Asian Journal of Chemistry Vol. 22, No. 3 (2010), 1982-1992

Solubility of Edible Films from Amaranth Flour Enzymatic Hydrolyzate Modified with Dialdehyde Starch

P. MOKREJS*, J. HRNCIRIK†, D. JANACOVA‡, V. VASEK‡ and R. CERMAK
Department of Polymeric Engineering, Faculty of Technology, Tomas Bata University, nam. TGM 275, 762 72 Zlin, The Czech Republic
Fax: (42)(057)6031563; Tel: (42)(057)6031230
E-mail: mokrejs@ft.utb.cz

The objective of the work is to prepare biodegradable and edible films based on Amaranth flour starch-protein hydrolyzate and evaluate the effect of chemical and physical modifications on solubility properties of films. Films were prepared by casting a 14 % (w/w) water solution of hydrolyzate containing 20 % (per hydrolyzate dry matter) glycerol and 0, 1 or 5 % (per hydrolyzate dry matter) additions of dialdehyde starch. After thermal exposure of films at 65 or 95 ºC (for 6 or 48 h) film solubility tests were performed in water at 6 or 37 ºC. It was found that chemical modification (added dialdehyde starch) and physical modification (thermal exposure) of films allows to markedly altering their water solubility. Results of water solubility tests can serve to practically utilizing starch-protein hydrolyzate of Amaranth flour for producing biodegradable packaging materials (foils, coatings) to be employed in the food industry, cosmetics or agriculture.

Key Words: Amaranth flour hydrolyzate, Dialdehyde starch, Edible films, Food packaging, Test of solubility.

INTRODUCTION

In latest years, new biodegradable (even edible) materials have been applied for packaging and protecting foodstuffs, pharmaceutics and other products. Biopolymers from renewable sources (proteins, polysaccharides, lipids) are successfully applied to produce biodegradable and edible films, foils, foodstuff packaging, separation layers or casings. Proteins employed are animal proteins, such as collagen, gelatine, casein, whey protein and keratin and vegetable proteins, such as maize zein, wheat gluten, soya protein and others[1]. Most used of the polysaccharides are starch, cellulose and their derivatives[2]. For the production of biodegradable materials solvents and

†Tomas Bata University, Faculty of Technology, Department of Food Engineering, nam. TGM 275, 762 72 Zlin, Czech Republic.
‡Tomas Bata University, Faculty of Applied Informatics, Department of Automation and Control Engineering, Nad Stranemi 4511, 760 05 Zlin, Czech Republic.

additives (plasticisers, cross-linking agents, antioxidants, antimicrobial preparations, vitamins and others) are added[3-5]. Supplied additives modify processing characteristics (for example, rheological properties or thermal stability) and utile properties of biopolymers *i.e.*, solubility, water sensitivity, adhesion to various substrates, mechanical properties, barrier properties (for example, for water vapour, CO_2 or O_2). In general, biopolymers may be processed by two different technologies depending on solvent content: (1) Casting–when large quantity of solvent (usually water) is present. In case a fine uniform coating is required, the technique utilises dipping (immersing the product in solution of film-making material) or spraying that solution onto product. (2) Extrusion (thermoplastification)–when only small quantity of solvent or plasticiser is present and the viscosity of the system is too high.

The most significant methods for modifying processing and utile properties of films are additions of plasticisers and cross-linking agents. A convenient cross-linking degree allows controlling film dissolution rate or degree of swelling and thus also rating of releasing active substance from such packaging[6,7]. This is utilized for controlled release of active substance, *e.g*. in the foodstuffs industry when (micro) encapsulating vitamins, spices, man-made sweetening, fragrant substances, *etc.*, in the pharmaceutics industry (medicines, vitamin supplements), cosmetics (*e.g*. anti-perspirants, bath salts and oils) or in agriculture (plant protection preparations). Cross-linking increases film tensile strength, decreases elongation at break; cross-linking agents improve barrier properties of films. Agents employed in cross-linking of proteins are *e.g*. formaldehyde, glyoxal, glutaraldehyde, hexamethylene diisocyanate, 1,3-butadiene diepoxide, 1,2,7,8-diepoxyoctane[8]. Formaldehyde is one of the most frequently used cross-linking agents but its toxicity limits its employment to non-food applications. Modifying films with formaldehyde increases film thermal stability (higher melting point), film solubility decreases and barrier properties for gases and liquids improve[9]. An accepted cross-linking agent for food and pharmaceutical applications is dialdehyde starch (DAS)[10] marked by a good cross-linking effect, which results in a strong decrease in film solubility and increase in tensile strength[11]. On the contrary, water vapour permeability slightly increases (due to high content of hydrophilic groups) and film colour turns yellow[12,13]. Cross-linking biodegradable film may also utilize physical cross-linking methods (thermal processing or UV irradiation). Even though such a strong cross-linking effect with proper chemical cross-linking agent is mostly not attained, these methods can regulate (reduce) film solubility and improve film barrier properties[14]. With films produced from wheat gluten it was proved that a marked drop in their solubility occurred and barrier properties improved after thermal treatment of films already at 55-75 ºC for 24 h. This cross-linking mode has the disadvantage of film colour changing to darker shades with increasing temperature[15]. In some cases it is possible to combine chemical and physical cross-linking methods to obtain desired properties of biodegradable films.

Added plasticisers reduce glass transition temperature (T_g), reduce breaking strength of films and improve their flexibility and degree of elongation at breaking. In thermoplastification processing of biopolymers, addition of plasticiser is essential to enable preparation of a 'dough-like' mass, which is processed at temperatures above T_g by usual plastic processing technologies combining action of temperature and pressure (*e.g.* extrusion) into a product whose shape is further stabilized by cooling[16]. The plasticising effect of hydrophilic plasticisers (glycerol and polyethylene glycols) for collagen hydrolyzate films assessed on the basis of T_g depression was studied by means of differential scanning calorimetry in previous work[17]. Contrarily, barrier properties of films to water, oxygen, oils and aromatic substances go down with added plasticisers[18,19]. The plasticiser most frequently employed, satisfactory for use in foodstuffs, is glycerol. An increasing glycerol addition is accompanied with reduced tensile strength of films, while elongation at break increases as well as water vapour and oxygen permeability. Films display better water solubility and high additions of glycerol cause film stickiness. Another plasticiser that may be used is sorbitol which, when added, allows to prepare clear films possessing higher tensile strength and lower elongation than films prepared with glycerol[20]. A further plasticiser acceptable for foodstuffs is sucrose. Coatings prepared from whey protein employing sucrose as plasticiser exhibited outstanding optical and surface properties that were far superior to those produced by glycerol or sorbitol used as plasticisers[21].

The goal of present paper is to prepare biodegradable and edible films based on Amaranth flour starch-protein hydrolyzate and evaluate the effect of chemical and physical modifications on solubility properties of films in water.

EXPERIMENTAL

Amaranth flour: Supplied by the AMR Amaranth Company (Hradec Kralove, Czech Republic); its composition is presented in Table-1. Dry matter was determined by drying a weighed quantity of sample in glass weighing bottle at 103 ± 2 ºC for 12 h and weighing after cooling in desiccator over dried silica gel. Inorganic solid was determined by incinerating a sample of flour in a ceramic crucible over a gas burner and then by annealing at 600 ºC in a muffle furnace and weighing after cooling. Total Kjeldahl nitrogen was determined by mineralizing a sample of flour by boiling (at *ca.* 440 ºC) for 0.5 h in sulphuric acid with added catalyst (Kjeltabs KWS). Nitrogenous substances were thus transformed into ammonium sulphate from which ammonia was released in an alkaline environment and then steam distilled and determined by titration. Coarse proteins were determined[22] by multiplying nitrogen content by conversion factor 5.70. Fat was extracted from the flour sample with *n*-hexane in a Soxhlet extraction apparatus for 4 h. After distilling off the solvent and drying the flask containing fat for 1 h and cooling, fat content was determined by gravimetry. Starch content was determined according to Czech Standard CSN[23] 56 0512-16. This determination is based on hydrolyzing starch into glucose by action of boiling diluted HCl. After clarification, glucose is determined by polarimetry.

The method for determining fibre as described in Czech Standard CSN ISO 5498[24] consists in eliminating accompanying substances from the sample by hydrolysis in an acid and alkaline medium; after 1.5 h hydrolysis in 1.25 % H_2SO_4, the undissolved solid fraction was washed with water and hydrolyzed for another 1.5 h in 1.25 % KOH. Non-hydrolyzed residue (fibre), after washing with water and drying at 103 ± 2 ºC for 6 h, was weighed.

TABLE-1
COMPOSITION OF AMARANTH FLOUR

Parameter	Value (%)
Dry matter	86.9
Inorganic solids[a]	3.6
Total Kjeldahl nitrogen[a]	2.8
Coarse proteins (nitrogen × 5.70)[a]	16.1
Fat[a]	9.8
Starch[a]	65.8
Fibre[a]	4.9

a = in dry matter.

Enzymes for Amaranth starch hydrolysis: Liquefaction of starch in Amaranth flour employed a combination of 3 commercial enzymatic preparations supplied by Novozymes A/S, Bagsvaerd, Denmark: BAN 480 L (α-amylase), AMG 300 L (glucoamylase) and CELLUCLAST 1, 51 FG (cellulase preparation). Enzymatic preparations were mixed in volume ratios BAN 480 L: AMG 300 L: CELLUCLAST 1, 51 FG = 4:3:3 and dosed in the quantity of 5 L per 1,000 kg flour dry matter. In our tests, we worked with weighed quantity 65 g flour; a stock solution of enzymes was thus prepared from concentrated enzyme solutions by pipetting 2 mL BAN + 1.5 mL AMG + 1.5 mL CELLUCLAST and the volume was filled with distilled water to 50 mL. When liquefying Amaranth flour starch, 3.25 mL was pipetted from the stock solution of enzymes (corresponding to dose of 5 L enzymes per 1,000 kg flour dry matter).

Powdery starch dialdehyde (DAS) supplied by Sigma-Aldrich (St. Louis, USA)-trade mark Polymeric Dialdehyde P 9265; glycerol (CAS No 56-81-5) was supplied by the Sigma Aldrich (Product No G9012); catalyst for mineralization-Kjeltabs KWS supplied by Thompson and Capper Ltd. (Cheshire, USA); 96 % H_2SO_4, KOH and NaOH p.a. were supplied by Petr Lukes (Czech Republic).

Drier WTB Binder E/B 28 (Germany), magnetic stirrer IKA RCT basic (Germany), incubator Binder BD23/RS422 (Germany), vacuum evaporator Laborota 4000 (Heidolph Instruments, Germany), electronic balance Kern 770/GS/GJ (Germany), Polarimeter Kruss P1000 (Germany) with polarimetric tube 200 mm, thickness meter TGL 7682-1 (Germany), silicone-rubber coated plate 270 × 210 mm (Tescoma, Czech Republic), filter paper Filpap KA-1 (Filtron, Czech Republic).

Preparation of starch-protein hydrolyzate of Amaranth flour: Enzymatic breakdown of polysaccharides of Amaranth flour proceeded under conditions already

proposed and optimized. Amaranth flour was mixed with water (at 22 ± 2 ºC) in ratio 1:20. Under laboratory conditions, 65 g flour dry matter was weighed into a 2,000 mL boiling flask and 1,300 mL distilled water was added. The flask containing mixture was kept in water bath and stirring with a shaft stirrer began (600 rpm), heating proceeded at a rate of 1.5 ºC min^{-1} until a temperature of 80 ºC was attained. Stock solution of enzymes (3.25 mL) was then added and the mixture was stirred for 10 min. Enzymes were inactivated by heating the mixture at 95 ºC for 5 min. Afterwards the mixture was cooled (under running cold water) to room temperature. Starch-protein hydrolyzate was subsequently separated from solid fraction (Amaranth protein concentrate) by filtering through polyamide cloth (pore diameter 150 µm) folded eightfold. Enzymatic breakdown as presented effects an 83 % conversion of starch and 32 % conversion of proteins into soluble fraction.

Preparation of films from starch-protein hydrolyzate of Amaranth flour: Starch-protein hydrolyzate was concentrated to 14 % dry matter content (w/w) on a vacuum evaporator (at 80 ºC). On cooling to 50 ºC, 20 % glycerol (related to hydrolyzate dry matter) was added and the solution was stirred for 20 min. Subsequently, solution pH was adapted to pH 11 ± 0.2 with (approx. 2 mL) 4 mol L^{-1} NaOH and dialdehyde starch was added (0, 1 or 5 % per hydrolyzate dry matter). Stirring continued for another 60 min under constant temperature 50 ºC. The solution (300 mL) was then cast hot onto a silicone-rubber coated plate (270 × 210 mm) which was then placed for 72 h in a forced-ventilation drier with temperature set at 35 ± 1 ºC. On evaporation of solvent (water), film was separated from silicone-rubber coated plate and its thickness was measured in 10 points. Test samples measuring 2 cm × 2 cm were cut from films.

Thermal exposure of films: Samples of films on Petri dishes were exposed for 6 or 48 h to thermal treatment in a drier (without forced air circulation) at 65 or 95 (± 0.5) ºC. On removal from drier, they were conditoned in a desiccator over dried silica gel for 72 h at 22 ± 2 ºC and then subjected to solubility tests.

Solubility tests of films: Solubility tests of film samples were conducted at temperatures of 6 ± 0.1 or 37 ± 0.1 ºC. A sample of film was placed in glass weighing vessel, weighed and covered with 35 mL distilled water cooled to 6 ± 0.1 or pre-heated to 37 ± 0.1 ºC. The glass weighing vessel was then placed in the incubator. After the prescribed dissolution time, the non-dissolved fraction of film sample was separated by filtration. This non-dissolved fraction was then dried on filter paper (in a Petri dish) at a temperature of 103 ± 1 ºC to constant mass, cooled in a desiccator over dried silica gel and weighed. Determination was performed with three samples, results present their arithmetic mean; standard deviation ranged within 5.0 %.

RESULTS AND DISCUSSION

Changes of film thickness after thermal exposure: Film prepared without added dialdehyde starch (DAS) was slightly sticky, transparent, light yellow and

quite flexible. The mean film thickness was 0.52 ± 0.03 mm. Thermal exposure of film samples at 65 or 95 ºC (6 or 48 h) produced a change in film colour toward darker shades of yellow and even to brown (film after 48 h exposure at 95 ºC); changes in film dimensions were not recorded.

Film prepared with 1 % addition of DAS was slightly sticky, semi-transparent, yellow and much more flexible than film without DAS and the mean film thickness was 0.57 ± 0.03 mm. Thermal exposure of film samples at 65 ºC changed film colour toward dark yellow and even orange (depending on exposure time); changes in film dimensions were not recorded. Thermal exposure of film samples at 95 ºC changed colour toward light brown and even dark brown (depending on exposure time) and an increased film thickness (swelling after length shrinkage) was recorded, approximately 1.5 times.

Film prepared with 5 % added DAS was again slightly sticky, semi-transparent, yellow-brown, very flexible; mean film thickness was 0.61 ± 0.03 mm. Thermal exposure of film samples at 65 ºC changed colour to light brown; changes in film dimensions were not recorded. Thermal exposure at 95 ºC produced a change in colour into dark brown shades and an increment even up to two-fold in film thickness (swelling) was again recorded.

Thermal characteristics of films: Dry matter content of films without addition of DAS, with 1 % or 5 % added DAS, of films without thermal exposure and of films exposed at 65 or 95 ºC (6 or 48 h) was determined by drying 3 test samples at 103 ± 2 ºC for 12 h and weighed after cooling in dessicator over dried silica gel. The arithmetic mean was calculated and the standard deviation ranged within ± 1.5 % (Table-2).

TABLE-2
DRY MATTER CONTENT OF FILMS

Film prepared with	Dry matter content of films (%)				
	Film without thermal exposure	Film thermally exposed at 65 °C		Film thermally exposed at 95 °C	
		for 6 h	for 48 h	for 6 h	for 48 h
0 % DAS	94.7	97.1	98.7	97.8	99.8
1 % DAS	95.6	97.5	98.2	97.1	99.4
5 % DAS	96.8	97.1	98.5	98.3	99.7

Thermal properties of films were studied by differential scanning calorimeter DSC 2010 and by thermogravimetric analyzer TGA Q500 (TA Instruments, New Castle, USA) in open crucibles. In both cases a quantity of approx 5 mg was weighed into the crucible and measurements were conducted under nitrogen atmosphere at a flow rate of 150 mL min^{-1} in a temperature interval 20-400 ºC, dT/dt = 10 ºC min^{-1}. DSC and TGA curves of films without added dialdehyde starch and without thermal exposure are presented in Fig. 1. The first co-ordinate on DSC (Fig. 1A) record shows a minimum at 141 ºC (E 1) and is associated with the release of 0.5 % of

sorbed water from film. Weight losses of film sample are well demonstrated on TGA curve (Fig. 1B). The second peak shows a minimum at 170.2 ºC (E 2) and is associated with the maximal rate of release of structurally bound water from film (1.8 %). Co-ordinate E 3 at 200.2 ºC indicates end of releasing of remained structurally bound water (3.6 %) and slant of further endothermal peak associated with start of evaporation of plasticizer (glycerol). The minimum of this endothermal peak is then at 219.4 ºC (co-ordinate E 4). At the temperature of 250 ºC glycerol is evaporated and thermal degradation of film begins. Peaks relevant to endotherm of crystal phase melting are not clear in thermograms, system is amorphous. Peaks corresponding to T_g are under room temperature.

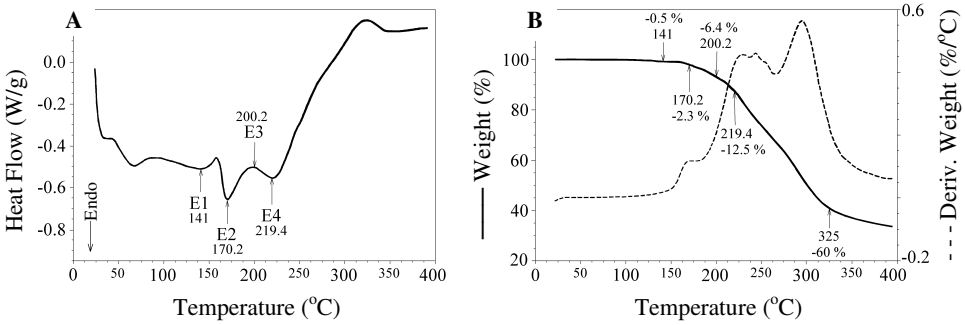

Fig. 1. DSC and TGA curves of films without added dialdehyde starch and without thermal exposure. A DSC curve of film, B TGA and DTGA curves of film

Solubility of films: The graphic presentations of film solubility without addition of DAS, with 1 % or 5 % added DAS, of films without thermal exposure and of films exposed at 65 or 95 ºC (6 or 48 h) are shown in Figs. 2-4. From the course of curves it is obvious that film solubility is notably affected by chain modifier (DAS) added during film preparation as well as by additional thermal exposure of prepared films.

Films without DAS: It was found with films without DAS (Fig. 2) that their additional thermal treatment produced a slowed dissolution rate. From solubility curves a trend may be traced showing film dissolution rate reduces with increasing temperature (65 or 95 ºC) and prolonging time (6 or 48 h) of additional thermal treatment. In the case of solubility test at 6 ºC (Fig. 2A) after dissolution lasting 5 min, dissolved quantities were 40.8 % film without thermal exposure, with film thermally exposed at 65 ºC it was 31.2 % film after 6 h exposure and 19.1 % film after 48 h exposure and at 95 ºC it was 12.6 % film after 6 h exposure and even a mere 10.1 % film after 48 h exposure. After 50 min dissolution, film without thermal exposure dissolved completely, with film thermally exposed 48 h at 65 ºC it was then 81.9 % film and only 53.4 % film thermally exposed 48 h at 95 ºC. In the solubility test at 37 ºC (Fig. 2B), films dissolved faster-film without thermal

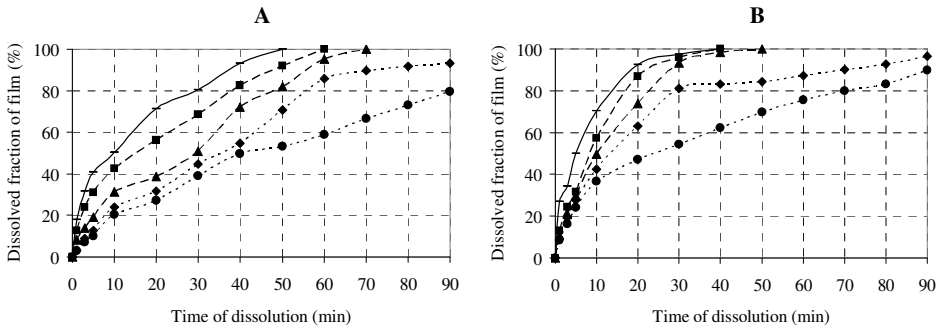

Fig. 2. Solubility in water of films without added dialdehyde starch

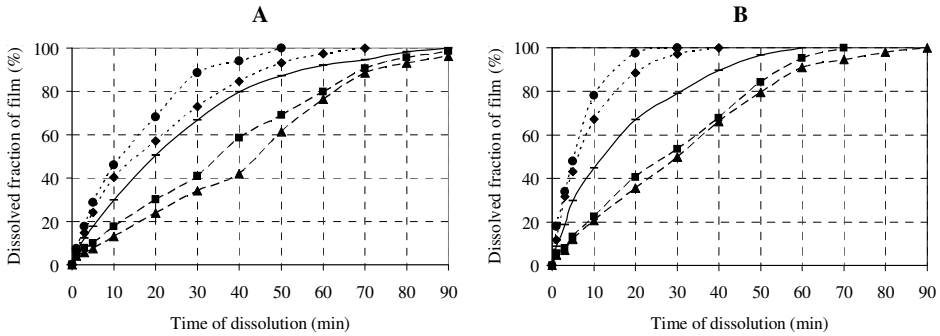

Fig. 3. Solubility in water of films containing 1 % dialdehyde starch

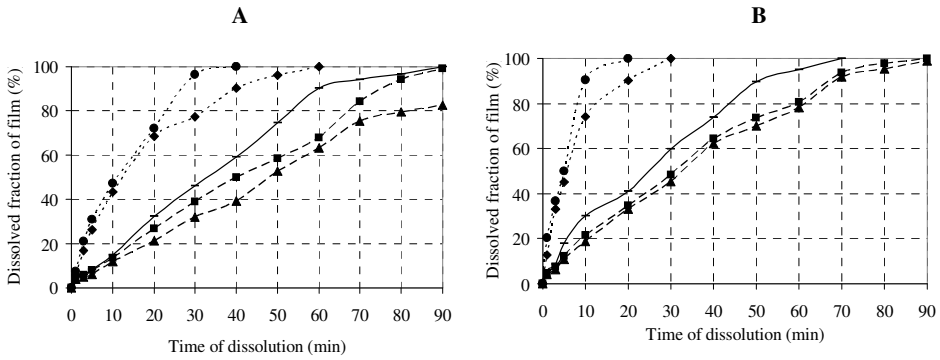

Fig. 4. Solubility in water of films containing 5 % dialdehyde starch, -film without thermal exposure, ■ film thermally exposed at 65 oC for 6 h, ▲ film thermally exposed at 65 ºC for 48 h, ♦ film thermally exposed at 95 ºC for 6 h, ● film thermally exposed at 95 ºC for 48 h, A solubility test at 6 ºC, B solubility test at 37 ºC

treatment already completely dissolved in 40 min, which is 10 min earlier than in solubility test conducted at 6 ºC. With films thermally exposed at 65 ºC, complete dissolution in the solubility test conducted at 37 ºC occurred 20 min earlier than in solubility test at 6 ºC.

Films with 1 % DAS (w/w): Somewhat different results pertain to film containing 1 % added DAS (Fig. 3). With this film, additional thermal treatment at 65 ºC brought about a markedly reduced film dissolution rate after both 6 h and 48 h. On the contrary, thermal exposure at 95 ºC produced an opposite effect-films after 6 h as well as 48 h exposure dissolved faster than films thermally unexposed. In the case of a solubility test at 6 ºC (Fig. 3A) after 5 min dissolving, dissolved quantities were 17.5 % film without thermal exposure, with film thermally exposed at 65 ºC solubility decreased to 9.9 % after 6 h exposure and merely 7.5 % after 48 h exposure, but with thermal exposure at 95 ºC it was 24.1 % after 6 h exposure and 28.7 % after 48 h exposure. Film without thermal exposure completely dissolved after 1.5 h dissolution, film thermally exposed 48 h at 65 ºC dissolved by 96.2 % after the same dissolution time. On the contrary, film thermally exposed for 48 h at 95 ºC completely dissolved after 50 min, which is 40 min earlier than film thermally unexposed. In case of solubility test at 37 ºC (Fig. 3B) films dissolved faster-film without thermal treatment completely dissolved after 1 h, which is 0.5 h earlier than in solubility test at 6 ºC. Film thermally exposed for 6 h at 65 ºC completely dissolved after 70 min in solubility test conducted at 37 ºC. In the solubility test conducted at 6 ºC, film dissolved by 98.6 % after 1.5 h. Film thermally exposed for 6 h at 95 ºC completely dissolved after 40 min (30 min earlier than in solubility test conducted at 6 ºC) and film thermally exposed 48 h at 95 ºC completely dissolved after 0.5 h (20 min earlier than in solubility test at 6 ºC).

Films with 5 % DAS (w/w): Film containing 5 % added DAS (Fig. 4) exhibits changes in film dissolution rate similar to film containing 1 % DAS, but with much greater differences. In case of solubility test at 6 ºC (Fig. 4A), dissolved quantities were 8.3 % film without thermal exposure after 5 min dissolution, with film thermally exposed at 65 ºC solubility mildly decreased to 7.9 % (6 h exposure) or to 6.2 % (with 48 h exposure); contrarily, film thermally exposed at 95 ºC dissolved more than 3 times more quickly-quantities dissolved after 5 min were 26.2 % (in the case of 6 h exposure) and 31 % film (with 48 h exposure). Film after no thermal exposure completely dissolved after 1.5 h, film thermally exposed for 48 h at 65 ºC dissolved by 82.5 % after 1.5 h; by contrast, film thermally exposed for 6 h at 95 ºC already completely dissolved after 1 h and on prolonging this thermal exposure to 48 h the film completely dissolved after 40 min. In solubility tests at 37 ºC (Fig. 4B) films dissolved more quickly-film with no thermal treatment completely dissolved after 70 min, which is 20 min earlier than in the solubility test conducted at 6 ºC. With film thermally exposed for 48 h at 65 ºC, almost complete film dissolution occurred in the solubility test after 1.5 h at 37 ºC (98.8 %) and 82.5 % film dissolution after 1.5 h at 6 ºC. Film thermally exposed for 6 h at 95 ºC completely dissolved after 0.5 h (0.5 h earlier than in solubility test at 6 ºC) and film thermally exposed for 48 h at 95 ºC dissolved completely after 20 min (20 min earlier than in solubility test at 6 ºC).

Conclusion

Partial enzyme hydrolyzate of Amaranth flour (*i.e.*, by-product of preparation of Amaranth protein concentrate) contains in dry matter *ca.* 32 % of hydrolyzed protein (based on initial content of protein in flour) and 83 % of hydrolyzed starch (based on initial content of starch in flour) and therefore provides systems with low viscosity by which it allows to easily reach substantial film (coating) thickness. With regard to hydrolysis of protein (reduced amount of amino groups), dialdehyde starch (DAS) has less cross-linking effect than it manifested during cross-linking of collagen hydrolyzate[6,7]. Enhancement of strength and barrier properties can be gained by increasing amount of protein (non-hydrolyzed), eventually by adding soya protein or gluten with necessity to use alcohol dissolvent[5,25].

We prepared films by casting a 14 % (w/w) solution of hydrolyzate with added 20 % glycerol (per dry substance of hydrolyzate) and various additions of dialdehyde starch 0, 1 or 5 % (per dry substance of hydrolyzate) at pH = 11 and 50 ºC. Water was evaporated at 35 ºC and films were peeled off after 72 h. After their thermal exposure at 65 or 95 ºC for 6 or 48 h, water solubility tests at 6 ºC or 37 ºC were performed. It was found that chemical modification (added dialdehyde starch, DAS) and physical modification (thermal exposure of film) strongly alter film solubility. Films without DAS exhibit decelerated film dissolution rate with growing temperature of additional thermal treatment (65 or 95 ºC) and prolonged time (6 or 48) of thermal treatment. With films containing 1 % added DAS, a slower film dissolution rate occurred after additional thermal treatment at 65 ºC. On the contrary, films thermally exposed at 95 ºC dissolved more quickly than thermally unexposed films. The chemical cause may be due to reduction ability of DAS in alkali environment which is unfavourable for formation of disulfide bonds as it is usual for wheat gluten[26,27]. Cleavage (most likely oxidizing cleavage) of cross-linking chains of DAS is also probable. Films containing 5 % added DAS recorded changes in film dissolution rate similar to those with films containing 1 % added DAS, but with much greater differences. Films had little gel phase or possibly it could be declined by chemical degradation (hydrolysis on protein, oxidizing cleavage on DAS). Films were unusually rapidly dissolved in *ca.* 0.5-1.5 h, compared to *e.g.* films prepared from collagen hydrolyzate (H). Films made from 80 % H + 20 % DAS with gel content up to 60 % released most of the dissolving part after approximately 10 h[28].

Starch-protein hydrolyzate of Amaranth flour has film-forming properties, which may be utilized for preparing biodegradable and edible films or coatings. Solubility test results of biodegradable and edible films prepared from Amaranth flour starch-protein hydrolyzate give a survey of their behaviour in an aqueous environment, which may serve to their employment in practical applications.

ACKNOWLEDGEMENT

The authors would like to thank to Ministry of Education of The Czech Republic for financial support to this work executed under MSM Grant No. 7088352102.

REFERENCES

1. T.R. Keenan, Handbook of Biodegradable Polymers, in eds.: A.J. Domb, J. Kost and D.M. Wiseman, Vol. 2, Chap. 16, CRC Press, Boca Raton, 307 (1997).
2. A.M. Clarinval and J. Halleux, Biodegradable Polymers for Industrial Applications, ed. R. Smith, Vol. 1, Chap. 1, CRC Press, Boca Raton, 4 (2005).
3. C. Bastioli, *Starch*, **53**, 351 (2001).
4. D. S. Cha and M.S. Chinnan, *Crit. Rev. Food Sci. Nutr*., **44**, 223 (2004).
5. J.W. Rhim, J.H. Lee and P.K.W. Ng, *LWT-Food Sci. Technol*., **40**, 232 (2007).
6. P. Mokrejs, D. Janacova, F. Langmaier, M. Mladek, K. Kolomaznik and V. Vasek, *J. Am. Leather Chem. Assoc*., **103**, 314 (2008).
7. P. Mokrejs, D. Janacova, M. Mladek, F. Langmaier, K. Kolomaznik and V. Vasek, *Res. J. Chem. Environ*., **12**, 13 (2008).
8. P.G. Dalev, R.D. Patil, J.E. Mark, E. Vassileva and S. Fakirov, *J. Appl. Polym. Sci*., **78**, 1341 (2000).
9. R.A. De Carvalho and C.R.F. Grosso, *Food Hydrocolloids*, **18**, 717 (2004).
10. R.H. Wilson, *Proc. Soc. Exp. Biol. Med*., **102**, 735 (1959).
11. H. Onishi and T. Nagai, *Int. J. Pharma*., **30**, 133 (1986).
12. P. Veiga-Santos, L.M. Oliveira, M.P. Cereda and A.R.P. Scamparini, *Food Chem*., **103**, 255 (2006).
13. J.W. Rhim, A. Gennadios, C.L. Weller, C. Cezeirat and M.A. Hanna, *Ind. Crop. Prod*., **8**, 195 (1998).
14. B. Mert and Z. Ustunol, *J. Food Sci*., **69**, 129 (2004).
15. P. Hernandez-Munoz, R. Villalobos and A. Chiralt, *Food Hydrocolloids*, **18**, 647 (2004).
16. P. Munzara, J. Zhang, S. Zhang and J-L. Jane, Protein-Based Films and Coatings", ed. A. Gennadios, Chap. 26, CRC Press, Boca Raton, 621 (2002).
17. F. Langmaier, P. Mokrejs, K. Kolomaznik and M. Mladek, *Thermochim. Acta*, **469**, 52 (2008).
18. J.H. Yang, J.A. Yu and X-F. Ma*, Carbohydr. Polym*., **66**, 110 (2006).
19. J.W. Park, W.S. Whiteside and S.Y. Cho, *LWT-Food Sci. Technol*., **41**, 692 (2008).
20. N. Laohakunjit and A. Noomhorm, *Starch*, **56**, 348 (2004).
21. S.I. Hong, J.H. Han and J.M. Krochta, *J. Appl. Polym. Sci*., **92**, 335 (2004).
22. J. Davidek, J. Hrdlicka, M. Karvanek, J. Pokorny, J. Seifert and J. Velisek, Handbook of Food Analysis, SNTL, Prague, 182 (1988).
23. CSN 56 0512-16, Methods of Milled Products Analysis: Part 16-Determination of Starch According to Ewers, Czech Standard (1995).
24. CSN ISO 5498, Agricultural Food Products: Determination of Crude Fibre Content-General Method, Czech Standard (1994).
25. Y. Ali, V.M. Ghorpade and M.A. Hanna, *Ind. Crops Prod*., **6**, 177 (1997).
26. N. Gontard, *J. Food. Sci*., **57**, 190 (1992).
27. T.J. Heralp, R. Gnanasambandam, B.H. McGuire and K.A. Hachmeister, *J. Food. Sci*., **60**, 1147 (1995).
28. F. Langmaier, P. Mokrejs, K. Kolomaznik and M. Mladek, *Waste Manage*., **28**, 549 (2008).

(*Received*: 5 May 2009; *Accepted*: 25 November 2009) AJC-8085