav-Westling et al. (28) and Lourdin et al. (39) indicated that TS of the films increases linearly with the amount of amylose.

Distinguished by physical properties of film-forming raw materials, films made of fibrous materials such as AG or HPMC offer stronger TS relative to films made of granular materials such as starch. The mechanical properties of materials are largely associated with distribution and density of intermolecular and intramolecular interactions allowed by the primary and spatial structures (40). For AG-based film, a three-dimensional network is formed by the association of the small domains in which double-helix linkages of the polymer chains were observed. During the preparation of starch films, retrogradation of gelatinized starch, a phenomenon in which molecules reassociate into double-helix structure to retrieve a crystalline order, could occur. According to Myllärinen et al. (29) the crystallinity was partial in amylose-based film; 23% of crystallinity was found in amylose containing 10% glycerin and stored at 54% RH. It could be suggested that the density of intramolecular interactions is more important in AG-based film than in starch films; consequently, film made of AG shows higher TS compared to starch films.

Percentage of elongation (%E) of samples was determined to estimate the stretchability of films. All of the films have lower %E than synthetic ones and protein films such as soy protein, wheat gluten (41), or corn zein (42) based films. From **Table 4**, physical properties of film-forming raw materials also influence %E of films. AG, HPMC, and AX films, composed of a fibrous biopolymer, are more stretchable than films made of starch. Indeed, CAS-based film, NRS-based film, and WRS-based film were stiffer and more brittle than AG-based film

and HPMC-based film. Particularly, AG-based film exhibits a high flexibility and is easy to handle. ESEM observations of cross section allow, to some extent, explanation of the differences in mechanical properties of the films; the better mechanical properties of AG-based film could be due to its more compact and homogeneous structure compared to that of NRS- and WRS-based films.

Conclusion. The moisture barrier properties of edible films made of AG, CAS, NRS, and WRS are comparable to those of other polysaccharides and their derivatives or some protein films. The WVP of the films depends on the water vapor pressure gradient and reaches a constant value at high environmental RH above 84%. Among these films, because AG-based film displays a higher moisture sorption at the same RH conditions, it seems to have better moisture barrier properties than CAS film. Due to their relatively high WVP values and their hygroscopicity, the potential use of these films as moisture barrier packaging or coating is restricted. However, some polysaccharides, when used in the form of a high-moisture gelatinous coating, will retard water loss from some foods during short-term storage; the above studied films could be used in the same way.

Except the WRS-based film, which exhibited poor mechanical properties, AG-, CAS-, and NRS-based films display interesting mechanical properties. Especially AG- and CAS-based films, plasticized with glycerin, are transparent, clear, homogeneous, flexible, and easily handled. Such mechanical properties give a promising utility for their use as packaging or coating because they can preserve not only the integrity of the products but also other functional properties of the films themselves. It should also be noted that the AG-based film was heat-sealable; however, an accurate study would be required to evaluate this property.

ACKNOWLEDGMENT

We thank Dr. Nathalie Cayot from ENESAD for her technical support in the characterization of starch and Dr. J. Relot from CMAB for her contribution in the ESEM observations. We also thank Dr. B. Colas and Dr. P. Cayot from Lab. IMSA-ENSBANA for their contribution in the characterization of mechanical properties of edible films and the chemical analysis of film-forming materials.

LITERATURE CITED

- (1) Guilbert, S.; Gontard, N. Le concept de l'emballage comestible. *AGORAL 1992 Nancy* **1992**, 1–25.
- (2) Greener-Donhowe, I.; Fennema, O. Edible Films and Coatings: Characteristics, Formation, Definitions, and Testing Methods. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994; pp 1–24.
- (3) Nisperos-Carriedo, M. Edible Coatings and Films Based on Polysaccharides. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994; pp 305–336.
- (4) Robinson, D. S. Food Carbohydrates. In *Food Biochemistry and Nutritional Value*; Robinson, D. S., Ed.; Longman Scientific and Technical: London, U.K., 1990; pp 3–111.
- (5) Roger, P.; Tran, V.; Lesec, J.; Colonna, P. Isolation and Characterization of Single Chain Amylose. *J. Cereal Sci.* **1996**, *24*, 247–268.
- (6) Roger, P.; Bello-Perez, L. A.; Colonna, P. Contribution of Amylose and Amylopectin to the Light Scattering Behaviour of Starches in Aqueous Solution. *Polymer* **1999**, *40*, 6897–6909.

- (7) Freitas, R. A.; Paula, R. C.; Feitosa, J. P. A.; Rocha, S.; Sierakowski, M. R. Amylose Contents, Rheological Properties and Gelatinization Kinetics of Yam and Cassava Starches. *Carbohydr. Polym.* **2004**, *55*, 3–8.
- (8) Juliano, B. O. Structure, Chemistry and Function of the Rice Grain and Its Fraction. *Cereal Foods World* **1992**, *37*, 772–774.
- (9) Sodhi, N. S.; Singh, N. Morphological, Thermal and Rheological Properties of Starches Separated from Rice Cultivars Grown in India. *Food Chem.* **2003**, *80*, 99–108.
- (10) Stanley, N. F. Agars. In *Food Polysaccharides and Their Applications*; Stephen, A. M., Ed.; Dekker: New York, 1995; pp 187–199.
- (11) Belitz, H. D.; Grosch, W. Carbohydrates. In *Food Chemistry*; Springer-Verlag: Berlin, Germany, 1999; pp 284–286.
- (12) Jarvis, C. E.; Walker, J. R. L. Simultaneous, Rapid, Spectrophotometric Determination of Total Starch, Amylose and Amylopectin. *J. Sci. Food Agric.* **1993**, *63*, 53–57.
- (13) AFNOR H-00-030, Matière en Feuille: Détermination du Coefficient de Transmission de la Vapeur d'Eau. In *Recueil de Normes Françaises*; Association Française de Normalisation: Paris, France, 1974.
- (14) ASTM E96-80. Standard Test Method for Water Vapor Transmission of Materials. In *ASTM Books of Standards*; American Society for Testing and Materials: West Conshohocken, PA, 1980.
- (15) Debeaufort, F.; Martin-Polo, M.; Voilley, A. Polarity, Homogeneity and Structure Affect Water Vapor Permeability of Model Edible Films. *J. Food Sci.* **1993**, *58*, 426–434.
- (16) McHugh, T. H.; Avena-Bustillos, R.; Krochta, J. M. Hydrophilic Edible Films: Modified Procedure for Water Vapor Permeability and Explanation of Thickness Effects. *J. Food Sci.* **1993**, *58*, 889–903.
- (17) Debeaufort, F.; Voilley, A.; Guilbert, S. Procédés de Stabilisation des Produits Alimentaires Par les Films “Barrière”. In *L'eau Dans les Aliments*; Le Meste, M., Lorient, D., Simatos, D., Eds.; Tec and Doc, Lavoisier: Paris, France, 2002; pp 549–600.
- (18) Hagenmaier, R. G.; Shaw, P. E. Moisture Permeability of Edible Films Made with Fatty Acid and (Hydroxypropyl)methylcellulose. *J. Agric. Food Chem.* **1990**, *38*, 1799–1803.
- (19) Cuq, B.; Gontard, N.; Cuq, J. L.; Guilbert, S. Functional properties of myofibrillar protein-based biopackagings as affected by film thickness. *J. Food Sci.* **1996**, *61*, 1–5.
- (20) McHugh, T. H.; Krochta, J. M. Permeability Properties of Edible Films. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994; pp 139–188.
- (21) Gennadios, A.; Weller, C. L.; Gooding, C. H. Measurement errors in water vapor permeability of highly permeable hydrophilic edible films. *J. Food Eng.* **1994**, *21*, 395–409.
- (22) Hwang, S. T.; Kammermayer, K. The Effect of Thickness on Permeability. In *Polymer Science and Technology: Permeability of Plastic Films and Coating to Gases, Vapors, and Liquids*; Hopfenberg, H. B., Eds.; Plenum Press: New York, 1974; pp 197–205.
- (23) Gennadios, A.; Weller, C. L.; Testin, R. F. Modification of Physical and Barrier Properties of Edible Wheat Gluten-based Films. *Cereal Chem.* **1993**, *70*, 426–429.
- (24) María, B.; Pérez-Gago, Krochta, J. M. Formation and Properties of Whey Protein Films and Coatings. In *Protein-Based Films and Coatings*; Gennadios, A., Ed.; CRC Press: New York, 2002; pp 159–180.
- (25) Gibson, L. J.; Ashby, M. F. Material Properties. In *Cellular Solids—Structure and Properties*; Gibson, L. J., Ashby, M. F., Eds.; Pergamon Press: New York, 1988; pp 42–68.
- (26) Wolff, I. A.; Davis, H. A.; Cluskey, J. E.; Gundrum, L. J.; Rist, C. E. Preparation of Films from Amylose. *Ind. Eng. Chem.* **1951**, *43*, 915–919.
- (27) Lawton, J. W. Effect of Starch Type on the Properties of Starch Containing Films. *Carbohydr. Polym.* **1996**, *29*, 203–208.
- (28) Rindlav-Westling, Å.; Stading, M.; Hermansson, A.-M.; Gatenholm, P. Structure, Mechanical and Barrier Properties of Amylose and Amylopectin Films. *Carbohydr. Polym.* **1998**, *36*, 217–224.
- (29) Myllärinen, P.; Buleon, A.; Lahtinen, R.; Forssell, P. The Crystallinity of Amylose and Amylopectin Films. *Carbohydr. Polym.* **2002**, *48*, 41–48.
- (30) Bell, L. N.; Labuza, T. P. Determination of Moisture Sorption Isotherms. In *Moisture Sorption: Practicle Aspect of Isotherm Measurement and Use*; Bell, L. N., Labuza, T. P., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1984; pp 33–56.
- (31) Gontard, N.; Guilbert, S.; Cuq, J. L. Water and Glycerol as Plasticizers Affect Mechanical and Water Vapor Barrier Properties of an Edible Wheat Gluten Film. *J. Food Sci.* **1993**, *58*, 206–211.
- (32) Gennadios, A.; Park, H. J.; Weller, C. L. Relative Humidity and Temperature Effects on Tensile Strength of Edible Protein and Cellulose Ether Films. *Trans. Am. Soc. Agric. Eng.* **1993**, *36*, 1867–1872.
- (33) Timmermann, E. O.; Chirife, J.; Iglesias, H. A. Water sorption isotherms of food and foodstuffs: BET or GAB parameters? *J. Food Eng.* **2001**, *48*, 19–31.
- (34) Al-Muhtaseb, A. H.; McMinn, W. A. M.; Magee, T. R. A. Water sorption isotherms of starch powders. Part 1: mathematical description of experimental data. *J. Food Eng.* **2004**, *61*, 297–307.
- (35) Debeaufort, F.; Voilley, A. Effect of surfactants and drying rate on barrier properties of emulsified edible films. *Int. J. Food Sci.* **1995**, *30*, 183–190.
- (36) Chung, H. J.; Chang, H. I.; Lim, S. T. Physical aging of glassy normal and waxy rice qtarches: Effect of crystallinity on glass transition and enthalpy relaxation. *Carbohydr. Polym.* **2004**, in press.
- (37) Krochta, J. M. Proteins as Raw Materials for Films and Coatings: Definitions, Current Status, and Opportunities. In *Protein-Based Films and Coatings*; Gennadios, A., Ed.; CRC Press: New York, 2002; pp 1–41.
- (38) McHugh, T. H.; Krochta, J. M. Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *J. Agric. Food Chem.* **1994**, *42*, 842–845.
- (39) Lourdin, D.; Valle, G. D.; Colonna, P. Influence of Amylose Content on Starch Films and Foams. *Carbohydr. Polym.* **1995**, *27*, 261–270.
- (40) Guilbert, S.; Gontard, N.; Morel, M. H.; Chalier, P.; Micard, V.; Redl, A. Formation and Properties of Wheat Gluten Films and Coatings. In *Protein-Based Films and Coatings*; Gennadios, A., Ed.; CRC Press: New York, 2002; pp 69–122.
- (41) Gennadios, A.; McHugh, T.; Weller, C. L.; Krochta, J. M. Edible Coatings and Films Based on Proteins. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994; pp 201–277.
- (42) Padua, G. W.; Wang, Q. Formation and Properties of Corn Zein Films and Coatings. In *Protein-Based Films and Coatings*; Gennadios, A., Ed.; CRC Press: New York, 2002; pp 43–67.

Received for review June 29, 2004. Revised manuscript received November 4, 2004. Accepted November 18, 2004. We thank the French Embassy in Vietnam for financial support.