This is to certify that the

thesis entitled

PRODUCTION OF BIOPOLYMERIC FILMS
AND COATINGS FROM WHEAT PROTEINS

presented by

Luis Martin Rayas

has been accepted towards fulfillment
of the requirements for

M.S. degree in Packaging

Dr. Ruben Hernandez
Major professor

Date 12/5/96

PRODUCTION OF BIOPOLYMERIC FILMS AND COATINGS FROM WHEAT PROTEINS

BY

Luis Martin Rayas

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

School of Packaging

1996

ABSTRACT

PRODUCTION OF BIOPOLYMERIC FILMS
AND COATINGS FROM WHEAT PROTEINS

By

Luis Martin Rayas

Over the last ten years, several new types of films have been developed that are degradable and/or edible. Advantages of degradable/edible protein-based films include: a) improvement in the mechanical-handling properties and structural integrity of packaged foods; b) bio-degradation in landfills; c) as edible coatings, increase the food value of the product; d) different nutrients and additives can be incorporated thus enhancing organoleptic properties and preventing deterioration of the packaged foods; and e) control of gas and moisture permeation from and into the product.

The biopolymeric films were produced by a new process developed at the School of Packaging, Michigan State University. The process consisted of treating wheat proteins by using selective solvents, separating insoluble materials, and casting of the solution into films.

The objectives of this study were: 1) development of a new method for separating the protein from wheat flour and 2) analysis of the mass transfer properties and photo-degradability characteristics. Sorption isotherm and permeability of oxygen as a function of equilibrium relative humidity (ERH) were

determined. Photo-degradability was evaluated by measuring changes in color and mechanical properties of the films.

The films produced were transparent, clear, strong, with mechanical properties similar to those of low-density polyethylene and with oxygen permeability similar to those of the polyamides (nylons). Mass transfer results indicated a significant dependence of the water sorption and oxygen permeability to the test equilibrium relative humidity, with greater effects at ERH greater than 40%. The photo-degradability study showed that the film's mechanical properties such as tensile strength and toughness increased with UV radiation exposure time. On the other hand, elongation values showed significant reduction in their values with an increase of UV radiation time.

Potential uses of these films are in the food and pharmaceutical industries. The films can be used both as a self-supporting films and coatings, specially for oxygen sensitive products, particularly at low relative humidities.

To my wife
Ana Teresa, with all my love

To my children
Luis Alberto, Anna Teresa, Diego Alejandro and Isabel Maria

To my parents
Luis and Irma, for their infinite love and support

ACKNOWLEDGMENTS

The author wants to profoundly thank Dr. Ruben J. Hernandez, major professor, for the privilege of working with him throughout the research and for the guidance, help, support, and friendship given during the stay at MSU. Sincere appreciation to Dr. Susan E. M. Selke and Dr. Jerry N. Cash, members of the committee for their guidance, time, and help throughout this research. Special thank to Dr. Rafael Gavara for the time dedicated to read the thesis and the valuable comments and suggestions made for it.

The author also wishes to thank the King Milling Company for the supply of raw material and the Center for Food and Pharmaceutical Packaging Research (CFPPR) for the economic support received during this study. Assistance and the use of facilities and equipment during this study from the Plastics, Mass Transfer, Materials Testing, Fresh Products Packaging and Permeation & Material Testing Laboratories, School of Packaging, Michigan State University is appreciated.

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NOMENCLATURE

a_w	water activity, dimensionless
C	constant, dimensionless
D	diffusion constant, $m^2\ s^{-1}$
D_w	water diffusion coefficient, $cm^2\ s^{-1}$
ERH	equilibrium relative humidity in which no net gain or lost of water occurs, %
F_t	oxygen flow rate permeating the film at time t, $cm^3\ m^{-2}\ s^{-1}$ or $mol\ m^{-2}\ s^{-1}$
F_∞	oxygen flow rate under steady conditions, $cm^3\ m^{-2}\ s^{-1}$ or $mol\ m^{-2}\ s^{-1}$
J	oxygen transmission rate (flux), $cm^3\ m^{-2}\ s^{-1}$ or $mol\ m^{-2}\ s^{-1}$
ℓ	film thickness (note: 1 mil = 0.001 in), mil or m
M_a	moles of water, mol
M_s	moles of solute, mol
m	water content, $(g\ H_2O)\ (g\ solids)^{-1}$
m_1	monolayer value, $(g\ H_2O)\ (g\ solids)^{-1}$
P	permeability (STP = standard temperature and pressure = 273.15 K; 1.013×10^5 Pa), m^3 (STP) $m\ m^{-2}\ s^{-1}\ Pa^{-1}$
p	water vapor pressure of food at temperature T, atm
p_0	water vapor pressure of pure water at temperature T, atm
S	solubility coefficient (STP = standard temperature and pressure = 273.15 K; 1.013×10^5 Pa), cm^3 (STP) $cm^{-3}\ Pa^{-1}$
t	time, s
Δp	partial pressure differential across the film, Pa
ε	elongation, dimensionless
ϕ	ration between flow rates at time t and at steady state, dimensionless
σ	stress, Pa

CHAPTER 1. LITERATURE REVIEW

CHAPTER 1. LITERATURE REVIEW

BIOPOLYMERIC MATERIALS

Biopolymeric materials are those polymers whose source is of biological nature, e.g., cellulose from wood, pectin from citric fruits, gluten proteins from wheat grain, and keratin from animal hair and feathers, among many others. When these biopolymers are used for a different function other than the natural, particularly towards the replacement of synthetic polymers, advantages such as bio-degradability and, in some cases edibility, are found.

Today, there is limited information available on degradable packaging materials, especially with respect to their degradability and gas barrier properties. C. Somerville had predicted that agricultural plastics (biopolymers) could become a reality within a decade (McWilliams, 1991). He indicated that price is the current main obstacle towards commercialization of these biopolymers. However, because they are truly biodegradable, and would help eliminate dependence of oil-based polymers, there is presently a growing commercial interest in biopolymeric materials.

According to Giusti *et al.* (1993), "the biological polymers of interest as biomaterials are those that constitute the fundamental parts of tissues and organs". However, they discussed limitations that are common in using biological materials, including those of some loss of the physico-mechanical properties such as fatigue behavior. This in part is due to the fact that when

biopolymers are separated from the living entity, they are no longer subject to repair or growth and thus they would behave similar to a synthetic material.

Thirty-seven years ago, Golding (1959) indicated that the use of natural biopolymeric products is possible as long as they were modified, e.g. chemically, in order to upgrade one or more properties and increase their usefulness. For instance, changes in the softening point, solubility, and chemical stability not present in the natural products are obtained by chemical treatment. Golding indicated that some of the biopolymeric products of industrial use include regenerated cellulosics, alginic acid derivatives, derivatives of natural rubber, and regenerated proteins. The last included polyamides from milk, soybeans, peanuts, and corn. Another advatage is that additives can be incorporated in edible films or coatings, which can be released into the food by diffusion (Wellinghoff, 1995).

Food applications for biopolymeric materials include coating fruits to delay ripening. For instance, whole apples with a sucrose polyester (SPE) solution (Semperfresh™), and storage at 39°F showed a lower ripening rate (Santerre *et al.*, 1988). In another study, Chai *et al.* (1991) found that the use of SPE also retarded apple ripening while increasing tissue firmness and titratable acidity. They found that for some apple cultivars, such as Golden Delicious and McIntosh apples, SPE treatments improved consumer acceptability ratings.

The mixing of different biopolymeric materials was performed by Lim and Jane (1993). They mixed starch and zein (5:1 and 9:1 wt/wt ratios) with cross-linking agents (formaldehyde and glutaraldehyde) to enhanced the physical

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strength and water-resistance of the biopolymeric blend. They also indicated that the optimum pH of the cross-linked starch-zein mixture was about 6.0.

Researchers at the Scottish Agricultural College in Edinburgh developed a film-forming polymer that could be sprayed onto plants to protect them from various diseases. The coating formed a barrier that reduced leaf penetration by fungal pathogens, but had no effect on plant growth. The polymers were based on naturally occurring compounds and were non-phytotoxic and permeable to gases. They also had good weathering properties and were biodegradable (Resource, 1995).

Selke (1996) indicated that the idea of replacing standard synthetic materials with degradable ones is not so simple. This author indicated that in landfills, conditions are not necessarily suitable for the decomposition of biodegradable materials. Therefore, efforts toward fabrication of biopolymers with the edible attribute are very desirable, so that disposal is not required.

In the next section, methods for fabricating degradable films by using wheat proteins as the biopolymeric material are discussed.

METHODS FOR PREPARATION OF WHEAT PROTEIN-BASED FILMS

Krull and Inglett (1971) developed films from whole gluten (wheat protein) both at laboratory and industry scales. Films were cast from 20% gluten in a solvent comprised of 60% ethanol, 20% lactic acid, and 20% water. Lactic acid is both a glutenin dissolving agent and plasticizer. Films prepared by Krull and Inglett (1971) were brittle, little water resistant, and showed lower tensile strength values than some synthetic films.

Gennadios and Weller (1990) prepared films using commercial Pro-80™ vital wheat gluten. They put 15 g of the gluten in 72 ml of 95% ethanol, then added 6 g of glycerol as plasticizer and boiled the solution to disperse the wheat gluten. They added 48 ml distilled water and increased the pH with 14 ml of 6N ammonium hydroxide to dissolve the glutenin fraction. The resulting films, after casting and drying, were strong, flexible, and translucent, but opaque. Good barrier properties to oxygen and carbon dioxide were obtained for the films, but they had very high water permeability values.

More recently, Gontard *et al.* (1992) developed films using commercial F 33000 vital gluten. These films were prepared from a solution of gluten in absolute ethanol, acetic acid, and water with glycerol added as plasticizer. The concentration values of gluten, ethanol, and the pH of the solution (adjusted with acetic acid) were varied to evaluate the effect of these variables on the films' properties. The significant findings were that pH and ethanol concentration of the film-forming solution were the two most important factors influencing film opacity, water solubility and water vapor permeability. Also, mechanical properties appeared to be strongly influenced by the concentration of gluten and the pH. They suggested that depending on the film use, a particular film-formation variable-combination may be chosen which could cover the basic properties to optimize.

Gontard *et al.* (1993) also investigated the influence of plasticizers and their effect on the mechanical and water vapor barrier properties of gluten films. They prepared the films using commercial F 33000 vital wheat gluten in a

concentration of 7.5/100 ml solution, 45 ml ethanol / 100 ml solution. The pH of the solution was adjusted to 4 with acetic acid. Lactic acid, polyols and other compounds were added as the variable to test in concentrations from 0 to 33.3 g/100 g dry film matter. They found that polyols (glycerol, sorbitol, propanediol) and lactic acid were the only substances that especially plasticized gluten film. Amphipolar substances (glycol monostearate, acetic ester of monoglyceride, sucrose ester of stearic acid and diacetyl tartaric ester of monoglyceride) had no real plasticizing effect. The hydrophobic substances (beeswax and fatty acids such as lauric, stearic, and oleic acids) had an anti-plasticizing effect on gluten film. The most effective plasticizer according to Gontard *et al.* (1993) was glycerol. Another significant finding was that water activity (a_w) and temperature were crucial parameters that affected gluten film properties. Gennadios *et al.* (1993) fabricated gluten films by following the technique of Gennadios and Weller (1990) in which 15 g wheat gluten, 72 ml 95% ethanol, and 6 g glycerol were mixed. Gennadios *et al.* (1993) described the temperature effect on the oxygen permeability of the gluten film. The oxygen permeability vs. temperature plot fitted the Arrhenius model as expected.

Crosslinking of Proteins

The term "cross-linking" denotes stable chemical bond association of generally large elements at specific places to create a new entity that has distinct properties as a result of the juncture (Pomeranz, 1987 and Friedman, 1977). In the case of proteins, cross-linking promotes changes in the chemical, functional, nutritional, biochemical, and physical properties. Cross-linking of

proteins has been reported by Lotan and Sharon (1977), Uy and Wold (1977), Harland and Feairheller (1977), Jane *et al.* (1993), and Mahmoud and Savello (1993).

Natural polymers, in contrast to synthetic polymers, are already formed in the source material. Because natural proteins are made of as many as 20 different monomers (amino acids) to build each molecule, they are more complex than most linear synthetic polymers. In addition, the linear polymeric chain of almost every natural protein has the property of being able to assume a specific three-dimensional folded conformation (Creighton, 1993). This would make the chemical complexity of protein macromolecules essentially limitless (Battista, 1958). However, proteins are structurally less complex since most chemical polymers are synthesized by polymerizing a mixture of monomers producing a distribution of chain lengths. In addition, if more than one type of monomer is present, an approximately random sequence of monomers is obtained. Proteins, on the other hand, are linear and unbranched and have precise lengths and exact sequences of amino acids. In fact, it is only the differences in length and sequence that distinguish one protein from any other, and make possible a diversity of structures and functions.

Mahmoud and Savello (1993) used transglutaminase to cross-link covalently concentrated proteins solutions of a 1:1 (wt/wt) mixture of α-lactalbumin and β-lactoglobulin to form gels. These gels were dehydrated and produced transparent films. An important finding by these authors was that although the protein films were insoluble in aqueous buffers at various pH and

heat treatments, these films were protease-digestible (bio-degradable). Golding (1959) reported that when casein is immersed in a 4 to 5 percent formaldehyde solution, the ε-amino group (of lysine), as well as the unsubstituted α-amino group, can bind one or two moles of formaldehyde, depending on the concentration of the latter. This author also reported that zein and casein plastics can be cured by immersing the plastics in formaldehyde solution. This reaction can be catalyzed by acids, HCl being most effective.

Lim and Jane (1993) reported that mixtures of starch and zein with cross-linking agents including formaldehyde and glutaraldehyde yielded plastic-like materials. They found that the effect of the cross-linking in the material was that of the enhanced physical strength and water-resistance. They indicated that their starch-zein plastics were economically feasible replacements for synthetic polyamides.

Cross-linking of proteins with glutaraldehyde was reported by Richards and Knowles (1968), and indicated that the protein cross-linking was an irreversible process. The cross-linked proteins resisted treatments with urea, semicarbazide, and wide ranges of pH, ionic strength and temperature. These authors and Chatterji (1989) reported the formation of a Shiff base by the interaction between the aldehyde and the amino groups of the amino acids. Chatterji (1989) attributed the change in color from pale yellow to deep orange to the formation of the Schiff (aldimine) linkage between the free amino groups of the proteins and glutaraldehyde. This author also reported that when native gelatin is treated with glutaraldehyde, the reaction exclusively involves the

lysines, to almost 100%. The scheme of the reaction is represented in Figure 1-1. Satyanarayana and Chatterji (1991) demonstrated that the cross-linking of gelatin granules with glutaraldehyde involves the ε-amino groups of the lysine residues of the protein and the aldehyde functionality of the glutaraldehyde yielded a versatile cross-linked matrix.

MASS TRANSFER IN POLYMERIC FILMS

Gas and vapor transport in polymeric materials is of great importance to the packaging industry because it is always present in a package system. No polymer film is known to provide a complete barrier to the transport of a gas or vapor molecule (Brown, 1981). The mechanism of mass transfer through a continuous polymer phase is by activated diffusion driven by a concentration gradient. The diffusant dissolves in the film matrix at the high concentration side, and then diffuses through the film towards the low concentration side. The overall phenomenon is influenced primarily by the chemical composition, size, shape, and polarity of the penetrating molecule and chemical composition and polymer-chain segmental motion within the film matrix. When the concentration of the permeant at both sides of the film is kept constant, the system eventually reaches a steady state after an initial period of transient flow. The determination of the permeability of edible films is carried out in a similar fashion as for non-edible films using continuous flow and quasi-isostatic methods (Donhowe and Fennema, 1994).

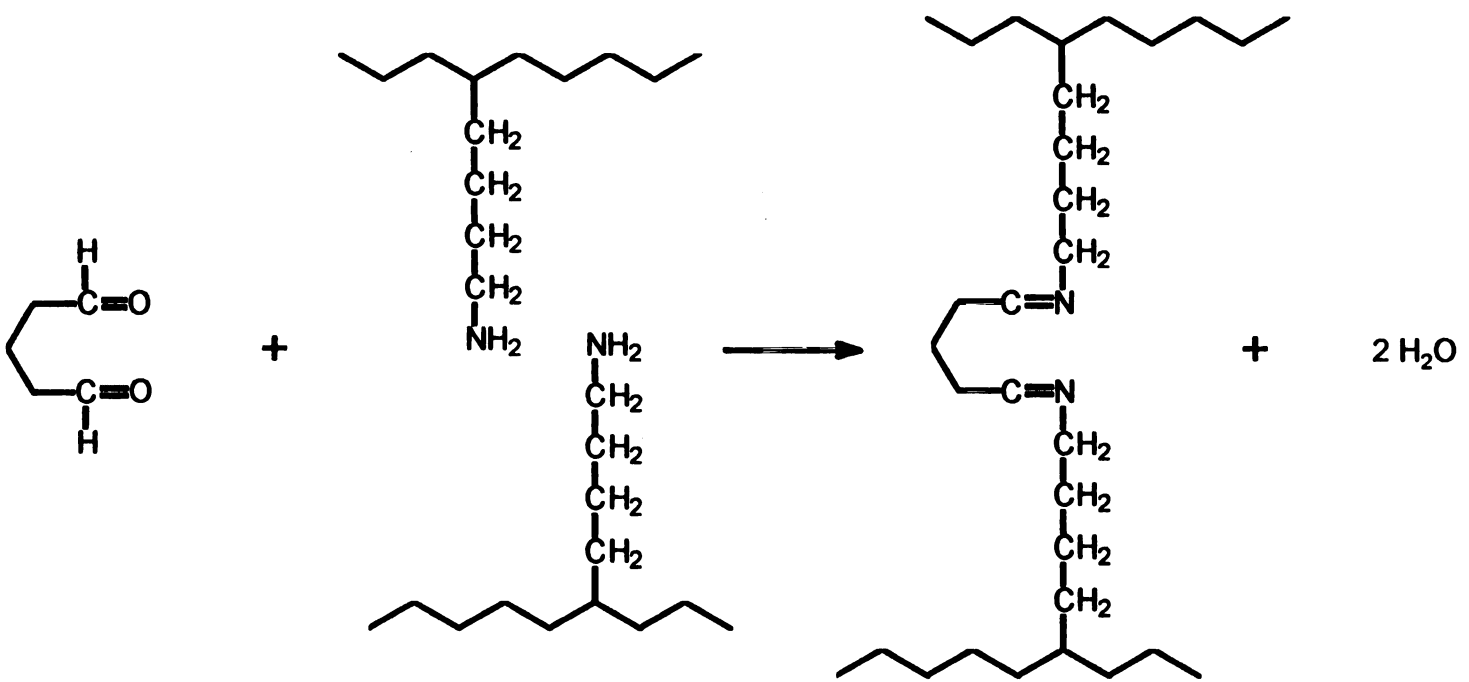

Figure 1-1. Reaction between the ε-amino group of lysine residues and glutaraldehyde.
[Adapted from Chatterji, 1989]

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The solution to the second Fick's law for a continuous flow permeation experiment for oxygen (Hernandez *et al.*, 1986) is given by the following equation:

$$\frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right)\left(\sqrt{\frac{\ell^2}{4Dt}}\right)\sum_{n=1,3,5}^{\infty} \exp\left(\frac{-n^2\ell^2}{4Dt}\right) \tag{1.1}$$

where F_t is the flow rate of oxygen permeating the films at time t, F_∞ is the oxygen flow rate under steady state conditions, ℓ is the film thickness, and D is the oxygen diffusion coefficient. By taking only the first term of the series, the permeation experiment up to a value of the flow ratio of 0.95 can be simplified to the following equation:

$$\phi = \frac{F_t}{F_\infty} = \left(\frac{4}{\sqrt{\pi}}\right)X^{1/2}\exp(-X) \tag{1.2}$$

where $X = \ell^2/(4Dt)$, and ϕ is the ratio between the flow rates at time t and at steady state. From a continuous flow permeability experiment, F_t values can be obtained as a function of time from t=0 to the steady state. The Newton-Raphson method can be used to evaluate X from Eq. (1.2) as a function of time. The diffusion coefficient D is determined from the slope of the straight line of the plot X^{-1} vs. time for values within the range of $0.05<\phi<0.95$ (Hernandez *et al.*, 1986). The oxygen permeability constant, P, can be determined directly from the steady state value of each permeability experiment:

$$P = \frac{F_\infty \ell}{\Delta P} \tag{1.3}$$

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where ΔP is the driving force given by the oxygen or permeant pressure gradient across the film.

According to Hernandez (1994), Gavara and Hernandez (1994), and Kester and Fennema (1986), the solubility (S) of the permeant into the film can be calculated by applying Fick's and Henry's laws with the following equation:

$$S = \frac{P}{D} \tag{1.4}$$

It is assumed, in Eq. 1.4, that D is independent of the concentration of the penetrant. Figure 1-2 presents a diagram of a setup for oxygen permeability studies using a diffusion cell, with carrier gas (nitrogen) and test gas (oxygen) flushed on either side of the cell and control of relative humidity of either gas. Setup similar to that of Figure 1-2 has been used by Gavara and Hernandez (1994). The equation used to calculate the oxygen permeability coefficient is:

$$P = DS = \frac{Q\ell}{At(\Delta P)} \tag{1.5}$$

where P is the permeability coefficient, D is the diffusion constant, S is the solubility coefficient, Q is the amount of oxygen passing through the film at standard temperature and pressure (stp), ℓ is the film thickness, A is the film area, t is time, and ΔP is the oxygen gas partial differential pressure. Note that Q/(At) is F_∞ in Eq. (1.3).

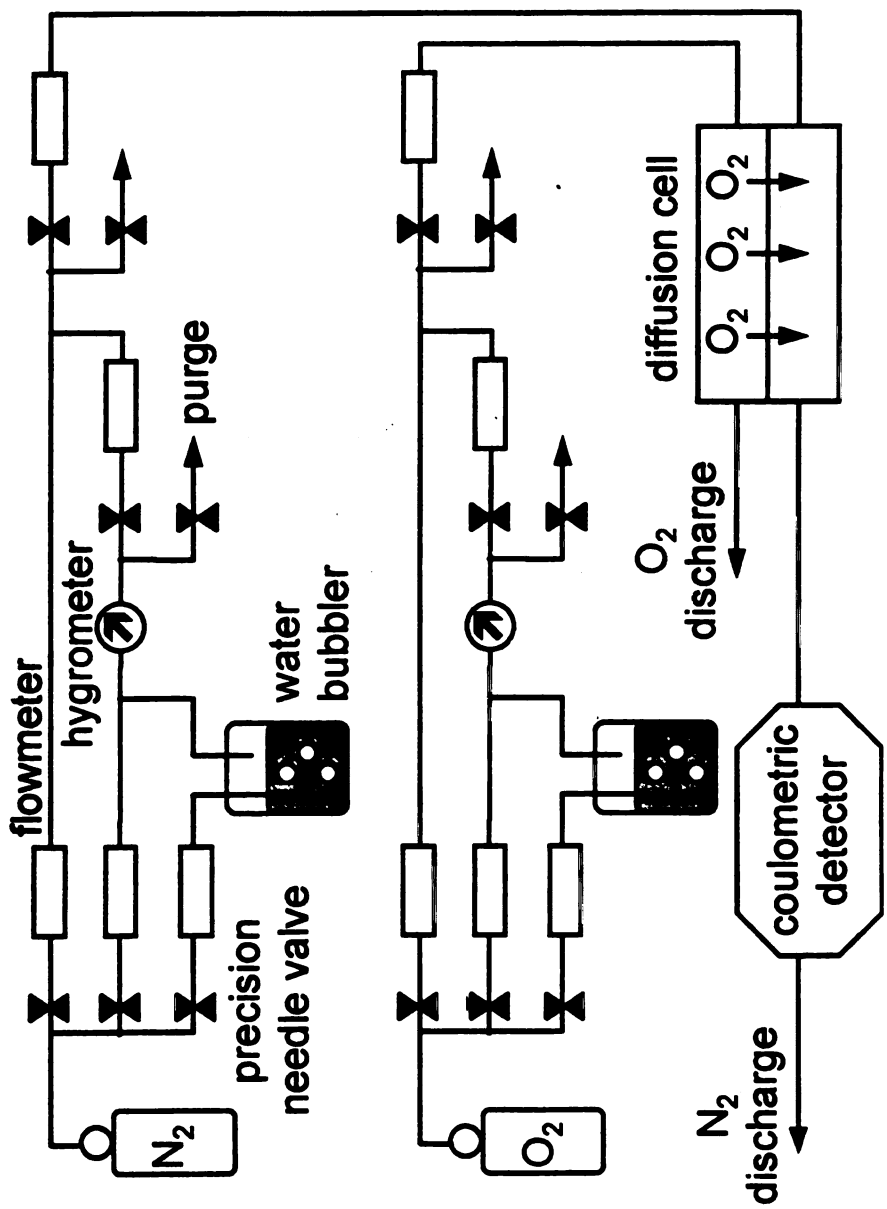

Figure 1-2. Diagram of an apparatus used to determine oxygen permeability as a function of % relative humidity

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PERMEABILITY OF GASES IN PROTEIN-BASED FILMS

Information on permeability of protein-based films (e.g., wheat gluten, corn zein, and wheat gluten/soy protein isolate) is limited (Gennadios *et al.*, 1993). These authors reported that wheat gluten films had a lower permeability compared to corn zein films under the same conditions. This was attributed to the more complex structure and closer polymer segments in the gluten films. They concluded that oxygen molecules can permeate more readily through a zein helical conformation (about 50%) than through the highly cross-linked gluten structure, and that the nature of interaction involves disulfide bonding. One of the major contributions of this study was that it gives practical comparisons between the three protein films with respect to other films. An important characteristic of these films was that the oxygen permeability values were lower compared to other polysaccharide and composite polysaccharide/lipid edible films. These values were also lower than some of the common plastic films used, such as low and high density polyethylene, polypropylene, polystyrene, and unplasticized polyvinyl chloride. Oxygen permeability values obtained at 0% relative humidity were lower for protein films than for Nylon-6 films. Oxygen permeability increased as relative humidity increased because of the hydrophilic nature of the protein molecules (Gennadios *et al.*, 1993).

Gontard *et al.* (1993), demonstrated that during hydration of a gluten film (increase of relative humidity), mechanical properties such as puncture strength and elasticity increased while water vapor transmission rate and extensibility

decreased. These results are comparable to synthetic moisture-sensitive films such as polyvinyl alcohol, polyvinyl acetate, cellophane, cellulose acetate, and ethylene-vinyl alcohol copolymers. The same behavior was also observed and determined for nylon-6 (a polyamide) by Gavara and Hernandez (1994). Overall, natural proteins had very good oxygen barrier properties but were moisture sensitive.

DEGRADATION OF POLYMERIC MATERIALS

The effect of environmental stresses on polymeric materials involves complex reaction processes which are often initiated by ultraviolet radiation and usually involve long exposure times. Kaplan *et al.* (1993) indicated that three major phenomena can influence degradation processes in polymers: biological, chemical and physical (Table 1-1). According to Narayan (1989), degradable plastics are materials that undergo bond scission in the backbone of a polymer through any of the variables shown in Table 1-1. This bond scission would occur in an environment at a rate which is reasonably accelerated, as compared to a control, leading to fragmentation or disintegration of the plastic. Bio-degradable plastics are those degradable plastics where the primary mechanism of degradation is through the action of microorganisms such as bacteria, fungi, alage, and yeast. Photo-degradable plastics are those degradable plastics where the primary mechanism of degradation is thorugh the action of sunlight.

Coma *et al.* (1994) carried out studies on the bio-degradability of natural polymeric materials. These authors used *Pseudomonas fluorescens* as the microorganism to degrade cellulosic materials and measured CO_2 evolved as an

Table 1-1. Variables affecting the degradation of polymeric materials*

PHYSICAL	CHEMICAL	BIOLOGICAL
weathering (including sunlight)	hydrolysis	bacteria & fungi
leaching	oxidation	predators
mechanical abrasion		higher organisms

* Adapted from Kaplan *et al.* (1993)

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indication of such degradation. In addition, recently the American Association for Testing and Materials (ASTM) proposed standards for determining biodegradation of plastic materials by either municipal sewage (ASTM D-5209) or controlled composting conditions (ASTM D-5338).

PHOTODEGRADATION STUDIES

Weathering is a broad term that is applied to the changes that take place in a polymer on exposure outdoors, and one way to measure it is by photodegradation studies. Weathering testers are apparatus used to measure this effect under accelerated conditions. The main agents of weathering are sunlight (particularly ultraviolet radiation), temperature, thermal cycling, and moisture (Nicholson, 1991). Searle (1989) indicated that the chemical effects of the absorbed radiation are promoted by temperature, humidity, and oxygen among other weathering factors. The main degradation is brought about by ultraviolet light, assisted by contributions from the visible and near-infrared portions of the electromagnetic spectrum. In particular the near-infrared radiation accelerates degradation reactions by raising the temperature.

All of the factors involved in weathering, including both the amount and intensity of sunlight, vary both seasonally and geographically. To understand fully and predict the weathering behavior of any polymer requires information about exactly how these factors vary and how they then contribute to the overall degradation process.

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Reaction Mechanisms:

Since UV light is the major source of damaging radiation for polymeric structures, these wavelengths are considered to be the basis for most photo-degradation reactions (Selke, 1990). Although absorption of light is a prerequisite to photochemical reaction, it is not a sufficient condition. For degradation to occur, the energy absorbed must be equal to or greater than the energy of the weakest bond in the molecule (Searle, 1989). Since each wavelength of light is associated with a discrete amount of energy, the wavelengths absorbed determine the types of bonds that can be broken. According to Searle (1989), the probability of bond breakage by wavelengths of sufficient energy depends on the intensity of the radiation, the absorption coefficient of the polymer for these wavelengths, and the photo-physical processes. Various types of bonds that may be present in biopolymers are listed in Table 1-2 in order of decreasing bond strengths.

Sunlight radiation has sufficient energy to break bonds weaker than a C-H bond in methane. A source which has shorter wavelength radiation than sunlight would be more effective in causing degradation because the shorter wavelengths are more likely to be absorbed and are capable of breaking many more bonds. However, the higher energy will usually cause differences in mechanism and type of degradation.

The photo-physical processes are responsible for elimination of radiation harmlessly by reemission processes such as fluorescence or phosphorescence or by radiationless decay processes (Searle, 1989). Most of the energy

Table 1-2. Bond energies in biopolymers*

BOND	BOND ENERGY (kcal/mole)
C≡N	209
C≡C	200
C=O	174
C=C	145
C=S	129
C-C (aromatic)	124
C-H (acetylene)	121
C-F	119
O-H	110
C-H (ethylene)	106
C-H (methane)	98
Si-O	89
C-O	87
S-H	87
N-H	84
C-C (aliphatic)	80
C-O (ether)	79
C-Cl	78
S=S	76
Si-H	75
Si-C	70
C-N (nitromethane)	68
C-S	66
O-O (peroxide)	64
N-N (hydrazine)	37

*Adapted from Searle (1989)

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absorbed by polymers is dissipated by photo-physical processes. Therefore, photochemical bond breakage is a very inefficient process in polymers. However, the molecular weight of a polymer is significantly reduced by the breakage of only one bond in the backbone of the polymer and radical chain reactions promote degradation rapidly.

Currently used polymers may be divided into three main groups depending on their sensitivity to photooxidation: highly photostable, moderately photostable and poorly photostable (Nowakowska, 1989). The last group consist of polymers such as polyolefins, polydienes, polystyrene and rubbers. In general, the photooxidation of the polymers is initiated by low energetic light wavelengths (λ's > 290 nm).

Impurities, which are responsible for many of the initial photodegradation steps, can be divided into two main groups depending on the mechanism of their accelerating action in polymer systems: photoinitiators and photosensitizers. According to Nowakowska (1989), photoinitiators (*I*) are defined as compounds which can reach the electronically excited states by absorption of radiation in the near-UV or visible region:

$$I + h\nu \rightarrow I^* \qquad (1.6)$$

and subsequently undergo or participate in various types of photochemical reactions resulting in radical formation, e.g.,

$$I^* \rightarrow R_1^\bullet + R_2^\bullet \qquad (1.7)$$

$$I + PH \rightarrow I\text{-}H + P^\bullet \qquad (1.8)$$

where: PH = polymer macromolecule. The low-molecular-weight radicals ($R_1^{\bullet}$ & $R_2^{\bullet}$) as well as polymeric radicals ($P^{\bullet}$), can initiate the further processes of polymer oxidation.

Photosensitizers (*S*) are compounds which after excitation, e.g.:

$$S + h\nu \rightarrow S^* \qquad (1.9)$$

can participate in photo-physical processes, mainly in energy transfer processes of the types:

$$S^* + PH \rightarrow S + (PH)^* \qquad (1.10)$$

$$S^* + {}^3O_2 \rightarrow S + {}^1O_2^* \qquad (1.11)$$

$$S^* + A \rightarrow S + A^* \qquad (1.12)$$

This results in formation of electronically excited states of the polymer macromolecule $(PH)^*$, oxygen molecule-singlet oxygen, or the molecule of another energy acceptor A^* present in the system, which can further act as a sensitizer or an initiator.

In some cases, the same chemical compound may, depending on experimental conditions, act either as a photosensitiser or as a photoinitiator. Most of the compounds show the dualism of either accelerating action (Nowakowska, 1989). From here, the term "photosensitized oxidation" can be used to indicate the oxidation processes induced by the photosensitizers and photoinitiators. According to this author, the photooxidation of hydrocarbon polymers in outdoor conditions always occurs via photosensitized processes. In general terms, the mechanism of photosensitized oxidation of polymers depends

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mainly on the chemical nature of both components of the system, the polymer and the photosensitiser.

Summarizing, free radicals are formed in polymers exposed to light, as a consequence of the excitation of absorbing groups in the polymer. This is a function of the energy of the light and of the structure of the polymer molecule, and the presence of adventitious impurities which sensitize photooxidation. In the presence of oxygen the plastics will simultaneously oxidize (photooxidation). Furthermore, consecutive thermal processes (oxidation) may be superimposed on the photooxidation reactions. The overall process is sometimes designated photothermal oxidation. For the matter of this discussion, only photooxidation processes will be considered.

Table 1-3 illustrates a set of possible reactions that may occur during photodegradation reactions where UV radiation has importance mainly in the chain initiation and chain branching reactions, specifically in equations (1.13), (1.16), (1.17), and (1.18) (Gugumus, 1993).

In the next section, some methods to determine weathering effects are discussed.

Testing Methods:

In addition to speed, one advantage that laboratory weathering testers have over actual outdoor exposures is reproducibility of results. To achieve this reproducibility, testers must have control of the critical parameters of light, moisture, and temperature.

Table 1-3. Oxidation reactions in polymers*

Chain initiation	
Hydroperoxides POOH Carbonyl compounds (C=O) → free radicals ($P^\bullet$, $PO^\bullet$, $HO^\bullet$, $HO_2^\bullet$,...) Catalyst residues (Ti, ...) Charge-transfer complexes	(1.13)
Chain propagation	
$P^\bullet + O_2 \rightarrow PO_2^\bullet$	(1.14)
$PO_2^\bullet + PH \rightarrow PO_2H + P^\bullet$	(1.15)
Chain branching	
$POOH \rightarrow PO^\bullet + {}^\bullet OH$	(1.16)
$POOH + PH \rightarrow PO^\bullet + P^\bullet + H_2O$	(1.17)
$2\ POOH \rightarrow PO_2^\bullet + PO^\bullet + H_2O$	(1.18)
$PO^\bullet + PH \rightarrow POH + P^\bullet$	(1.19)
$^\bullet OH + PH \rightarrow H_2O + P^\bullet$	(1.20)
Chain termination	
$P^\bullet + P \rightarrow P\text{-}P$	(1.21)
$P^\bullet + PO_2^\bullet \rightarrow POOP$	(1.22)
$PO_2^\bullet + PO_2^\bullet \rightarrow POOP + O_2$	(1.23)
$PO_2^\bullet + PO_2^\bullet \rightarrow$ nonradical products + O_2	(1.24)

* Adapted from Gugumus (1993)

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Scott (1989) indicated that if one conducts a test using any of the laboratory accelerated testing devices currently on the market with the intent of correlating the results to those obtained by exposing replicate specimens outdoors, the probability of achieving success should be greatly enhanced if the appropriate factors of the average climate for the geographical area in which the outdoor exposure is made are programmed into the laboratory test. Programmed Environmental Testing is the concept of conducting accelerated laboratory weathering tests based on specific geographical outdoor exposure parameters. The particular device used in this study is described next.

Accelerated Weathering Tester with Solar Eye Irradiance Control

The Q-U-V Accelerated Weathering Tester with Solar Eye Irradiance Control is a modern apparatus which falls in the description for the fluorescent UV-condensation type. There are two main differences: The first is that this type of apparatus uses UVA lamps, which are lamps whose spectra closely resembles that of the sunlight. The second, is that there is a very close control of the irradiance coming from the UVA lamps, thus making it appropriate for testing materials simulating actual outdoor weathering.

Among other systems, "Sunshine Carbon Arc", "Enclosed Carbon-Arc", Xenon Arc", and "Fluorescent UV-Condensation", are included. For a more detailed explanation of any of these, see Scott (1989).

Outdoor Weathering

Outdoor weathering of polymer samples is performed on racks oriented to the south (northern hemisphere) with the exposure surface inclined usually at

45°. The samples may be mounted on suitable backings, preferably stainless steel, or on frames. Outdoor weathering under glass is another possibility. The standards to be used are: DIN 53386-1982, ISO 4607-1978, ASTM D-1435-85.

Measurement of incident energy is achieved with pyroheliometers and expressed as Joule cm^{-2}. In this way, the radiation from the ultraviolet to the infrared region is registered but the short-wavelength UV radiation mainly responsible for polymer degradation is not measured separately. This is one of the reasons for the variation of weathering results with geographical location and season.

On outdoor weathering, samples are usually motionless. However, in the so-called EMMA or EMMAQUA devices, the samples are moved to follow the sun to ensure continuous vertical sunlight. Through additional focusing of the incoming light, "accelerated outdoor weathering" is achieved, used so far mostly in the USA (Gugumus, 1993). The considerations discussed show that photooxidative degradation is heavily dependent on the season. Therefore, the time of the beginning of the outdoor weathering may influence the results considerably, especially for short-lived articles, and for this reason it should always be mentioned.

In conclusion, photo-degradation of polymeric materials occurs primarily by the combination of these two factors: 1) difficulty to obtain pure polymeric systems, free of photoinitiators and/or photosensitizers; and 2) radiation from sun with capability to produce or promote bond scission. Therefore, the study of

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photo-degradation of biopolymers, as new materials to make films and coatings, is necessary to establish practical and potential uses.

CHAPTER 2. FABRICATION OF WHEAT PROTEIN FILMS

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CHAPTER 2. FABRICATION OF WHEAT PROTEIN FILMS

INTRODUCTION

Although degradable films can be successfully formed using carbohydrates, lipids, and proteins, today there is limited information available on degradable packaging materials produced from these goods. In the United States, approximately 57 million metric tons of wheat are produced annually. At the farm gate, this figure contributes more than $7 billion to the US Gross Domestic Product. Given that edible/degradable films from wheat proteins, if commercialized, could replace some synthetic packaging materials, their future seems assured. It is estimated that growth of 4% in the global consumption of plastic resins from about 101 million metric tons in 1993 to about 123 million metric tons in 1998 will be observed, with the US consumption estimated to rise from about 30.5 to about 36 million metric tons in these same period (Modern Plastics Encyclopedia, 1995). From these figures, packaging is the single largest market for plastics, and most processed (and many non-to mildly processed) foods are packaged. Therefore, the possibility of finding a market for edible/degradable films seems promising.

Wheat proteins present unique characteristics for use as edible or degradable films. First, they account for 8-15% of the dry weight of wheat kernels and about 70% of the total protein is contained in the wheat endosperm, the major source of wheat flour (Gennadios *et al.*, 1993). Second, the presence of high molecular weight groups of proteins is highly advantageous in order to

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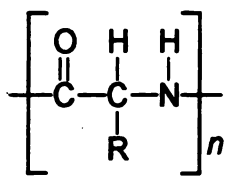
form a film with desirable properties. Third, the gas permeability characteristics of films obtained from proteins in general have been determined to be low (Donhowe and Fennema, 1994 and Rayas, 1995).

CHARACTERISTICS OF WHEAT PROTEINS

Wheat Protein Chemistry

Proteins are sometimes referred as nylon-2 (Sperling, 1992), and have the following general structure:



(2.1)

There are 20 common types of amino acids found in protein structures. The structure of the "R" group, depends on the amino acid present in the repetitive unit. These amino acids follow very specific sequences, but they are copolymers in the broad sense of the term (Sperling, 1992).

Wheat flour proteins are very unique and therefore could be studied apart from other cereal proteins. Krull and Wall (1969), reported the composition of wheat proteins based on solubility and identified the following types: albumin (water soluble), globulin (salt solution soluble), gliadin (70% ethanol soluble), and glutenin (dilute acid soluble) proteins. Gluten comprises about 80% w/w of total protein and is formed by the gliadin and glutenin fractions. Chen and Bushuk (1970) reported the components of hard red spring wheat using the classical protein fractionation procedure of Osborne (see Table 2-1), in which

Table 2-1. Fractional solubility of the endosperm proteins of hard red spring wheat*

Component	Protein content (%)	Fraction of total protein (%)
Water-soluble fraction	53.3	11.9
Salt-soluble fraction	76.2	5.2
Alcohol-soluble fraction	89.7	28.5
Acetic-acid-soluble fraction	70.2	16.6
Residue	6.4	34.0

* Adapted from Chen and Bushuk (1970)

components were analyzed for protein content and the fraction that each one represented as compared to the total sample weight.

Each of these fractions have different amino acid compositions which in turn gives them their solubility and functional characteristics. Krull and Wall (1969) reported that concentrations of ionizable amino acids (acidic: glutamic, aspartic; and basic: lysine, histidine and arginine) are low for gliadin and glutenin but high for albumin and globulin. Glutamine accounts for more than 35% of the total amino acids present in wheat gluten (Hoseney, 1994), corresponding to similar values reported by Wall and Beckwith (1969), who indicated that about 37% of the total amino acid content in wheat gluten proteins was glutamine, and Krull and Inglett (1971) who reported that about 37% of glutamine was present in gluten. However, Gontard *et al.* (1993) reported that about 45% of the amino acid present in wheat gluten proteins is glutamine. Another important amino acid in gluten is proline, because of its influence in the structural conformation of the proteins. This amino acid comprises about 12.5% and 13.9% in glutenin and gliadin proteins, respectively. When a proline residue is present in an α-helix, it imparts a twist to the polypeptide chains. In addition, it is known that proline residues are frequently found in flexible regions of proteins (Branden and Tooze, 1991), which suggests that the gluten proteins are elastic.

According to Krull and Wall (1969), physical bonds (i.e. hydrogen bonding, electrostatic interaction, and hydrophobic interaction) can be broken by the use of specific solvents, changing the pH of the solution, increasing the temperature or altering the salt content. Also, using an acetic acid solution (pH

3.8) gliadin had approximately 23% helix as compared to 14% for glutenin. They also indicated that gliadin has a more stable structure as compared to glutenin due to internal disulfide bonds, and found that glutamine residues and non polar amino acids are responsible for the aggregation of gluten proteins in aqueous media. In this study, Krull and Wall (1969) also reported that amide groups are the primary sites of association of wheat flour proteins through hydrogen bond interaction, which in part give wheat dough cohesive and elastic characteristics.

MOLECULAR WEIGHT

Albumins and globulins in flour are commonly referred to as the soluble proteins (Kent, 1983). Albumin proteins of wheat, according to Kent (1983), have molecular weights of 17,000 to 28,000. They are responsible in part for the differences in baking characteristics among flours. This author also indicated that globulins are essential for proper baking performance. Both albumin and globulins have metabolic and structural functions.

The insoluble proteins (gliadin and glutenin) are regarded as the storage proteins of wheat and are contained primarily in the endosperm. The gluten fraction is composed of two main groups of proteins: a) gliadin, soluble in 70% ethanol, and b) glutenin, soluble in dilute acetic acid. According to Hoseney, (1994), the gliadins are a heterogeneous group of proteins which have an average molecular weight of about 40,000 and are single-chained. Kent (1983) mentioned reported values of 42,000 to 47,000 for the molecular weight of the gliadins. These proteins are extremely sticky when hydrated, with little or no

resistance to extension. Gliadins are thought to be responsible for dough cohesiveness.

The molecular weight of the peptide chains of the glutenin fraction is on the order of 20,000 (Kent, 1983). Hoseney (1994) indicated that the range of the molecular weights of the glutenin subunits is from 16,000 to about 133,000. They are a heterogeneous group of proteins bonded together by disulfide bonds into macrounits with molecular weight from about 100,000 to several million (Hoseney, 1994 and Kent, 1983). Their average complex molecular weight is about 3 million. A physical characteristic of this group of proteins is that they are resilient but not cohesive, therefore these proteins are known to give dough its property of resistance to extension (Hoseney, 1994).

WHEAT FLOUR PRICING INFORMATION

Wheat prices vary depending on the crop production for every year. The year of 1996 was a year in which the average price observed of about 0.12 $/lb was abruptly interrupted by a peak held from about May through July. This jump was due to a smaller-than-expected forecast for the 1996 corn crop. According to Milling & Baking News (1996), "when wheat prices jumped upward in sympathy with corn, a few buyer stepped forward to cover October-December requirements. Prices were however back to their normal range and are currently in a downtrend since grain stocks are building and are predicted to build again next year.

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The objective of this study was to develop a method to make degradable films from wheat proteins, providing characteristics similar to that of synthetic films. Characterization of the resulting films including color, oxygen permeability, and tensile properties was also conducted. Cost of the film based on wheat flour usage was estimated using current wheat flour prices.

MATERIALS AND METHODS

FLOUR

A commercial enriched bleached wheat bread flour, "Bakers and Chefs," made of a blend of hard spring and winter wheat class flours and distributed by North Arkansas Wholesale Co. Inc. (Bentonville, AR) was used to prepare all films.

FLOUR CHARACTERIZATION

Flour moisture contents of the samples were determined by an oven method 44-15A (AACC 1992). Flour protein content was determined by the micro-Kjeldahl method 46-13 (AACC 1992). Falling Number was determined according to standard method 56-81B (AACC 1992) to evaluate for any sprouted wheat present in the flour sample. A Farinograph was used to evaluate dough properties of the flour sample.

FILM PREPARATION

Two hundred grams of flour at 14% moisture basis (mb) was added to 330 ml of hexane and stirred for 10 minutes at room temperature. Centrifugation followed at 1,700 x G for 10 minutes at room temperature. To the precipitate, 650 ml of a 0.5 M NaCl solution was added, stirring for 15 minutes at room temperature. Centrifugation was carried out at 1,700 x G for 15 minutes, with disposal of the supernatant. To the precipitate, 600 ml distilled water was added, in order to wash out any remaining NaCl, and continuous mixing for 10 minutes was performed. Centrifugation followed for 15 minutes at 1,700 x G. This last process was repeated once more. After the final centrifugation, the

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precipitate was mixed with 500 ml of a 70% (v/v) ethanolic solution. The pH was adjusted to 4.0 with a 1:1 glacial acetic acid:lactic acid (v/v) solution. Once the pH was adjusted, mixing continued for one hour at room temperature, after which the slurry was centrifuged at 1,700 x G for 15 minutes at room temperature. Separation of the starch was achieved and asserted by the intense blue color present upon addition of an iodine solution to the white precipitate. About 400 ml of supernatant was collected to make the film-forming solution. A cross-linking agent consisting of 0.25 ml of a 37.5% formaldehyde solution (Mallinckrodt Specialty Chemicals Co., Paris, KY) was added at this time. It was assumed that the molecular weight of the biopolymer was first decreased by breaking disulfide bonds by the solvents and heat used, unfolding the proteins, as demonstrated by Rayas (1995). However, upon the cross-link reactions, a permanent increase to several million dalton was assumed to occur, although no studies were carried out to investigate the extent of this. Cross-linking studies have been investigated elsewere (Gruenwald, 1993; Nicholson, 1991; Sperling, 1992; Mandelkern, 1993). The film-forming solution was concentrated by boiling it down to approximately 30% solid content. Three grams of glycerol (plasticizer) were added. The solution was then placed into a spreader with a layer thickness regulator (Desaga Co., Heidelberg, Germany) heated at 80°C to avoid setting of the film-forming solution inside the spreader cavity.

The viscous film-forming solution was then cast to a thickness of 1.0 mm on a clean glass surface. The film was dried in air at 12±1% relative humidity and 22°C for 24 hours, and later removed from the glass and placed into a

desiccator containing Drierite (W.A. Hammond Drierite Co., Xenia, OH. USA). Films were kept in the desiccator at a constant moisture content until the tests were performed. Film thickness was measured by a Micrometer Model 549 (Testing Machines, Inc., Amityville. NY, USA). A simplified flow diagram of the film-making process is presented in Figure 2-1.

FILM CHARACTERIZATION

a) Color analysis

The physical measurement of color for the films was done using Color Hunter Lab instrument 45/0 ColorQUEST (Hunter Associates Laboratory, Inc., Reston, VA. USA). Values for "L," "a," and "b" corresponded to 0 to 100% (black to white), negative-to-positive (green-to-red), and negative-to-positive (blue-to-yellow), respectively. Protein, PET, EVA, LDPE, and PVC films were tested.

b) Oxygen permeability

Barrier characteristics of the films were tested by measuring their oxygen permeability at 25°C, according to ASTM method D-3985 (ASTM 1992). Relative humidity was measured to be <2% when at dry conditions. Sample films of 11x11 cm with thickness of 4.826×10^{-5} m (1.9 mil) were prepared. An Oxtran 200 permeability tester apparatus (Modern Controls, Inc., Minneapolis, MN. USA), equipped with an Endocal temperature control bath, model RTE 100 (Neslab Instruments, Inc., Newington, USA), was used. Measurements were conducted using dry oxygen and dry nitrogen as test and carrier gases, respectively.

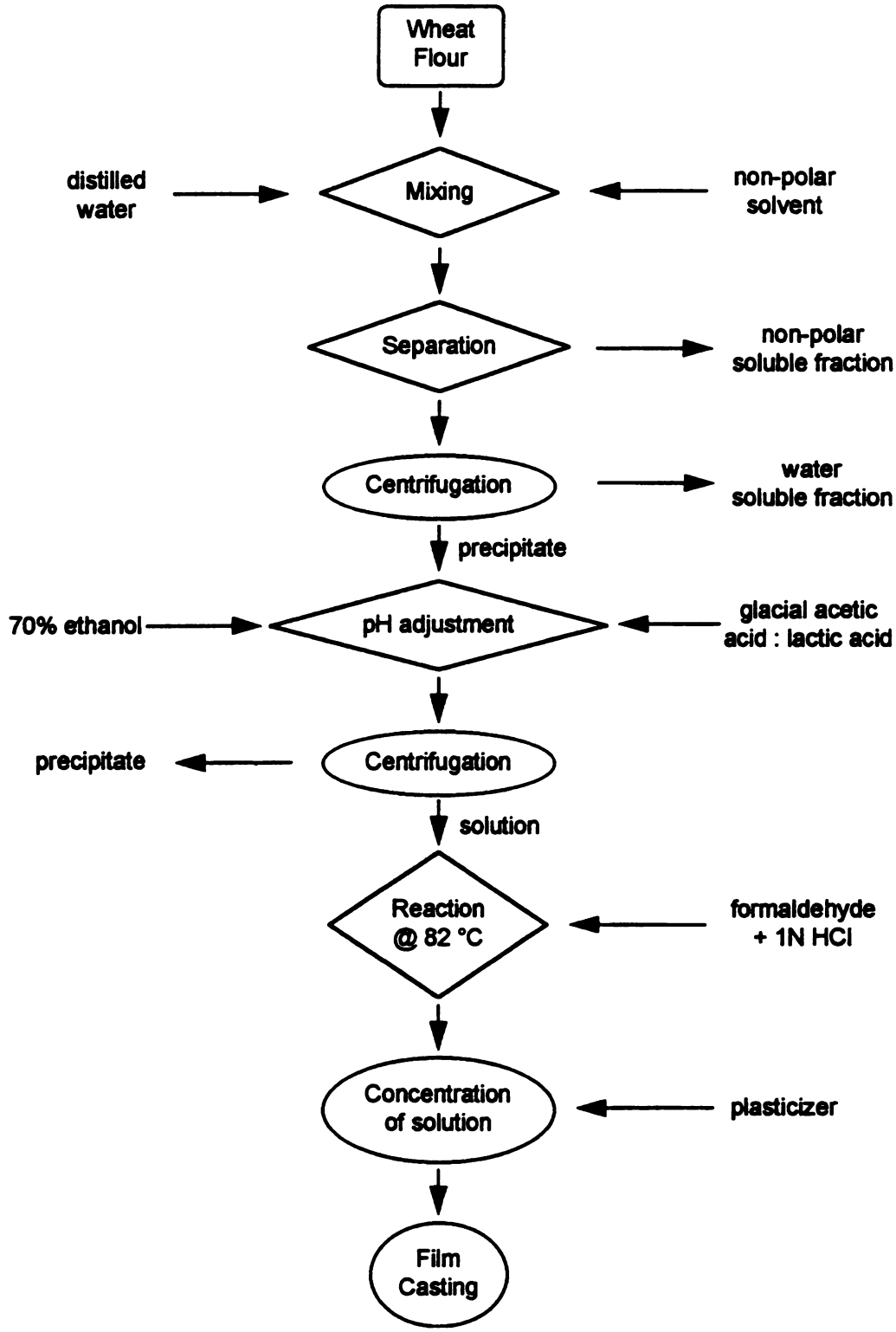

Figure 2-1. Diagram of the protein-based film-making process

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c) Tensile properties

Tensile measurements were carried out on sample films according to ASTM method D-882 (ASTM 1992). An Instron Model 4201 apparatus (Canton, USA) equipped with a 1 kN static load cell was used. Sample films were cut to 25.4 mm wide using a JDC Precision Sample Cutter model 25 (Thwing Albert Instrument Co., Philadelphia, PA, USA). The grip separation in the Instron was set to 50.8 mm (2 in), with cross head speed of 508 mm/min (20 in/min). Prior to the tensile measurements, the films were conditioned at 22±1°C and 50±2% relative humidity for 48 hours.

FILM COST ANALYSIS

Film cost was estimated using current wheat flour prices. Flour price used was 0.11 $/lb, which corresponded to the average price for the month of October 1996. A year before (October 1995), the price was 0.15 $/lb. The 12-month price range was 0.1050-0.1806 $/lb. As explained earlier, currently wheat flour prices are in a downtrend, the grain stocks are building and it is estimated that will build again next year (Milling & Baking News, 1996).

RESULTS AND DISCUSSION

FLOUR CHARACTERIZATION

The summary of the characteristics measured for the flour used to make the films is presented in Table 2-2.

Table 2-2. Characteristics of the commercial flour used in this study[1]

Moisture (%)	Protein[2] (%)	Falling Number (s)
12.11 ± 0.08	13.56 ± 0.09	241 ± 11

[1] Mean values ± standard deviation of five replications.
[2] Adjusted to 14% moisture basis.

Results indicated that the flour was in the lower recommended moisture level of 12 to 13% so that minimum oxidation or mold growth would occur. From this, it was expected and assumed that no significant detrimental changes that could vary the results would be found in the flour. As a preventive action, it was stored at 4 °C in sealed containers while not in use. The end purpose of this flour was to serve as a bread flour, and was labeled as a "blend of hard spring and winter wheat", therefore, it was expected that it would have the level of protein content found as typical of these flour classes. It was probable that the flour had added vital wheat gluten. This was assumed because flour companies usually add vital wheat gluten to the blend of wheat flours sold to adjust or

replace the original protein content of the flours, depending on final use (McDermott, 1985 and Magnuson, 1985).

As for the falling number, the value fell within the range indicated by Perten (1988) for unsprouted wheat. From this result, it was anticipated that possible problems associated with the α-amylase activity, such as presence of free sugars in the FFS (non-enzymatic browning, etc.), would be minimum, or absent from this study. In addition, since flours milled from sprouted wheat may present not only α-amylase but protease activity (since both are enzymatic changes involved in the development of the new plant), it was assured that the flour used in this study contained the proteins with minimum or no degradation (i.e., no change in molecular weight of the biopolymer). Overall, the determination of α-amylase as a variable is expected to play a minor role in the process since one of the objectives of the preparation of the film is to separate the starch by centrifugation. Its importance falls in the indirect determination of any change in molecular weight of the proteins.

Dough resistance to mixing during the successive stages of its development is measured with the Farinograph. This property may be important to know since it could relate to and be used as an indirect tool to select in the future from different types of flour to make the biopolymers using the procedure of this study (i.e. flour that yield films with desired characteristics). This could be particularly true because during the fabrication of the film, mixing of the film-forming solution is done and protein-protein interactions are affected by this mixing process, which could be similar to those found during dough mixing.

It was observed from the farinogram (Figure 2-2) that the shape of the curve is typical of strong flours. This behavior agrees with a report by D'Appolonia (1984) and Lorenz (1984) indicating that, with an increase in protein content, a definite strengthening of the farinogram curve occurs. Also, by comparing the shape with those presented in a study by Preston and Kilborn (1984), it was established that the flour could be considered strong. This was further validated since the values for MTI of 40 (Table 2-3), fell between the 30 (strong) to 80 (medium strength) range, classification developed by these authors. The calculated values obtained from the curve in Figure 2-2 for water absorption, dough development time (mixing time), and stability are also reported in Table 2-3. Since absorption is correlated positively by the protein content and the damaged starch (D'Appolonia, 1994), and the flour contained added gluten, it was expected that the higher protein level in it yielded a slightly higher value of the maximum typical value expected. The absorption values for this type of flour indicated a resemblance with the observations made by Shuey (1984) in which absorption generally increases, when all other variables are kept constant, with an increase in the protein content of the flour. This indicated that the commercial flour required, respectively, greater amounts of water, as compared to other flours, to give the same consistency. Finally, stability showed great tolerance to mixing with a value of 7.25 minutes.

FILM CHARACTERIZATION

The developed process has the following tangible advantages over published procedures:

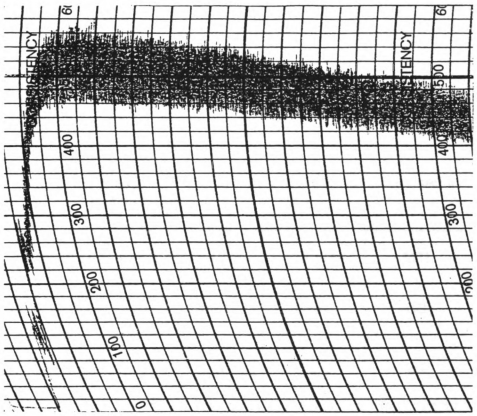

Figure 2-2. Farinogram of the commercial wheat flour used to make the films
*increments are 0.5 min

Table 2-3. Farinograph results for the commercial flour used in this study*

Water absorption (%)	Mixing time (min)	Stability (min)	MTI (BU)
67.05	2.50	7.25	40

* Values obtained from Figure 2-2

- The flour disperses easily (no lumps formed) as compared to gluten.
- After centrifugation, mostly soluble material is present in the supernatant which ultimately leads to films with greater homogeneity.
- No granular texture is observed in the final films.
- The films obtained using the new method are transparent, comparable to synthetic films.

Biodegradability

Another important observation made from the film, was that of being bio-degradable. Altough this was not an objective of this study, it was done to corroborate the expected bio-degradability, particularly after cross-linking of the wheat proteins was performed. Two sample pieces of the film were put in a beaker, across from each other. Each one of them was placed towards the bottom the beaker corner so that a part of it was on the bottom floor and the other part on the wall. A small quantity of tap water was added in the beaker and then it was held at room temperature for 3 weeks. Within one week, colonies of mold were growing on the film surface and totally covered the film. By the second to third weeks film integrity was totally lost. At the third week, addition of more water and mixing promoted complete disintegration of both film samples. This experiment demonstrated that the film upon cross-linking was bio-degradable. No analytical data was obtained from this experiment, only the visual data reported above.

a) Color Analysis

The results for the Hunter Color analysis are presented in Table 2-4. Some common synthetic plastics were measured and included in order to compare where the protein film values fall among them.

Table 2-4. "L", "a", and "b" values* for synthetic and protein-based films

Color value	FILM				
	Protein (5 mil)	PET (0.5 mil)	EVA (0.8 mil)	LDPE (1.25 mil)	PVC (1 mil)
L	92.55	94.64	94.83	96.93	97.59
a	-1.13	-0.20	3.01	-0.07	-0.02
b	7.76	0.69	-1.06	-0.16	0.19

* average of 5 measurements.

It was observed that the whiteness, "L" value (indirectly related to the transparency as it was setup for this study) of the protein film was similar to those of their synthetic counterparts, especially if the thickness of the films measured is taken in account. As for the "a" value, it was observed that the values fall to the green area of the spectrum although the value was so small that it was not apparent in visual comparisons. On the other hand, the "b" with a value of 7.76 towards the yellow side of the spectrum, was significantly higher than their synthetic counterparts. The color could be barely picked up by the naked eye, particularly if comparing the plastics side-to-side. However, it is important to consider that the thickness of the protein films analyzed was about five times that of the synthetic plastics.

b) Oxygen Permeability

The oxygen permeability values of the protein film studied, with a thickness of 1.9 mils (4.826×10^{-5} m) and literature values for some common synthetic films, are listed in Table 2-5. The oxygen permeability value was 2.0×10^{-18} m^3 m m^{-2} s^{-1} Pa^{-1} and was the same order of magnitude as the values reported for biaxially-oriented nylon (BON), with an average of and 6.8×10^{-18} m^3 m m^{-2} s^{-1} Pa^{-1}. The similarity of values may be explained by the similar chemical structure of these materials based on the polyamide functionality (Sperling, 1992).

From Table 2-5, only polyvinylidene chloride (PVDC) and ethylene vinyl alcohol (EVOH) were better oxygen barriers than the protein films. In addition, values in the order of nylons or lower are generally considered to be good oxygen barriers (Osborn and Jenkins, 1992).

c) Tensile Properties

Results showed that the wheat protein films tested had average tensile strength and elongation values of about 2,200 lb in^{-2} and 290%, respectively. These values fell in the range of those found for low density polyethylene (Table 2-6). It is important to note that the measurements were carried out at 50% relative humidity, and lower values would be expected at lower relative humidity conditions.

This may lead to think of possible uses of the film as a supporting structure, including those of pouches, small bags, particularly if used around those relative humidities. In addition, it is anticipated that if used at lower

Table 2-5. Oxygen permeability through films made of wheat protein[1] and other common synthetic[2] films

Film	Oxygen Permeability Coefficient (m^3 m m^{-2} s^{-1} Pa^{-1})
Wheat Protein	$1.270\text{-}2.634 \times 10^{-16}$
LDPE	2.240×10^{-15}
HDPE	$4.497\text{-}8.994 \times 10^{-16}$
EVA	$3.148\text{-}4.047 \times 10^{-15}$
OPP[3]	$4.497\text{-}7.195 \times 10^{-16}$
PET[3]	$1.350\text{-}2.698 \times 10^{-17}$
PVC	$0.135\text{-}2.698 \times 10^{-16}$
PVDC	$0.450\text{-}4.497 \times 10^{-19}$
OPS[3]	$0.899\text{-}1.574 \times 10^{-15}$
BON[3]	$4.497\text{-}8.994 \times 10^{-16}$
EVOH	$4.497\text{-}8.994 \times 10^{-20}$
PC	$0.809\text{-}1.349 \times 10^{-15}$

[1] Average of 2 replications ± deviation
[2] Adapted from Jenkins and Harrington (1991). Measured at 77°F and 0%RH.
[3] Biaxially oriented

Table 2-6. Tensile properties of films made of wheat protein[a] and other common synthetic[b] films

Film	Tensile Strength (lb in^{-2})	Elongation (%)
Protein[c]	2100-2300	260-325
LDPE	1200-4550	100-650
HDPE	3200-4500	10-1200
Cellulose acetate	4500-8000	25-50
EVOH	5400-13650	180-330
Nylon-6	6000-24000	300[c]
PC	9100-10500	110-150
PP	4500-6000	100-600
PS	5200-7500	1-3

[a] Range of 3 replications
[b] Adapted from Modern Plastics Encyclopedia (1996)
[c] Conditioned @ 50% RH

relative humidities as a primary package, inside a box, another bag, etc., it may compared to other plastics such as HDPE, cellulose acetate, or even PP.

FILM COST ANALYSIS

The cost was calculated based on an average basis (see below) taking into account total flour used in each experiment and the respective weight, thickness and area of the resulting film. These figures were estimated to have an accuracy of ± 10%, based on the observed values. For instance, for the average film, using the following basis:

Flour used = 100 g	Weight of protein in film = 6.85 g
Area of film = 22 x 40 cm^2	Thickness of film = 2.9 mils

The proximate density of the film was calculated using the following formula:

$$(6.85) / [(22)(40)(2.9)(.001)(2.54)] = 6.85 / 6.48 = \mathbf{1.06\ g/cm^3}$$

This value corresponded to the fact that when a piece of film was immersed in beaker containing distilled water, it slowly sinked to the bottom of it.

Using the current wheat flour price of 0.11 $/lb of flour, or 0.2423 $/kg, thus the price per film as described above was calculated as follows:

(0.2423 $/kg)(0.1 kg/film) = **0.02423 $/film**,

and, the price per square meter of a one-mil thickness wheat protein film is **0.0459 $/m^2-mil**. In addition, when taking into account the proximate density of the biopolymer of 1.06 g/cm^3, the price of the biopolymer is **0.0017 $/g, or 1.7056 $/kg.**

This price may appear to be expensive, but one must take into account that there is also about 85% of wheat starch that is separated during the fabrication process. This starch can potentially be recovered thus decreasing the total cost of the film and film process. Also, given that thickness of the film is 1 mil, one kilogram of plasticized biopolymer would yield about 670 m^2 of the wheat protein film.

CONCLUSIONS

The films produced by this method are homogeneous, clear, transparent, and strong. This was attributed to the fact that the protein solution from which films were cast did not contain lipid-soluble compounds or starch. These wheat flour components are removed from the solution both by dissolution as well as by centrifugation processes. The starch can be completely recovered as a by-product. In addition, a large percentage of the solvent can be re-used from the supernatant containing the proteins through evaporation. This could potentially help to develop an industrial process which would be efficient in terms of costs and environment.

From the mechanical standpoint, the films were strong, with similar tensile properties to LDPE. Potential applications of these films are in the food and pharmaceutical industries for wrapping products and protecting them from contamination, oxygen, and, if edible, increasing the nutritive value of the food products.

CHAPTER 3. MASS TRANSFER PROPERTIES OF BIOPOLYMERIC FILMS

CHAPTER 3. MASS TRANSFER PROPERTIES OF BIOPOLYMERIC FILMS

INTRODUCTION

WATER SORPTION ISOTHERM OF POLYMERIC FILMS

Water activity or equilibrium relative humidity (ERH) is related to water content in organic materials by their sorption isotherms. In general, a biopolymer stored in a room at constant temperature in a closed environment develops a unique water vapor pressure which depends on the initial water content and the temperature (Badui, 1981). There are two basic methods for measuring sorption isotherms: in the first a sample of known water content is placed in a closed system and a sensor measuring vapor pressure or relative humidity in the headspace reports water activity; in the second method, a sample of known initial water content is placed in a constant humidity enclosure and the final water content is determined after equilibration (Karel, 1975).

The relationship between water activity with other factors is given by the following equation:

$$a_w = \frac{ERH}{100} = \frac{p}{p_0} = \frac{M_a}{M_a + M_s} \qquad (3.1)$$

where a_w is water activity, ERH is percent equilibrium relative humidity, p is water vapor pressure of sample at temperature T, p_0 is water vapor pressure of pure water at temperature T, Ma is moles of water, and Ms is moles of solute.

Typical isotherms in food systems are "S" shaped. A portion of the total water content present in food is strongly bound to specific sites. These sites

include the carbonyl and amino groups of proteins, hydroxyl groups of polysaccharides, and other on which water can be held by hydrogen bonding, by ion-dipole bonds, or by other strong interactions (Karel, 1975).

According to Karel (1975) not all of the water present in food systems can be considered as free water. For instance, water bound on specific sites (i.e., monolayer water) is not. According to this author, knowledge of the sorption behavior of biological systems is useful in concentration and dehydration processes for two reasons: (a) it is of importance in design of the processes themselves, because it has an important impact on the ease or difficulty of water removal, which depends on the partial pressure of water over the food and on the energy of binding of the water in the food; and (b) water activity affects food stability and therefore must be brought to a suitable level at the conclusion of drying and maintained within an acceptable range of activity values during storage.

A way of estimating the contribution of adsorption at specific sites to total water binding is by the use of the Brunauer-Emmet-Teller (BET) isotherm. The equation is as follows:

$$\frac{a_w}{m(1-a_w)} = \frac{1}{m_1 C} + \frac{(C-1)}{m_1 C} a_w \qquad (3.2)$$

where a_w is water activity, m is water content, m_1 is the monolayer value, and C is a constant. This equation is based on oversimplified assumptions, but extremely useful in estimating the "monolayer value", which can be considered

as equivalent to the amount of water held adsorbed on specific sites (Karel, 1975).

From the profile of weight gain versus time, the diffusion coefficient of water in protein films can be determined. The raw data is gathered when water gain is recorded as a function of time until constant weight, and water content is determined by the difference in weight between dry and humidified atmospheres. From sorption curves the diffusion coefficient, D, can be determined by the following equation (Hernandez and Gavara, 1994):

$$D = \frac{0.049\ell^2}{t_{0.5}} \qquad (3.3)$$

where ℓ is the film thickness in centimeters and $t_{0.5}$ is the time in seconds when half of the total water uptake is attained.

OXYGEN PERMEABILITY OF POLYMERIC FILMS

Gas and vapor transport through polymeric materials is of great importance to the packaging industry because a main function of a package is to protect its contents. No polymer film is known to provide a complete barrier to the transport of a gas or vapor molecule (Brown, 1981). The overall phenomenon is influenced primarily by the chemical composition, size, shape, and polarity of the penetrating molecule, and chemical composition and polymer-chain segmental motion within the film matrix. The determination of the permeability of edible films is carried out in a similar fashion as for non-edible

films, i.e., continuous flow and quasi-isostatic methods (Donhowe and Fennema, 1994).

The objectives of the present study were to determine mass transfer properties of wheat protein films. Sorption isotherm, sorption curves and their corresponding diffusion coefficients, and oxygen permeability as a function of the relative humidity were obtained and discussed.

MATERIALS AND METHODS

FILM

All films were prepared according to the methodology of Chapter 2.

WATER SORPTION ISOTHERM

A sorption isotherm curve was obtained at 25°C. The moisture weight gain was carried out on a CAHN D-200 Digital Recording Balance (CAHN Instruments, Cerritos, CA). The arm of the electrobalance was encased in a Controlled Temperature/Humidity Chamber model SM8SH (Thermotron Industries, Holland, MI). Nitrogen gas was humidified at different levels by means of bubbling it in water. Different relative humidities were obtained by controlling the amounts of the mixed dry/moist gas passing through the balance (see Figure 3-1). The relative humidity was measured by using a Hygrocheck™ hygrometer (Hanna Instruments, Woon Socket, RI).

The films were placed in one arm of the electrobalance which in turn was placed inside the controlled temperature/humidity chamber. The initial weight was recorded and then dry nitrogen gas (measured to have <2% RH) was flushed for at least 48 hr. to further dry and equilibrate the film sample until a flat base-line was obtained. The assumption of the film with no removable water was made and it was at this point that the film was considered dry. All sorption curves were obtained starting from dry condition. Then, each sorption curve was obtained by humidifying of the nitrogen gas as described above (obtaining a specific ERH%). When a new curve was to be obtained, the film was again

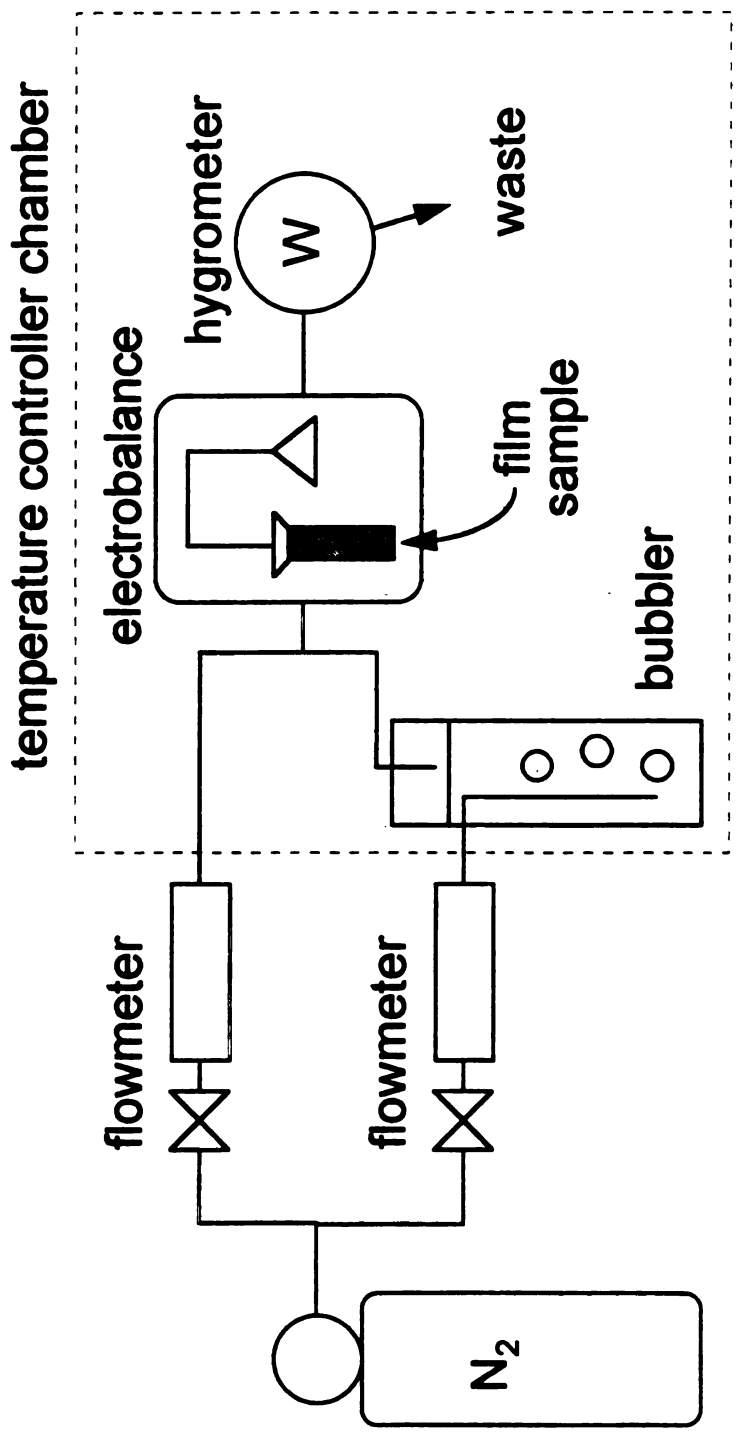

Figure 3-1. Diagram of the sorption apparatus

equilibrated with the dry nitrogen gas to dry conditions, and a new level of humidity of the gas was then tested.

Oxygen Permeability as a Function of Relative Humidity

Oxygen permeability was measured according to the standard method ASTM D-3985 (ASTM, 1992). This property was determined as a function of the relative humidity at room temperature (25°C). An Oxtran-200 permeability tester apparatus (Modern Controls, Inc., Minneapolis, MN) was used to determine the oxygen flux (J). A schematic of the apparatus is shown in Figure 3-2. The temperature was controlled using an Endocal temperature control bath model RTE 100 (Neslab Instruments, Inc., Newington, NH).

Statistical Analysis

Data obtained were treated according to the Student-Neuman-Keul comparison test and the two-way analysis of variance calculated using MSTAT-C statistical program (Michigan State University, 1990).

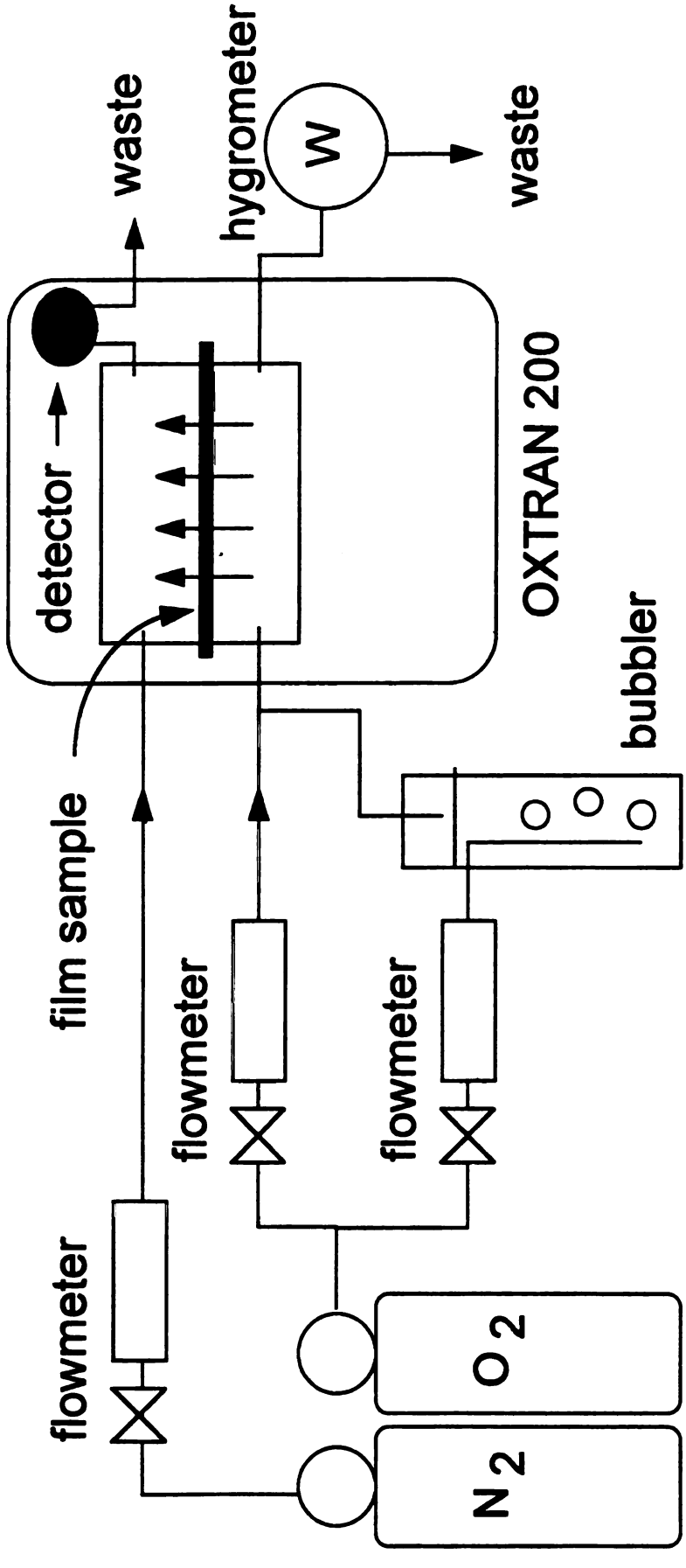

Figure 3-2. Diagram of apparatus setup for measurement of oxygen permeability as function of the relative humidity

RESULTS AND DISCUSSION

WATER SORPTION ISOTHERM

The sorption isotherm for the protein films was obtained at 25°C (Figure 3-3). As expected, as the relative humidity increased, the moisture content also increased, particularly above 40% ERH. This may explain the actual observation that the film can be maintained in up to 40% ERH environments without changing significantly its physical tangible attributes. Values of the moisture content reached values of more than 100% for ERH of 71.3%. This figure correlated with the oxygen permeability dependency on relative humidity as explained later below. The sorption isotherm provides a basis to select food applications for this film.

Using a least square difference statistical analysis, the parameters of the BET isotherm were evaluated as shown in Figure 3-4. In addition, the monolayer value (m_1) for water was calculated from this plot using equation 3.2.

From this analysis, the values of m_1C and C-1 were calculated as follows, using the BET equation:

$m_1C = 1 / 0.0366 = 27.3224$, and

$C-1 = 4.8165\ (m_1C)$

Therefore,

$C = 1 + (4.8165)\ (27.3224) = 132.5984$, and

$m_1 = 27.3224 / C = 27.3224 / 132.5984 = 0.2061$ g H_2O / g solids.

From these results, when substituting the parameters obtained, theoretical data agreed very well with experimental results. In addition, it was

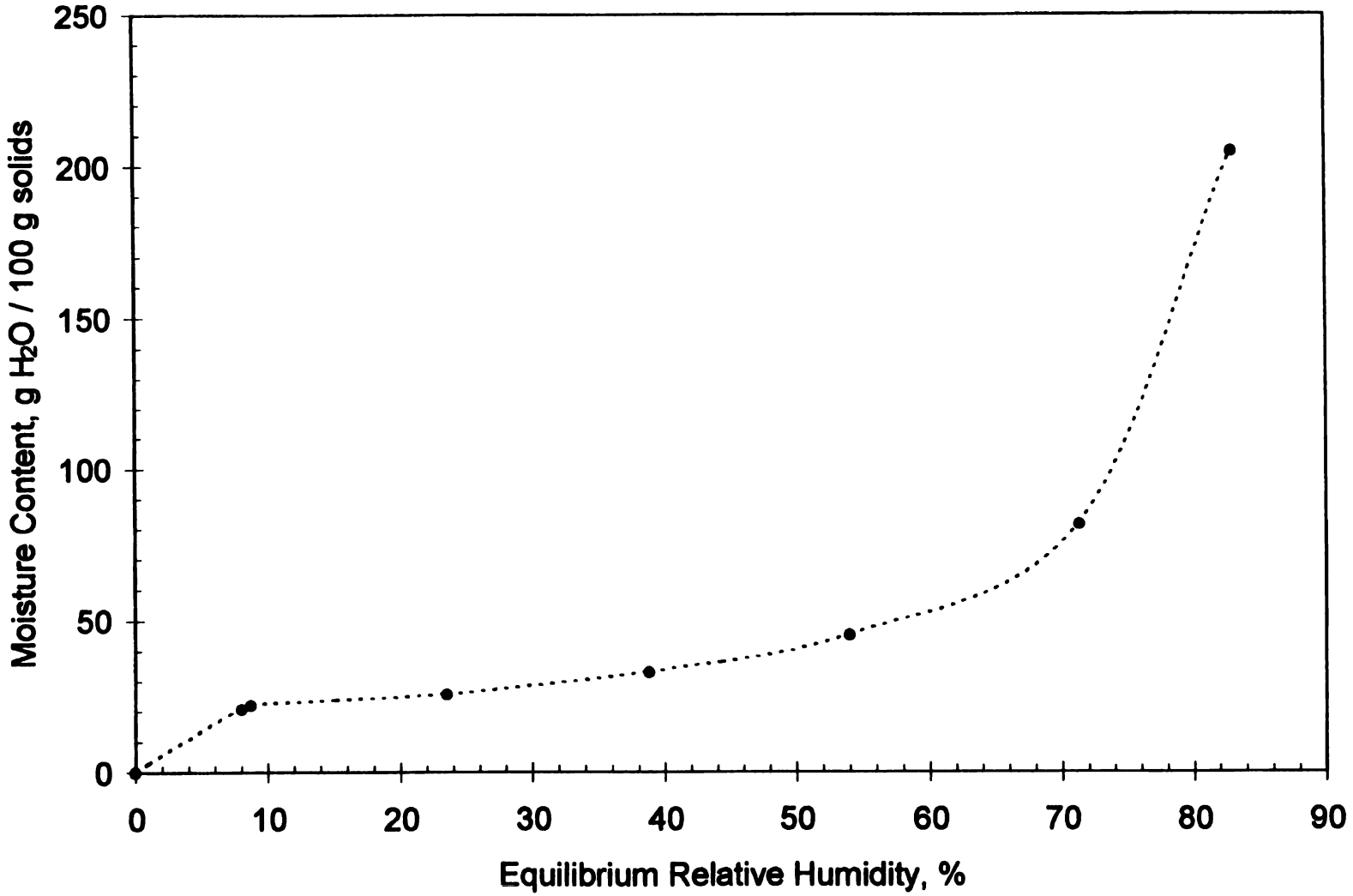

Figure 3-3. Sorption isotherm of protein film at 25°C

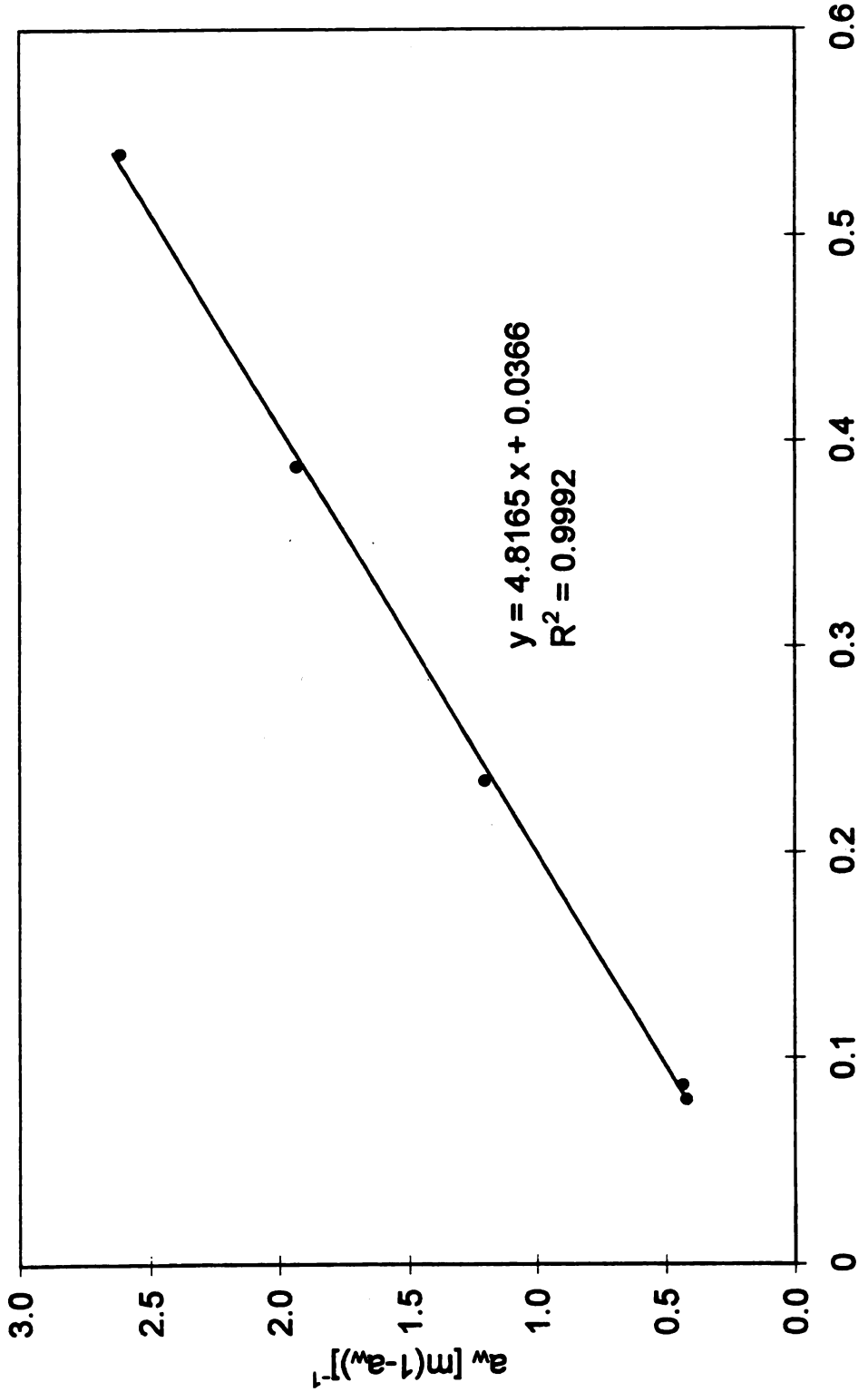

Figure 3-4. Brunauer-Emmet-Teller plot for determination of the "monolayer value" for water in the protein film

observed that the value for monolayer water is low (about 0.2 g H_2O / g solid), as compared to other food systems (Table 3-1), which ranged from 2.89 to 5.0 grams of water per gram of solid. This is an indication of strong interactions of the protein molecules among themselves, helped by the cross-linked structure (macromolecule), which limited the amount of water able to interact strongly. About 0.2 g of water per gram of solid in the film forms the monolayer value, being water interacting strongly at specific sites, such as the carbonyl and amino groups of the protein film (Karel, 1975).

From the sorption curves, the diffusion coefficient of water, D_w, was obtained at three conditions: 1) 22°C from dry (see Materials and Methods) to 62.0 ERH%; 2) 32°C from dry to 30.0 ERH%; and 42°C from dry to 20.4 ERH%. D_w was also determined from three more curves obtained at 25°C from dry to 21.8, 57.1, and 82.6 ERH%, respectively. Figures 3-5, 3-6, 3-7, 3-8, 3-9, and 3-10 show the water vapor uptake as a function of time for the conditions specified, respectively. A comparison of the shape of the curves obtained at 25°C and at different temperatures is presented in Figure 3-11. Table 3-2 summarizes these results. As ERH% increases, equilibrium moisture content (EMC) increases as for any hydrophilic polymer. Rayas (1995) showed that formaldehyde-cross-linked wheat protein films can hold more than 250% its weight when immersed in water. This capability of holding water may be due to the high ability of the molecules to open and sorb water within the hydrophilic structure. Therefore, at low relative humidity, an equilibrium was quickly reached and a steady state was thus rapidly obtained. On the other hand, by

Table 3-1. Water monolayer values (m_1) for experimental protein film and other foods or model systems

System	Temperature (°C)	m_1 (g H_2O / g solid)	RH (%)
Formaldehyde-cross-linked wheat protein film	25	0.21	6.5
Cellulose-lipid system*	37	2.89	19.5
High fat-high protein system*	55	3.89	31.0
Salmon*	37	5.00	20.0

* Adapted from Karel (1975)

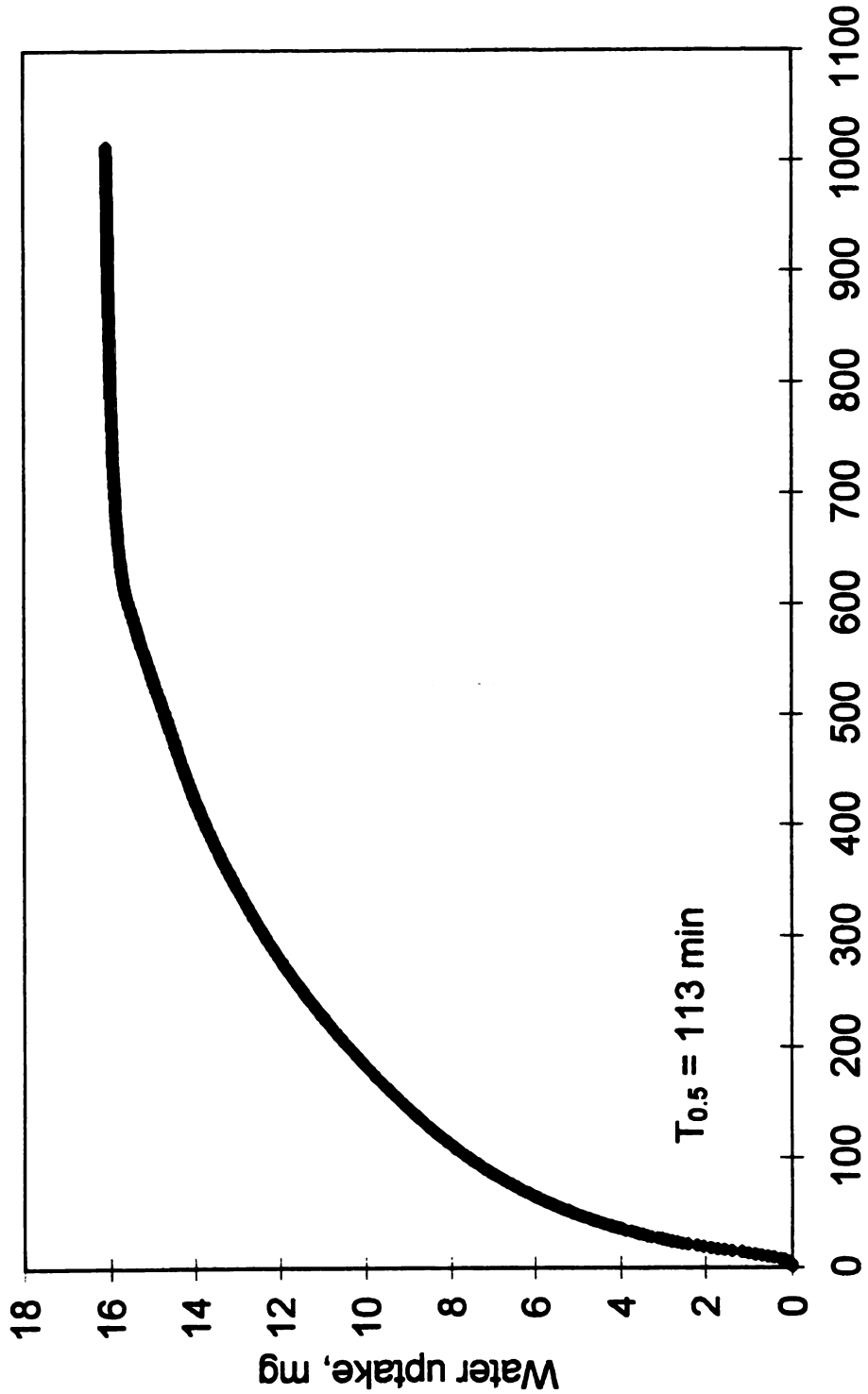

Figure 3-5. Sorption curve for protein film at 22°C from dry to 62.0% ERH

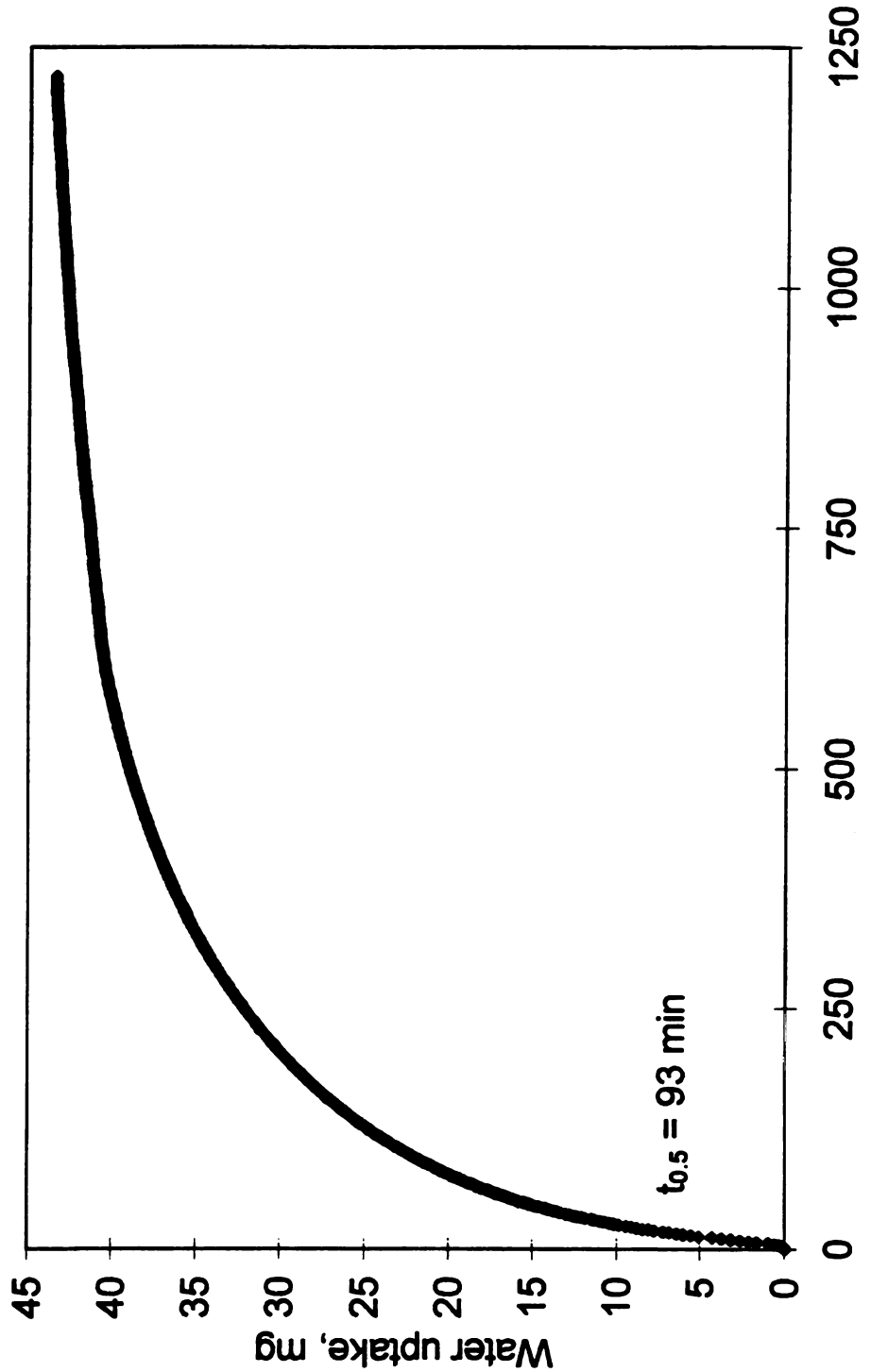

Figure 3-6. Sorption curve for protein film at 32°C from dry to 30.0% ERH

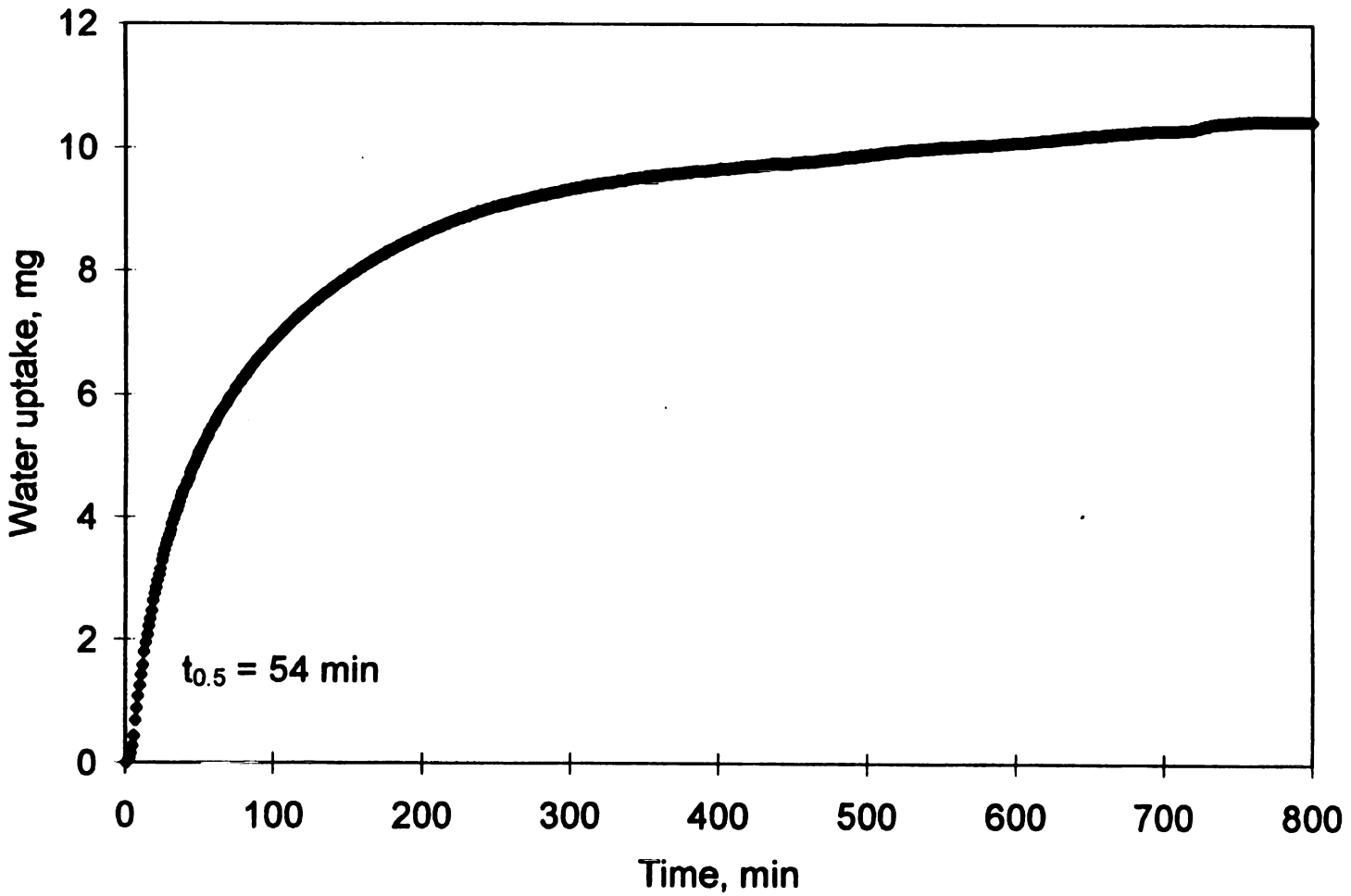

Figure 3-7. Sorption curve for protein film at 42°C from dry to 20.4% ERH

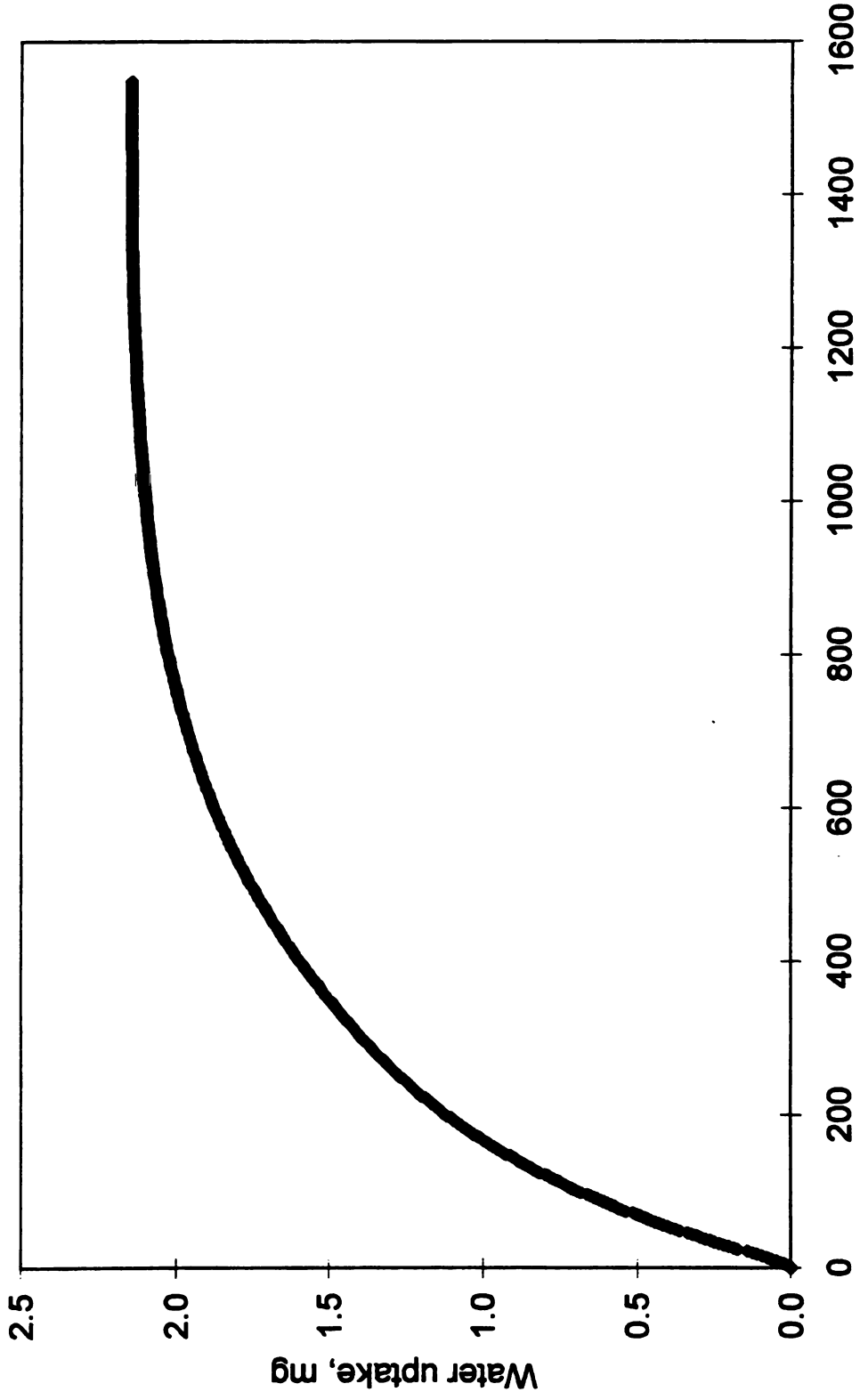

Figure 3-8. Sorption curve for protein film at 25°C from dry to 21.8% ERH

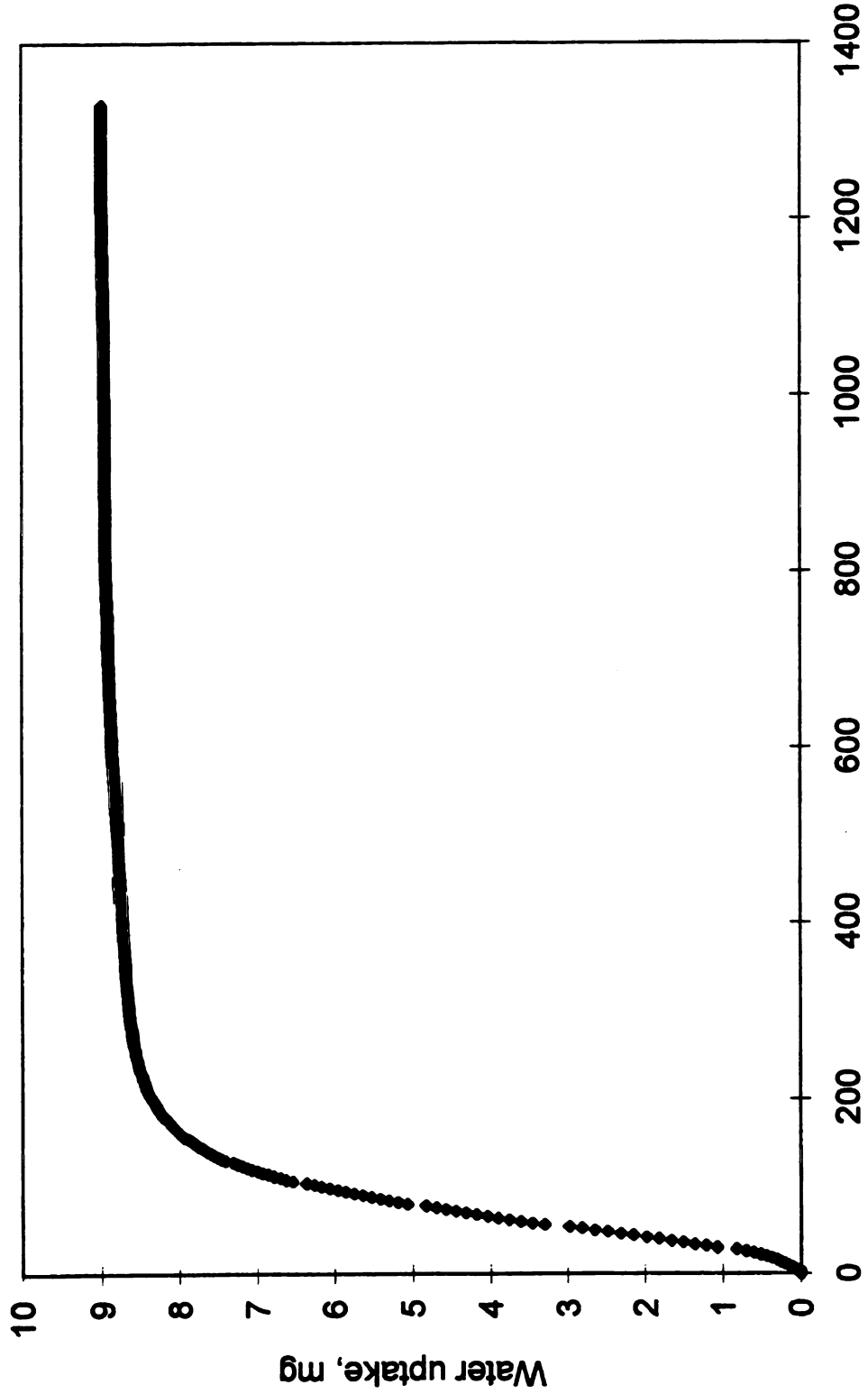

Figure 3-9. Sorption curve for protein film at 25°C from dry to 57.1% ERH

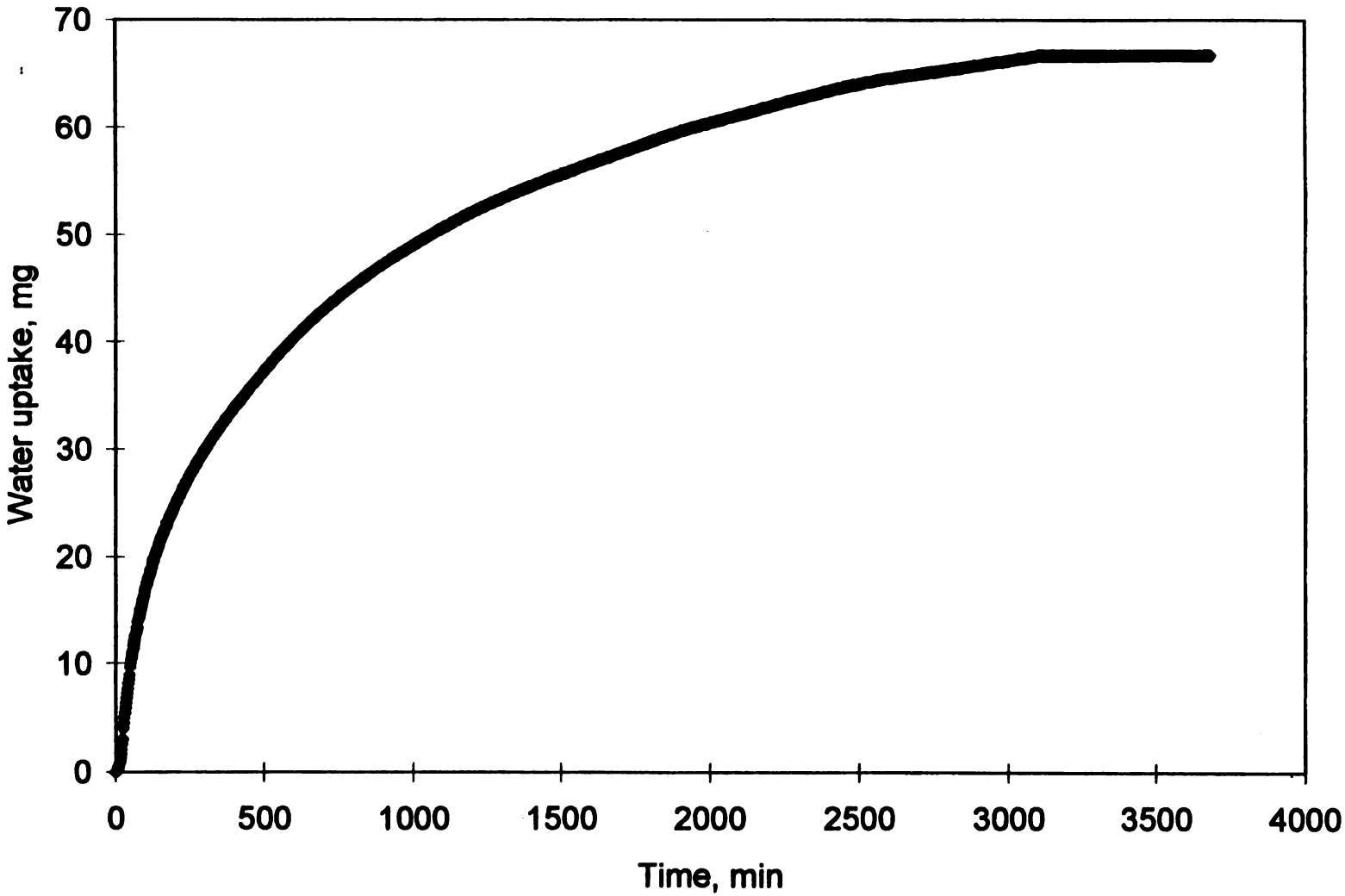

Figure 3-10. Sorption curve for protein film at 25°C from dry to 82.6% ERH

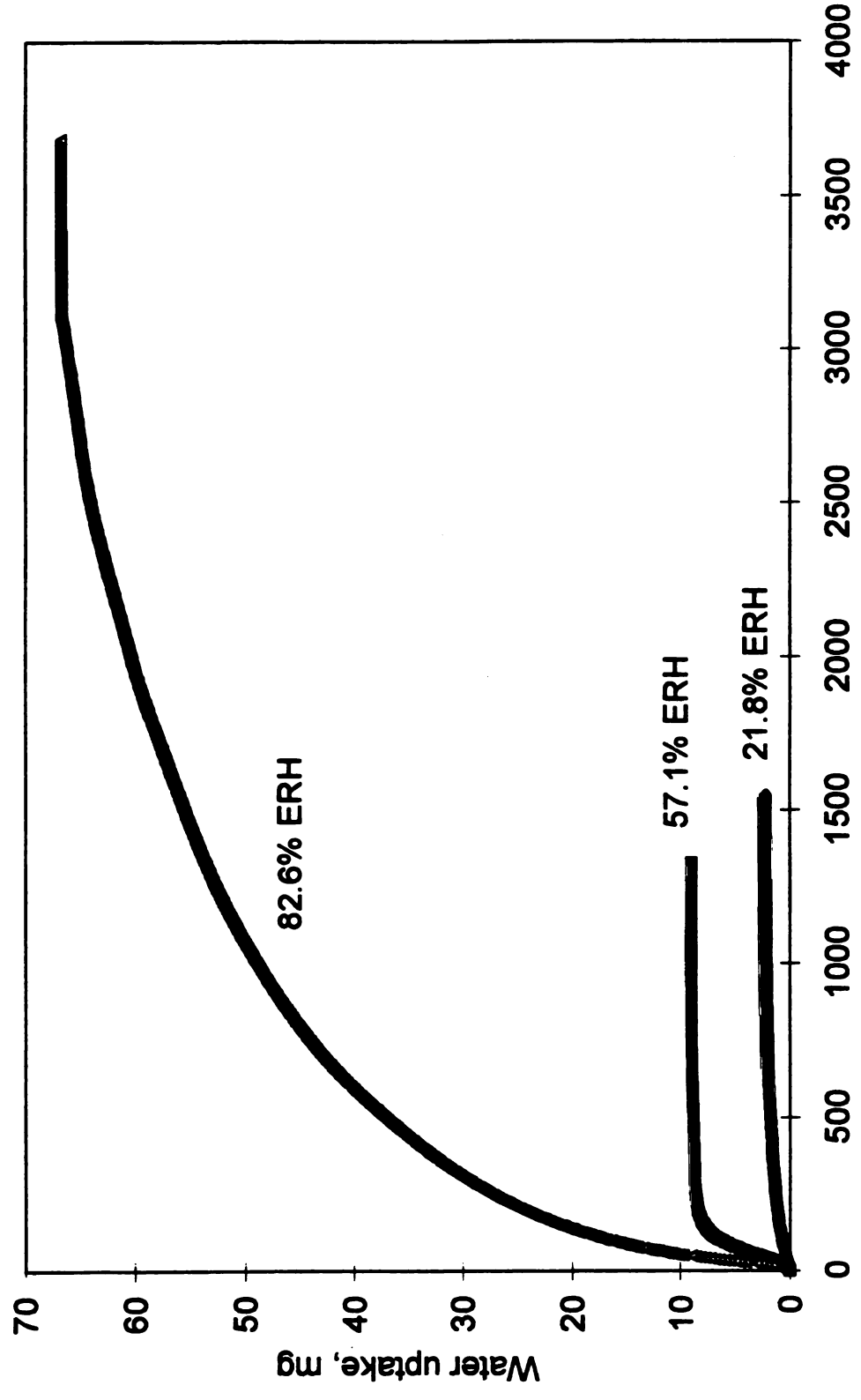

Figure 3-11. Comparison of sorption curves of protein films at 25°C from dry to corresponding ERH

Table 3-2. $T_{0.5}$ and diffusion coefficient, D, of degradable plastics

Temperature °C	*ERH* %	$t_{0.5}$ min	D $cm^2\ s^{-1}$
22	62.0	113	1.7×10^{-10}
25	21.8	186	1.0×10^{-10}
25	57.1	71	2.6×10^{-10}
25	82.6	389	4.8×10^{-11}
32	30.0	93	2.0×10^{-10}
42	20.4	54	3.5×10^{-10}

comparing the time to reach equilibrium at high relative humidities (Figures 3-5 and 3-10) and low relative humidities (Figures 3-7 and 3-8), the increased time of the first may be due to opening of the structure (i.e., break hydrogen bonds) by the water, as indicated by Hernandez and Gavara (1994) in a similar study based on nylon-6.

OXYGEN PERMEABILITY

Oxygen permeability was determined at 25°C as a function of the relative humidity. A plot of oxygen permeability vs. relative humidity is presented in Figure 3-12. The oxygen permeability values were significantly influenced by the relative humidity. This was congruent with observations made by Gavara and Hernandez (1994) in which they observed this type of behavior with another polyamide, nylon-6.

The effect of water is more intense above 30% relative humidity measurement. Below this value little change in oxygen permeability, if any, was observed. The water sorption isotherm also shows the steepest increase at a value between 30-40 %ERH (Figure 3-3). This suggests that with an increase in the amount of water molecules sorbed into the film, which act as a plasticizer in the film, the oxygen permeability is thus increased, as also reported by Hernandez (1994) and McHugh and Krochta (1994). These authors concluded that the type of effect in this type of experiment is exponential, which appeared evident in Figures 3-3 and 3-12.

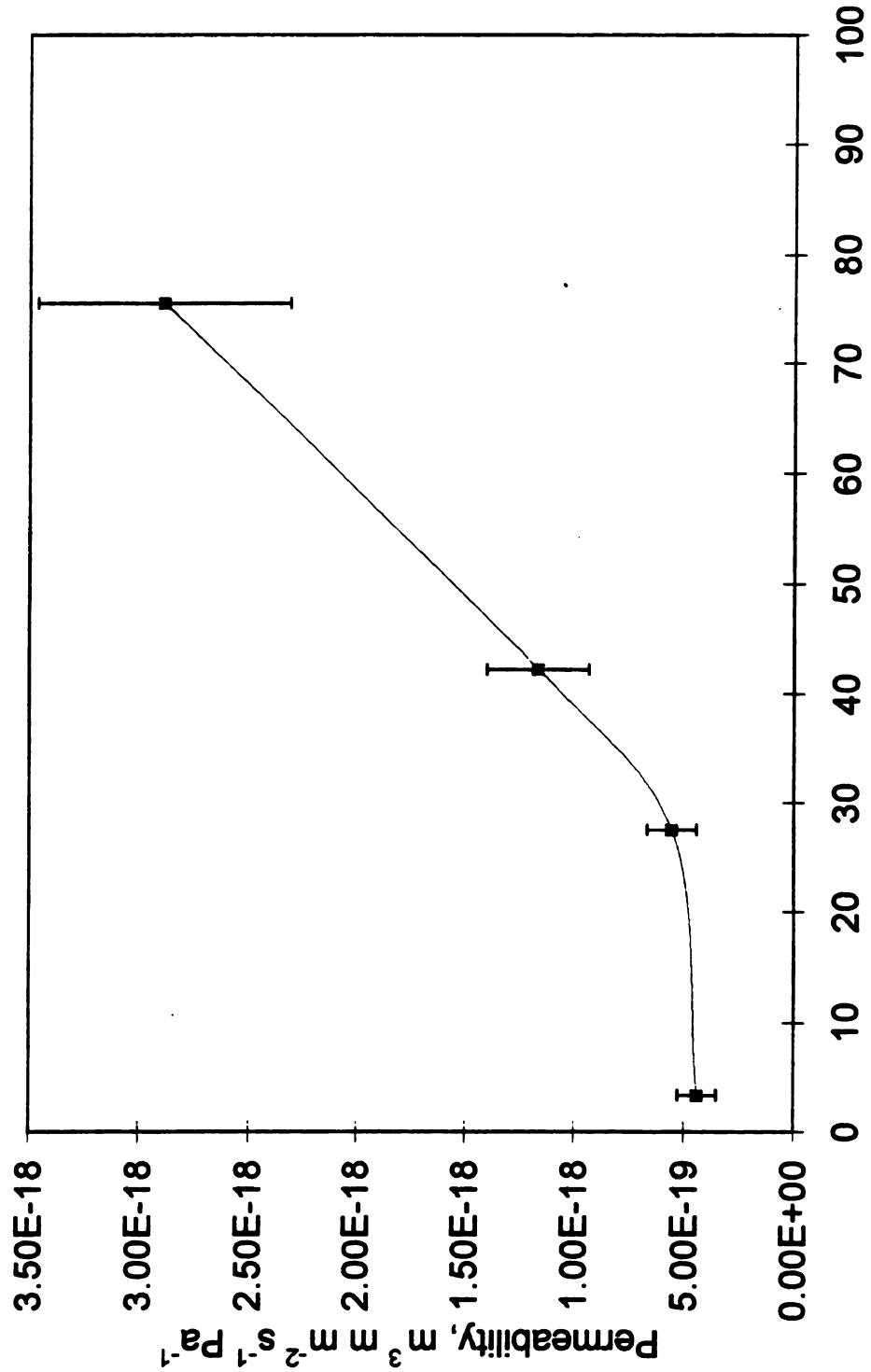

Figure 3-12. Oxygen permeability as function of relative humidity at 25°C

CONCLUSIONS

The values of the sorption isotherm indicated that a significant uptake of water occurred at relative humidities of 35-40 % and above. This was correlated with the oxygen permeability results, since the last were significantly higher at relative humidity values greater than 40%. It was also concluded that water had a plasticizing effect on the film, opening the structure by means of hydrogen-bonding interactions. A greater effect on the water effects was observed at relative humidity greater than 40%.

The food and medical industries would benefit from the use of this material. Products coated or wrapped by these biopolymers may have an increase in their shelf-life or their quality improved due to the polymer's low oxygen permeability. In addition, it would impart a clean shiny luster to some products (e.g. candies, snacks, etc.) while also retard oxidative reactions (pharmaceutical pills, peanuts, nuts, seeds, etc.) and would supplement nutritional value (from the biopoymer's protein). Paper and paperboard coated with this biopolymer will show increased strength, gloss, and better barrier while maintaining their recyclability characteristics.

CHAPTER 4. PHOTODEGRADABILITY STUDIES OF WHEAT PROTEIN FILMS

CHAPTER 4. PHOTODEGRADABILITY STUDIES OF WHEAT PROTEIN FILMS

INTRODUCTION

Degradable plastics, according to Narayan (1989), are "plastic materials that undergo bond scission in the backbone of a polymer through chemical, biological and/or physical forces in the environment at a rate which is reasonably accelerated, as compared to a control, and which leads to fragmentation or disintegration of the plastic". Selke (1996) indicated that bio-degradable plastics are those whose mechanism of degradation is by biological means, in which the polymer chain backbone is broken down so that different compounds are the end product. Selke (1996) suggested that the end compounds are carbon dioxide, methane, water, and humus-type materials, and that "biodegradation involves a total loss of the chemical identity of the starting material." Coma et al. (1994) studied the bio-degradability of natural polymeric materials, based on cellulosic compounds.

Photo-degradable plastics are "those degradable plastics where the primary mechanism of degradation is through the action of sunlight" (Narayan, 1989). Selke (1996) defined photo-degradation as "a significant loss in mechanical properties within a reasonable period of time after exposure to sunlight." The main difference with bio-degradation is that in photo-degradation the chemical identity of the polymer is not destroyed, but rather changes in molecular dimensions are observed, mostly reduction in size.

There are two main reasons for which photodegradation of plastics should be considered. The first, which is of most interest for the manufacturers of goods made of polymeric materials, is that of undesirable changes in properties of these commodities that lead to failure of the product. The second, is the disposal problems relevant to our society, that is, garbage and litter disposal.

Weathering testers are apparatus used to measure degradation of materials in an accelerated way. Laboratory weathering testers are characterized by the reproducibility of results. To achieve this reproducibility, testers must have control of the critical parameters of light, moisture, and temperature. According to The Q-Panel Company (1992), control of irradiance in a laboratory tester is particularly important because:

1. Changes in light intensity may affect the rate of a material's deterioration, and
2. Changes in light wavelength, or Spectral Power Distribution (SPD), may affect both the speed and the type of material degradation.

The objective of the present study was to study the effect of ultraviolet (UV) radiation, referred to as photodegradation, as related to color changes and tensile properties of the films. This was with the purpose of investigating potential uses of these films in applications which require products to be exposed to sunlight or artificial light, e.g. during loading or unloading or displaying in the stores. This in turn provided information that can be used as

valuable data to calculate the shelf-life of food or pharmaceutical products or the film itself.

MATERIALS AND METHODS

FILM

All films were produced by following the methodology of Chapter 2.

PHOTODEGRADATION MEASUREMENTS ON THE FILM

Photodegradability studies were performed by using the ASTM D-4329 method (ASTM 1992). UV radiation was used and the surface of the film samples were irradiated by placing them on a Q-U-V Accelerated Weathering Tester with Solar Eye Irradiance Model QUV/SE (The Q-Panel Co., Cleveland, OH., USA). 40-watt UV-A lamps were used. The peak emission of these lamps was 340 nm with a cutoff at 295 nm. This is comparable to natural outdoor weathering equivalent to Florida's noon-time, summer sunlight. The irradiance of the lamps was set to 0.72 $W/m^2/nm$ and the temperature to 70 ± 1 °C during all the tests. The relative humidity was monitored during the test and was found to be 12 ± 2%.

Samples of films were taken out every 24 hours for 10 days. Control films were prepared by wrapping them in aluminum foil and introduced in the weathering machine with the others. The control films were removed at the 10th day along with the last samples. Two types of test were performed to account for the photodegradability of the films: a) measurement of color changes and b) measurement of tensile properties.

COLOR CHANGES

The color changes observed during the 10 days of the test were evaluated by HunterLab ColorQUEST equipment. The film samples were equilibrated at 50 % RH prior to testing. The analysis consisted of obtaining the Hunter "L", "a", and "b" values. The "L" goes from zero (total blackness) to 100 (total whiteness) and thus corresponds to the lightness of the sample. The letters "a" and "b" correspond to color variations as represented in the diagram of Figure 4-1.

TENSILE PROPERTIES

The tensile properties measured were tensile strength, elongation (%) and toughness as related to the films taken out from the Q-U-V Tester at 24-hour intervals. The films were analyzed using an Instron Model 4201 equipped with a 1 kN static load cell (Canton, USA.). This test was done in conformance to test method A (static weighing, constant-rate-of-grip separation test) of the standard ASTM method D882-91 (ASTM, 1992). This method employs a constant rate of separation of the grips holding the ends of the test specimen.

The film samples were cut to 1 inch widths using a JDC Precision Sample Cutter model 25 (Thwing Albert Instrument Co., Philadelphia, PA, USA) and the edges cut with scissors to about 4 in of length. The separation between the grips of the Instron was set to 1 in. The cross head speed for the test was set to 20 in/min. The method indicates that a width-thickness ratio of at least eight shall be used. All films were conditioned at 22±1°C with 50±2% relative humidity for 48 hours. Testing was performed at these conditions. Tensile strength

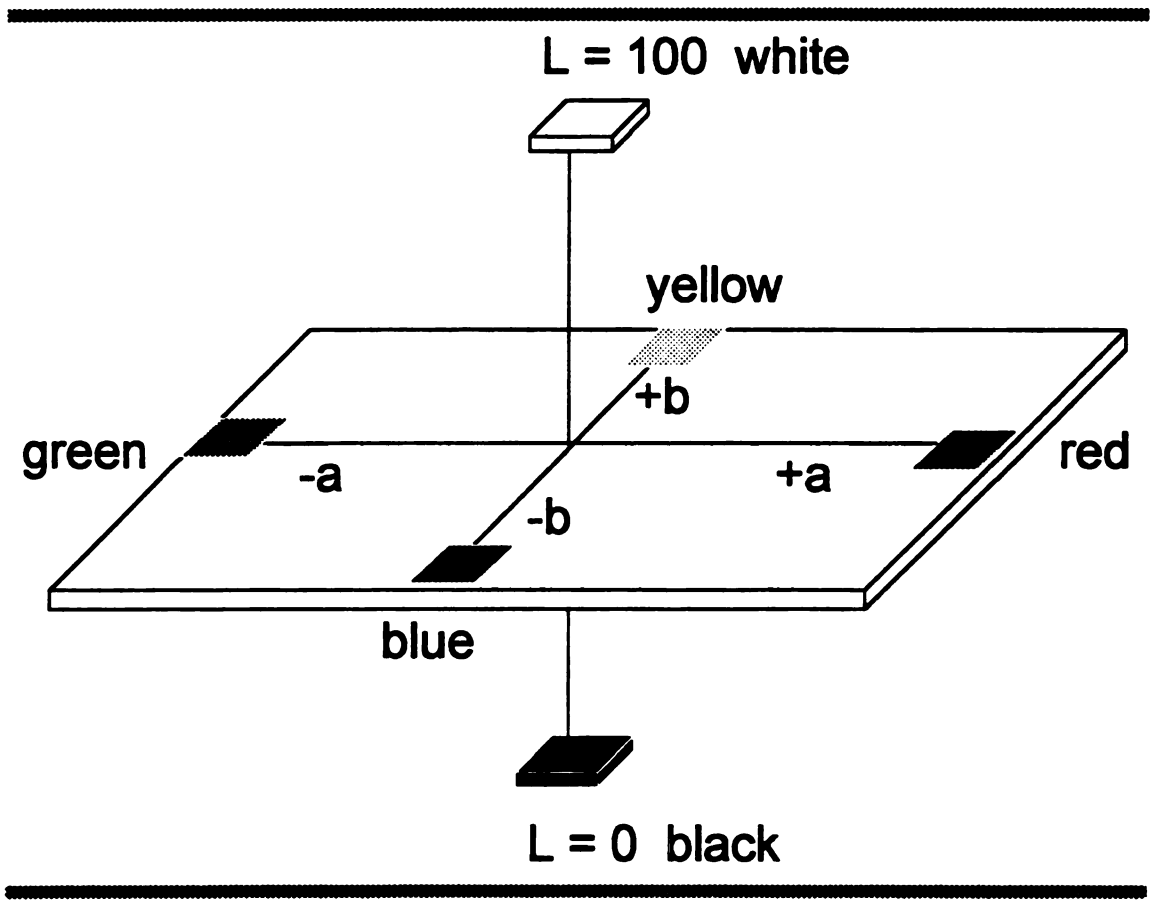

Figure 4-1. Three-dimensional analysis of color (L-a-b System)

(maximum stress in the stress-strain graph) and % strain (or % elongation) are reported.

Toughness is a measure of the energy required to break the material (Grulke, 1994). Energy is the product of force times distance and can be estimated as the area under the stress-strain curve for unnotched tensile bar samples, in accordance to equation 4.20:

$$Energy\ /\ Volume = \int_0^{\varepsilon_{max}} \sigma d\varepsilon \qquad (4.20)$$

where ε is strain or elongation and σ is the stress in the stress-strain plot.

STATISTICAL ANALYSIS

Data obtained were treated according to the Student-Neuman-Keul comparison test and the two-way analysis of variance using MSTAT-C statistical program (Michigan State University, 1990).

RESULTS AND DISCUSSION

PHOTODEGRADABILITY OF THE FILM

The changes in color, as noted by the changes in the values for L, a, and b, are presented in figures 4-2 to 4-4. These results showed that UV radiation significantly reduced transparency of the films as measured by the Hunter Lab System for the time and conditions used during the test. There is a statistical correlation between changes in transparency and UV exposure.

This was evidenced by the control film (wrapped in aluminum foil) introduced in the Q-U-V machine that did not show a significant change in the "L" value as compared to the film that was not introduced in the machine (labeled as "zero" hours).

The change in the "a" value also showed significant increase towards the green color of the films. This was due to both UV radiation and temperature effects. The change in the "b" value showed significant increase in the yellowness of the films. Statistical analysis inferred that both UV radiation and also temperature effects were responsible for this change in color.

The color changes can be summarized as follows: Although the color and transparency changes were found to be significantly different for all cases, there are marked differences in the order of magnitude observed for each of them. For instance, photodegradation studies showed that at the conditions of testing (exposure to UV 340/295 nm, 70 °C, 12% RH, 0.72 $W/m^2/nm$), the protein films increased yellowness more than 9 times that of greenness and over 4 times that of the reduction of transparency. That is, "L" changed from about 86 to 82.5 (3.5

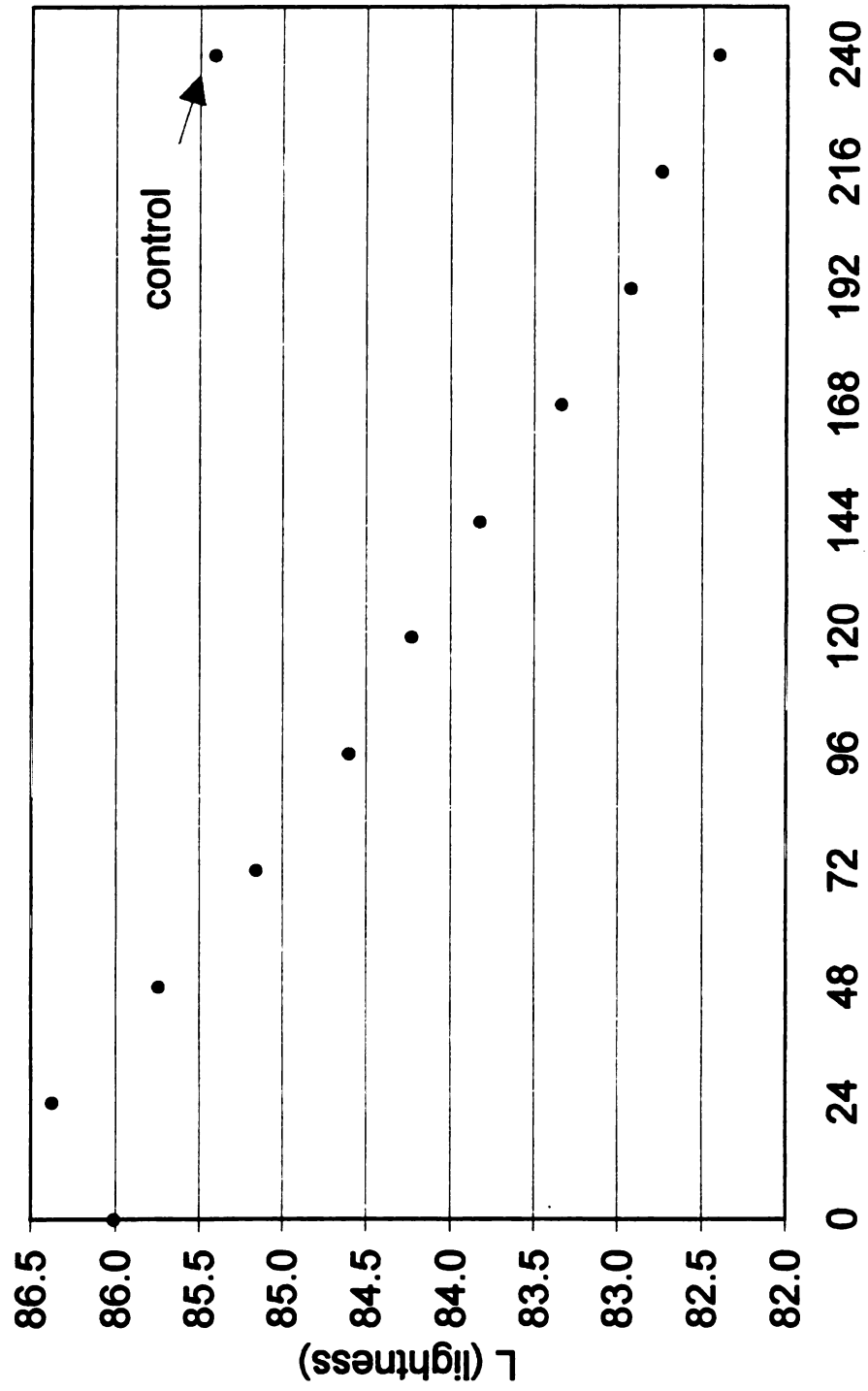

Figure 4-2. Lightness change of protein films during the photodegradation process

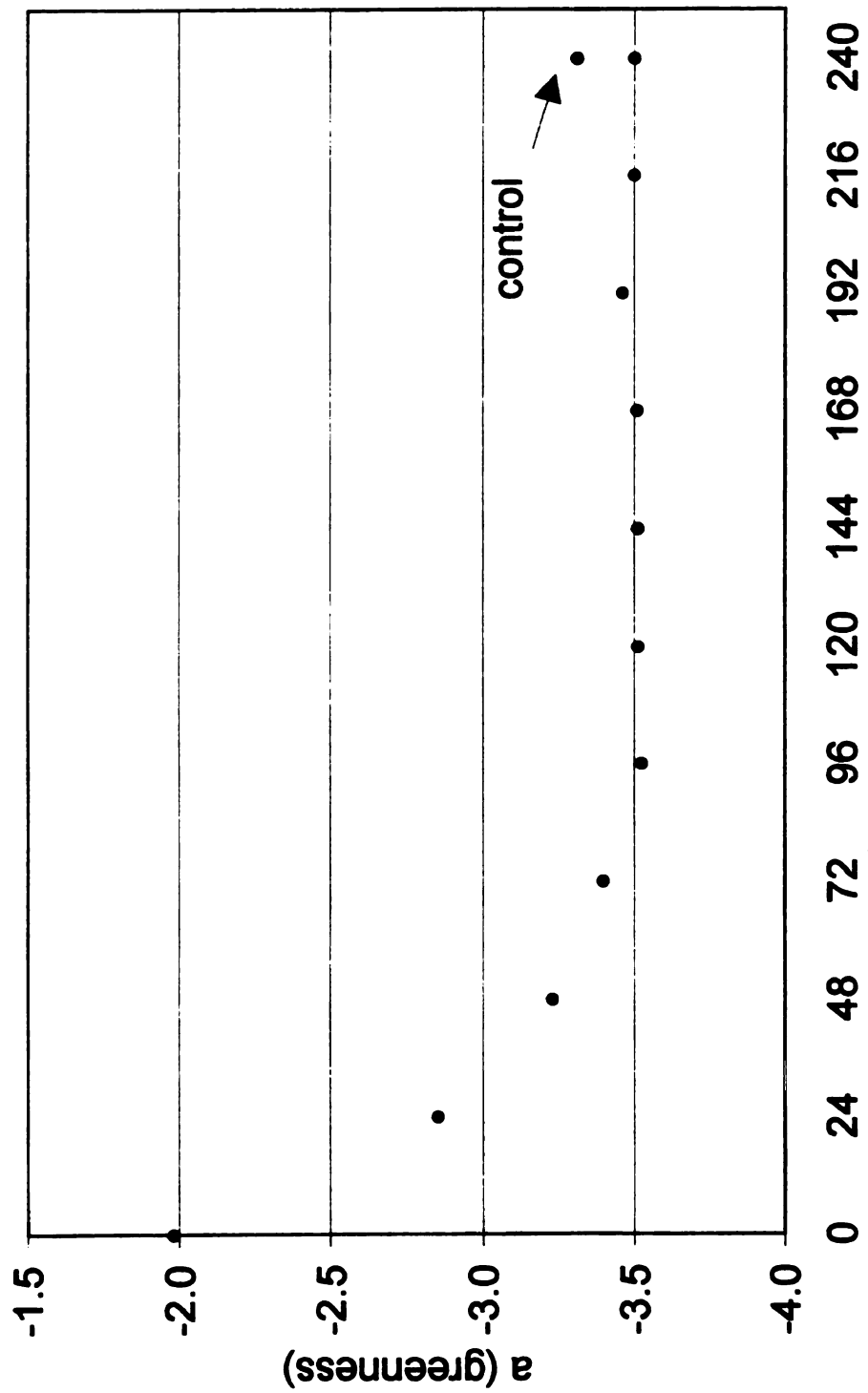

Figure 4-3. Greenness color change of protein films during the photodegradation process

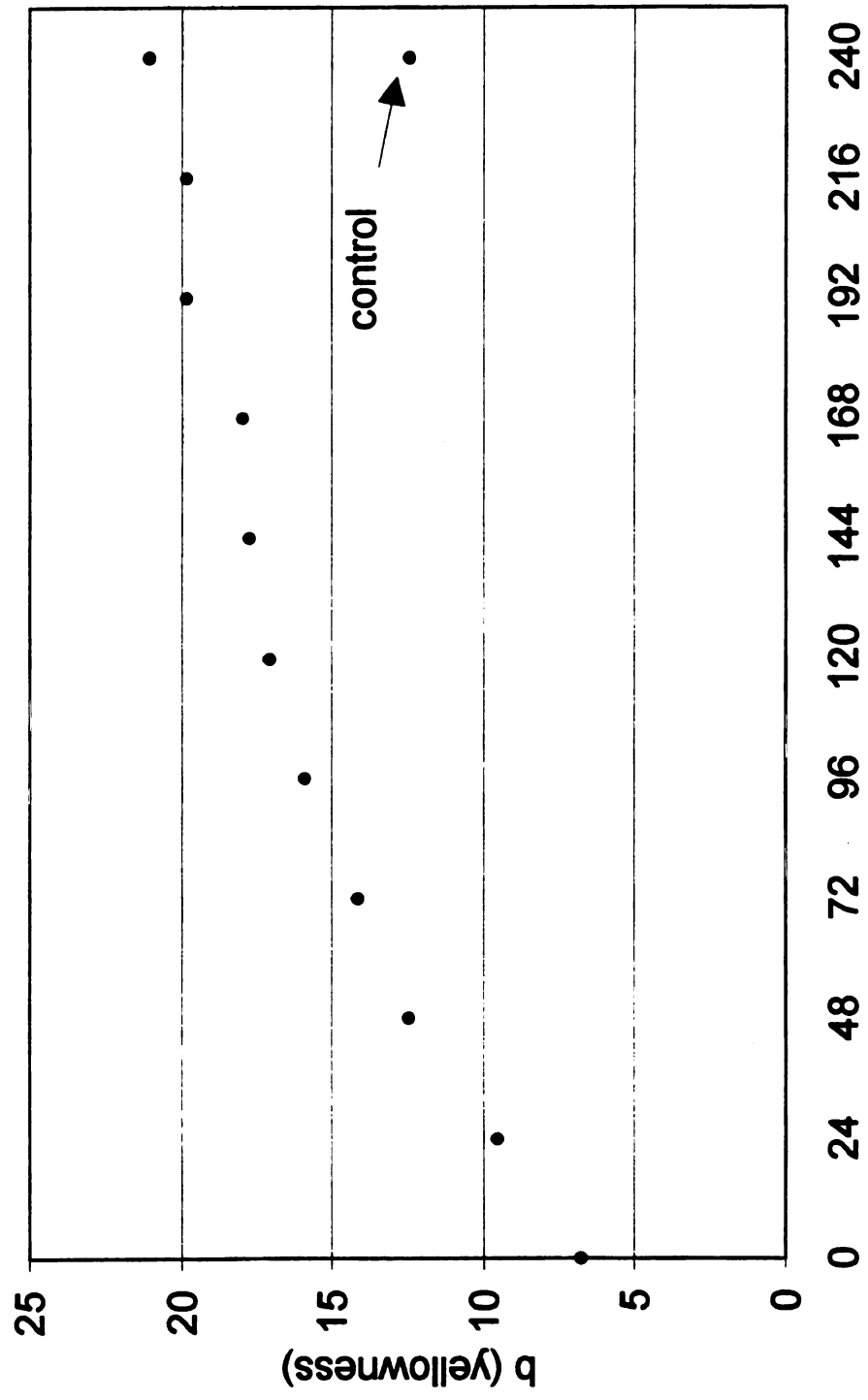

Figure 4-4. Yellowness color change of protein films during the photodegradation process

units), "a" changed from about -2 to -3.5 (1.5 units), and "b" changed from about 7 to 22 (15 units) . This indicated that photodegradation can be considered to have an effect, in terms of color, on the increase in yellowness, while having a minor effect on the reduction of transparency and greenness of the wheat protein-based films. A comparison showing values of "L", "a" and "b" for what protein-based and other synthetic films tested using the Hunter Lab system was already presented in Table 2-4.

TENSILE STRESS MEASUREMENTS

The change in tensile strength (TS), is represented in Figure 4-5. Tensile strength was significantly increased during the photodegradation study over the time and conditions of testing. This increase in tensile strength was attributed by statistical analysis to be caused by the UV exposure effect, rather than that of the temperature effect. Figure 4-5 shows this observation in that the control film indicated by 10* did not change significantly its tensile strength values.

Elongation at break values were observed to be reduced by exposure to UV radiation. This reduction in elongation was not conclusive as to whether the change was induced by the UV radiation or temperature effect. It was observed that the control film (unexposed to the UV radiation, but subjected to the Q-U-V machine's condition) had a significant reduction in the elongation values, similar to that of the exposed films. This effect can be observed in Figure 4-6 that the control film (noted as 10*) did have a significant change in strain (elongation) values similar to those films kept for longer times. It was concluded that a

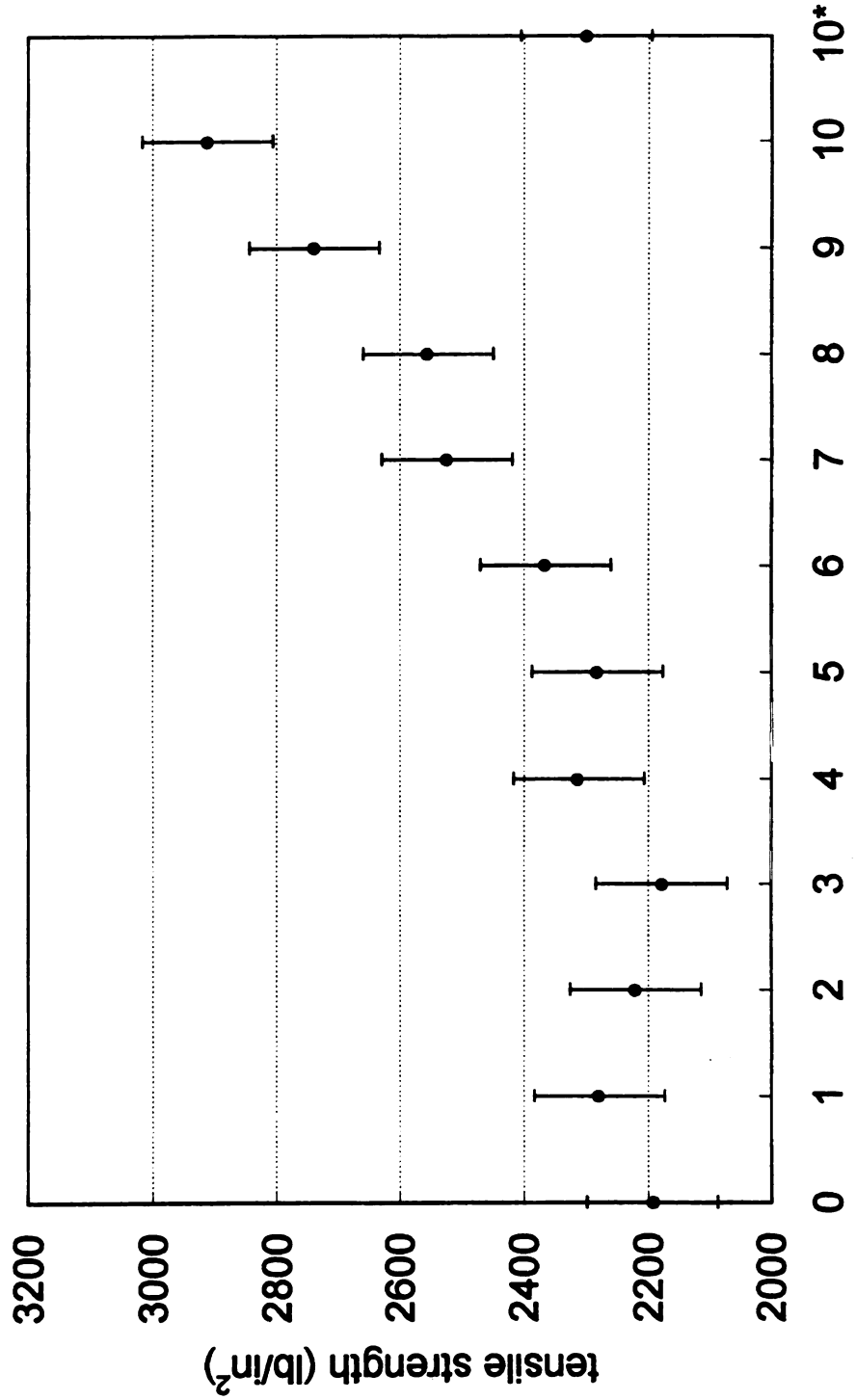

Figure 4-5. Tensile strength change of protein films during the photodegradation process - 10* is the control film (tensile strength was measured the 10th day)

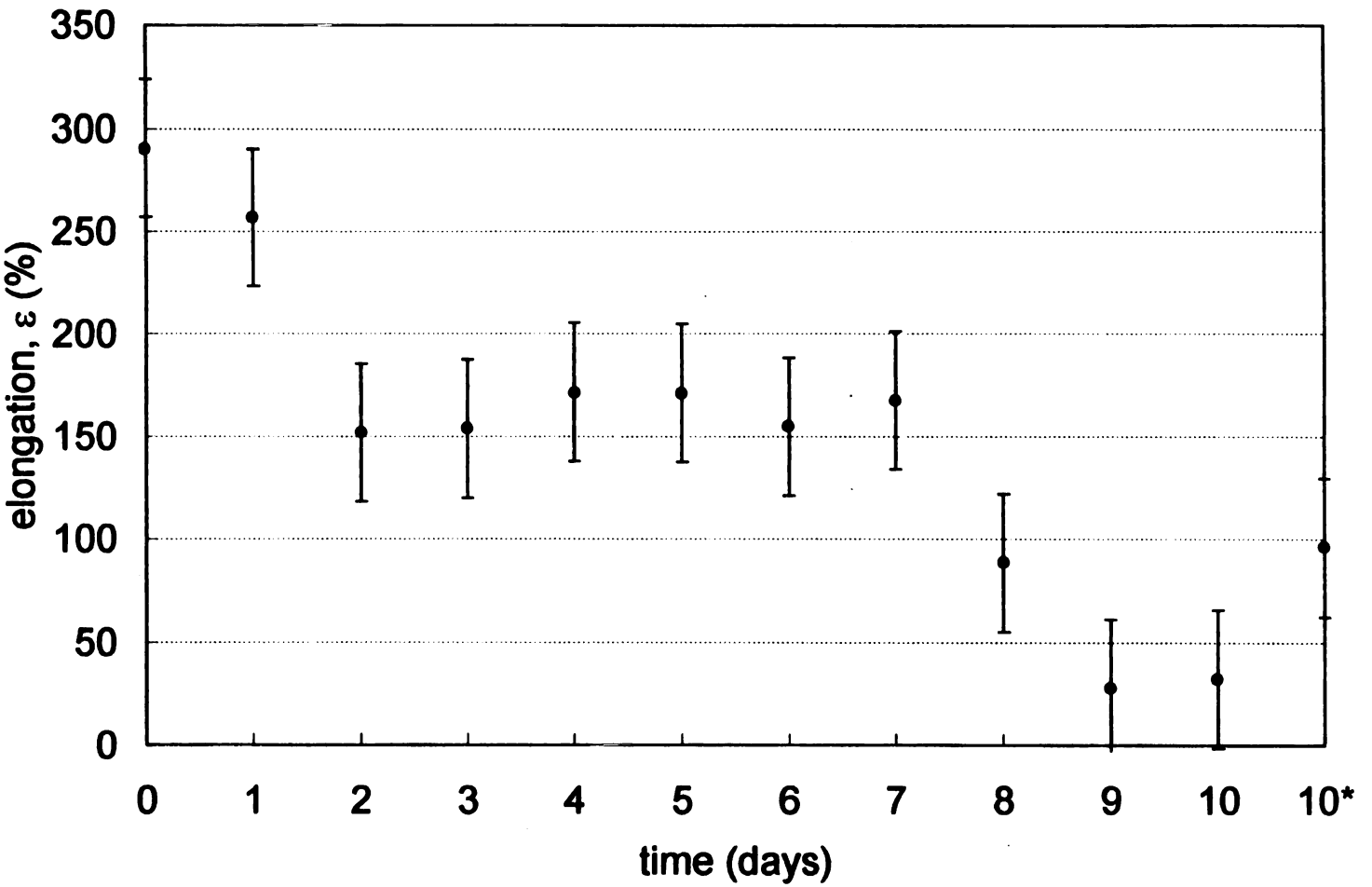

Figure 4-6. Elongation at break change of protein films during the photodegradation process - 10* is the control film (elongation at break was measured the 10th day)

significant reduction in the elongation values was attained within the first two days with no significant change in those values after that day.

Finally, toughness, a measurement of the area under the stress-strain curve showed a significant reduction during the time of exposure to UV radiation during the time and conditions of the study. Statistical analysis showed that this reduction in toughness was attributed primarily to the temperature effect rather than that of the UV-radiation effect. Figure 4-7 confirms this observation by showing that the values of the 10* film (control) was similar to that of the exposed films. It is clear that if the temperature did not have a significant effect on this property, the values for the control film would have remained similar to that of the unexposed films.

In general, for proteins, it has been reported by several authors (Pomeranz, 1987 and Levine and Slade, 1990) that heating of wheat protein films even just above 55 °C may have significant effect on the conformation of the proteins. This may even result in dynamic catalysis of disulfide exchange, which, in turn, results in

1. progressive depletion of the least stable cross-links and catalytically effective thios and
2. eventual establishment of permanent local cross-links in the gliadin fraction of the gluten, a permanent long-range (cooperative) network by HMW glutenins, and residual catalytically ineffective thiols.

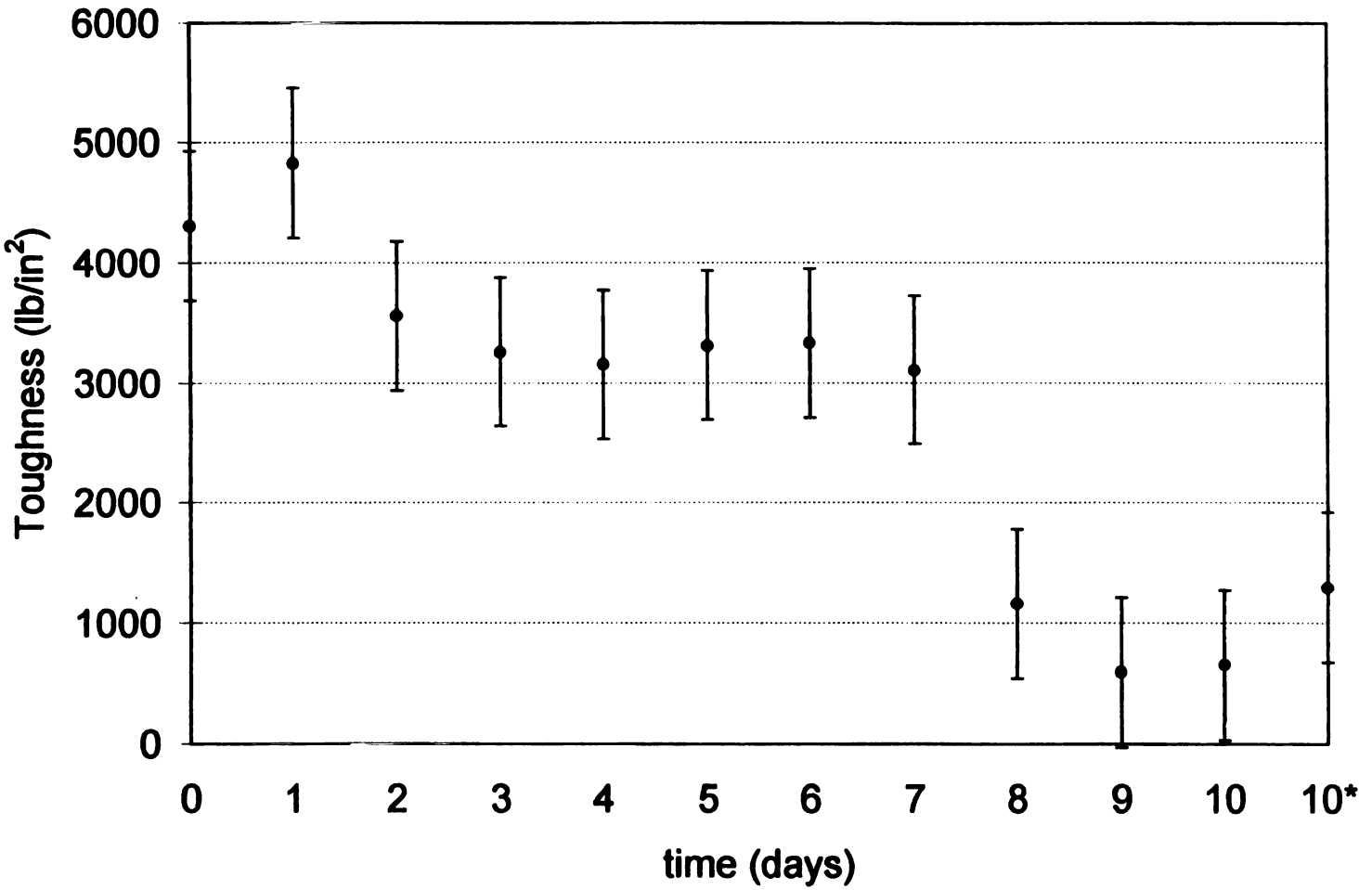

Figure 4-7. Toughness change of protein films during the photodegradation process - 10* is the control film (toughness was measured the 10th day)

This may explain in part the observations of an increase in the tensile strength while a reduction in the strain observed during the photodegradation study.

CONCLUSIONS

Films produced in this study were transparent and smooth in texture. Photodegradability studies showed that the lightness (Hunter L-value) and the yellowness were the most affected parameters by the effect of UV radiation. This indicated that loss of clarity was due to the increase in yellowness of the film, which was evident even by comparing the non-radiated and radiated samples using the naked eye. On the other hand, loss of transparency was also observed, and related to the lightness value measured.

Values of tensile strength were also affected by the UV radiation. The main change observed was the decrease in values for elongation and toughness and an increase in the tensile strength values. This may suggest that chemical changes occurred in the protein structure that promoted strong interactions (i.e. cross-linking) due to rearrangement and reaction of the structure due to the UV radiation. Further, the accelerated weathering test performed on these films may have result in the usage of temperatures at which important changes in protein structure or chemical reactions induced by heat had happened. This is of concern, particularly because these changes may in some instances be not totally comparable to those observed during normal weathering of the biopolymeric films.

SUGGESTIONS FOR FUTURE WORK

There is great potential to direct studies toward practical uses of these edible and degradable films. Applications which are suggested include easy to oxidize dry fatty products (e.g., peanuts, nuts, potato chips, etc.) or oxygen sensitive pharmaceutical products (e.g. capsules, pills, etc.). There is also potential for testing the ability of the films to be used as instruments for carrying specific additives, such as colorants, antioxidants, flavorings, etc.

In terms of testing, a more in depth study of the dimensional change (e.g., thickness) as affected by the relative humidity is needed. This is in order to further explain possible relationships of the sorption characteristics as affected by this variable.

In addition, a thermal degradation study of the film is suggested in order to explain how other variables may be affected, and to what extent, by this factor.

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