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Thermal study and solubility tests of films based on amaranth flour starch–protein hydrolysate

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Abstract The study deals with the effect of chemical and physical modifications on thermal properties and solubility properties of films based on amaranth flour starch–protein hydrolysate. Biodegradable and edible films were prepared by casting a 25% (w/w) solution of hydrolysate containing 20% glycerol and various additions of dialdehyde starch (0, 1 and 5%). After thermal exposure of films at 65 and 95 °C (for 6 and 48 h), thermal properties of films were studied employing differential scanning calorimetry and thermogravimetric analysis. Film solubility tests were performed in an aqueous environment at 25 °C. Chemical and physical modifications of films markedly affect their thermal properties and solubility.

Keywords Amaranth flour · Biodegradable films · Dialdehyde starch · DSC · Hydrolysate · Solubility test · TGA · Thermal exposure

Introduction

Edible films and coatings are generally defined as thin layers of edible material applied onto a food product to prolong life and preserve natural organoleptic properties of foodstuffs. Such a coating or film acts as a barrier against water vapour, CO_2, oxygen, aromatic substances or oils. Even though use of edible coatings and films to protect foodstuffs is not a new concept, there is considerable interest by consumers, producers and even governmental organisations in development and manufacture of new and enhanced-quality packing layers [1].

Biodegradable and edible films and coats are based on animal proteins (for example, collagen, gelatine, keratin, whey, casein), vegetable proteins (for example, maize zein, wheat gluten, soy protein), polysaccharides (in particular, starch, cellulose and their derivatives, chitin, chitosan, pectins), lipids and waxes [2, 3]. Modifying mechanical properties (particularly to increase flexibility) utilises plasticizers (for example, glycerol or sorbitol) which are added to mixtures for preparing films or coatings [4]. Additives also used are particularly antioxidants, antimicrobics, nutritive substances, gustatory additives and colorants, which improve qualitative and organoleptic properties of films and coatings [5–7]. Cross-linking agents are added to limit solubility of films and coatings and to improve mechanical properties [8–10]. At present, research teams strive to find possibilities of replacing animal proteins with vegetable proteins and thus create potential for a completely new market for agricultural commodities or waste arising from agricultural production.

Protein-based films exhibit quite high water vapour permeability levels—approx. 2–4 times greater than current plastics (polyethylene, polypropylene, polyethylene terephthalate or polyvinyl chloride) [11]. Limited resistance of proteins to water vapour permeation is given by content of hydrophilic groups in proteins as well as by content of hydrophilic plasticizers. An addition of lipids may reduce water vapour permeation. Films based on polysaccharides exhibit much better mechanical properties than films based on proteins. Starches containing a greater

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amylose fraction provide films of better mechanical and barrier properties than starches with a majority proportion of amylopectin. Polysaccharides also break down more slowly, which is exploited to regulate film dissolution rate. Films composed of proteins and polysaccharides are designated composite films, and their preparation especially intends improving mechanical properties (tensile strength) and barrier properties of films. Examples of this may be, for example, composite films of gelatine and chitosan, whose optimum ratio can achieve required mechanical properties (tensile properties, modulus) and barrier properties (water vapour permeability, gas permeability) [12].

Edible films and coatings are successfully used in the food industry for all sorts of meat, including poultry and fish. These layers of coat display a number of advantages, for example, they reduce losses in moisture, retain meat juices, act as barrier to oxygen (reducing oxidation of fats), limit rise of micro-organisms and losses of aromatic substances, improve appearance of the product on sale and its shape, and may serve as carriers of aromatic substances [13]. Coatings of natural polymers (for example, of collagen) are successfully used as protective layers on fruit and vegetables especially aiming to prevent losses in moisture content and to improve appearance. Improved appearance is most required with citrus fruits. Coatings are used to slow down metabolic reactions of fruit and vegetables after harvest ripening, for example, in ship transport [14–16]. Another highly lucrative field of applying protective films is fried foodstuffs because the growing proportion of fried foods occurs with an increased consumption of fats, which is undesirable from the health point of view. Applying a protective layer (film) on foodstuffs before frying may achieve up to 40% reduction of oil absorbed in frying [17, 18].

Development of biodegradable materials is not only focused on food applications, but also on their utilisation in agriculture. At present, plastic films are employed in agriculture with the aim of protecting plants, catching water and nutrients and creating a superior micro-environment for plants as well as protection against external climatic influences. Due to this, consumption of plastic films is swiftly increasing. Biodegradable materials prepared, for example, from starch, collagen, or polymers prepared by combining natural and synthetic polymers are qualitative variant equalling products from synthetic plastics. An example may be biodegradable containers used to grow young seedlings or strips employed as seed carriers. Biodegradable films made from proteins provide young plants with initial protection and, after breakdown, enrich soil with important nutrients (nitrogen) [19].

Amaranth has lately been the object of keen interest especially in the food industry. Amaranth grain may yield as much as 18% high-quality protein possessing a very well balanced composition of essential amino acids. Protein content in amaranth grains is higher, as opposed to proteins of current cereals [20]. Starch content in amaranth ranges from 48% to approx. 62%. Grains of starch from amaranth are very small, of 1–3 μm diameters, angular polygonal shape. Starch bonds very strongly but is highly sensitive to action by amylases [21]. Total fat content of cereal amaranth is 5.4–17.0% containing almost 50% linolenic acid [22, 23]. An important component of amaranth fat is squalene, whose content is 8 times greater than in olive oil [24].

Objective of the work

Within the scope of a project focused on obtaining proteins from untraditional sources, we concentrated on isolating proteins of amaranth flour. Seed proteins are usually isolated by using the sequential solvent method [25]. We developed the method of separating protein by enzymatic degradation of polysaccharides (starch), their liquefaction into soluble glucose, and enrichment of solid phase with vegetable protein. Separated amaranth protein may be effectively applied in production of functional foodstuffs and quality supplements. We also focused on employing the solution of starch–protein hydrolysate to prepare biodegradable and edible films. The objective of research in this study was to evaluate the effect of chemical and physical modifications on thermal properties and solubility properties of films based on amaranth flour starch–protein hydrolysate.

Materials and methods

Amaranth flour was supplied by the AMR Amaranth Company (Hradec Kralove, The Czech Republic); its composition is presented in Table 1.

Apparatus and equipment comprised: Drier WTB Binder E/B 28 (Germany), magnetic stirrer IKA RCT basic (Germany), incubator Binder BD23/RS422, 23l (Germany), electronic balance Kern 770/GS/GJ (Germany), thickness

Table 1 Composition of amaranth flour

Parameter	Value (%)
Dry matter	86.91
Inorganic solids in dry matter	3.57
Total Kjeldahl nitrogen in dry matter	2.82
Coarse proteins (nitrogen × 5,70) in dry matter	16.07
Fat in dry matter	9.81
Starch in dry matter	65.79
Fibre in dry matter	4.85

meter TGL 7682-1 (Germany), silicone plate 270 × 210 mm (Tescoma, The Czech republic), filter paper Filpap KA-1 (The Czech Republic).

Stock solution of enzymes: Liquefaction of starch employed a combination of three commercial enzymatic preparations supplied by Novozymes A/S, Bagsvaerd, Denmark: BAN 480 L (α-amylase), AMG 300 L (glucoamylase) and CELLUCLAST 1,51 FG (cellulose 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).

Other chemicals: 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 Co, U.S.A. (Product No G9012); NaOH p.a. was supplied by Petr Lukes (The Czech Republic).

Preparation of starch–protein hydrolysate of amaranth flour

Enzymatic breakdown of polysaccharides (starch) of amaranth flour proceeded under conditions we had to this purpose already proposed and optimised. Amaranth flour was mixed with water (at 22 ± 2 °C) 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 put over water bath and stirring of its contents with a shaft stirrer began (600 rpm), heating proceeded at a rate of 1.5 °C min^{-1} until a temperature of 80 °C was attained. Stock solution of enzymes (3.25 mL) was then added; the mixture was stirred for 10 min and cooled (under running cold water) to room temperature. Starch−protein hydrolysate was subsequently separated from solid fraction (concentrated protein) by filtering through polyamide cloth folded eightfold. Enzymatic breakdown as presented effects an 83% conversion of starch and 32% conversion of proteins.

Preparation of films from starch–protein hydrolysate of amaranth flour

Starch–protein hydrolysate was concentrated to 25% dry matter content (w/w) on a vacuum evaporator (at 80 °C). On cooling to 50 °C, 20% glycerol (related to hydrolysate 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 and 5% per hydrolysate dry matter). Stirring continued for another 60 min under constant temperature 50 °C. The solution was then cast onto a silicone plate (270 × 210 mm) which was then placed for 72 h in a forced-ventilation drier with temperature set at 35 ± 1 °C. On evaporation of solvent (aqueous phase), film was separated from silicone plate, evaluated in sensory manner and its thickness was measured in 10 points. Test samples measuring 2 by 2 cm were prepared from films by mechanical separation.

Thermal exposure of films

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

Thermal study of films

Temperature co-ordinates of characteristic peaks and mass loss were determined by differential scanning calorimeter DSC 2010 (TA Instruments, New Castle, USA) in open aluminium crucibles and by thermogravimetric analyser TGA Q500 (TA Instruments, New Castle, USA) in open platinum 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 °C, dT/dt = 10 °C min^{-1}. Each test was performed threefold and arithmetic mean calculated, standard deviation ranged within ±2.0%.

Solubility tests of films

Solubility tests of film samples were conducted at temperature of 25 (±0.1) °C. A sample of film was placed in glass weighing vessel, weighed and covered with 35 mL distilled water preheated to 25 (±0.1) °C. 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 °C to constant mass and weighed. Determination was performed with three samples, results present their arithmetic mean.

Results and discussion

Film prepared without added dialdehyde starch (DAS) was slightly sticky, transparent, light yellow, and quite flexible; mean film thickness was 0.52 ± 0.03 mm. Thermal exposure of film samples at 65 and 95 °C (6 and 48 h) produced a change in film colour toward darker shades of yellow and even to brown (film after 48-h exposure at 95 °C); changes in film dimensions were not recorded. Film prepared with a 1% addition of DAS was slightly sticky, semitransparent, yellow and much more flexible than film without DAS; mean film thickness was 0.57 ± 0.03 mm. Thermal exposure of film samples at 65 °C produced a change in 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 °C produced a change in colour toward light brown and even dark brown (depending on exposure time) and an increased film thickness (swelling) was recorded, approximately 1.5 times. Film prepared with 5% added DAS was again slightly sticky, semitransparent, yellow-brown, very flexible; mean film thickness was 0.61 ± 0.03 mm. Thermal exposure of film samples at 65 °C produced a change in colour to light brown; changes in film dimensions were not recorded. Thermal exposure at 95 °C produced a change in colour into dark brown shades, and an increment even up to twofold in film thickness (swelling) was again recorded.

Results of DSC and TGA measurements of films based on amaranth flour starch–protein hydrolysate are arranged for facilitated orientation in tabular manner (Table 2). DSC and TGA curves of films without thermal exposure are presented in Fig. 1. From the indexes for the transitions in films (E1, E2, E3 and E4) the difference between films containing 1 and 5% added dialdehyde starch and film without added DAS is apparent. Similar alterability was recorded with the effect of thermal exposure of films.

Analyses of DSC and TGA records of films containing no added dialdehyde starch (Fig. 1a, b) allow drawing conclusions as follow. With films without thermal exposure the first co-ordinate on DSC record (E 1) shows a minimum at 141 °C and is associated with the release of negligible amount of sorbed water from film. The second peak (E 2) shows a minimum at 170.2 °C and is associated with the release of structurally bound water from film; this is well demonstrated by an approx. 2.3% drop in mass of film sample at this temperature on the TGA curve (see Fig. 1b). At 200.2 °C, the DSC record (Fig. 1a) indicates an obvious slant towards a further endothermal peak (co-ordinate E 3) associated with release of plasticizer (glycerol). The minimum of this endothermal peak is then at 219.4 °C. These changes are well documented by TGA analysis, where 6.4% loss of sample mass appeared at 200.2 °C, and a 12.5% loss of sample mass was recorded at 219.4 °C (see Fig. 1b). Above the temperature of 219 °C thermal degradation of film begins (co-ordinate E 4). This stands with results of works dealing with thermal stability of plant proteins and starch based films which detected thermal degradation starting around the temperature of 230 °C (proteins) and 250 °C (starch) [26–28]. In addition, in our previous work results of DSC measurements of dried hydrogels of collagen hydrolysate cross-linked with dialdehyde starch proved that temperatures above 230–240 °C are a hazardous region [29]. At a temperature of 325 °C, marked thermal breakdown of the sample is obvious—an approx. 60% loss in sample mass (Fig. 1b). Indexes for the transitions in films that were subjected to thermal exposure at 65 °C show a certain shift. Thermal co-ordinate associated with release of plasticizer was recorded at temperatures 15–18 °C higher and thermal co-ordinate corresponding to the onset of film decomposition was recorded at temperatures approx 34 °C higher (depending on time of thermal action—6 and 48 h) than with sample without thermal treatment. Thermal exposure of films at 95 °C again elevated film co-ordinate associated with release of plasticizer—9 to 33 °C higher; thermal co-ordinate corresponding to the onset of film decomposition also shifted to a higher temperature—29 to 37 °C higher (depending on time of thermal action) than with sample without thermal treatment. Sample mass loss at 325 °C was within limits 63–65%.

Characteristic peaks and thermal co-ordinates of DSC and TGA records of films containing 1% (Fig. 1c, d) and 5% (Fig. 1e, f) added dialdehyde starch, and differences applying to films without added DAS may be summarised as follows. With added DAS, index for the transition associated with evaporation of remaining sorbed water (E 1) and index for the transition corresponding to the minimum of endothermal peak associated with the release of structurally bound water (E 2) from film shifted to lower levels (see DSC curve in Fig. 1c, e). While films without added DAS displayed co-ordinate E 1 in a range from 139.1 to 156.2 °C (depending on time and temperature of film thermal exposure), films with 1% added DAS displayed co-ordinate E 1 ranging from 113 to 135.4 °C (depending on time and temperature of film thermal exposure). Films containing 5% added DAS had E 1 ranging from 113.4 to 127.8 °C (depending on time and temperature of film thermal exposure). Minimum of the endothermal peak (E 2) with films containing no added DAS was within limits 169.9–174.8 °C (depending on time and temperature of film thermal exposure), with films containing 1% DAS in limits 136.1–152.8 °C (depending on time and temperature of film thermal exposure), and with films containing 5% added DAS within limits 137.3–150.4 °C (depending on time and temperature of film thermal exposure). As DAS additions grow, both indexes

Table 2 Results of thermal analysis of films based on amaranth flour starch–protein hydrolysate

Indexes for the transitions	Film without thermal exp.		Film thermally exposed at 65 °C				Film thermally exposed at 95 °C			
			For 6 h		For 48 h		For 6 h		For 48 h	
	T (°C)	–Δm (mass %)	T (°C)	–Δm (mass %)	T (°C)	–Δm (mass %)	T (°C)	–Δm (mass %)	T (°C)	–Δm (mass %)
Film without added dialdehyde starch										
E 1	141.0	0.5	152.4	3.2	152.4	2.1	139.1	0.3	156.2	0.9
E 2	170.2	2.3	169.9	4.7	174.8	3.9	172.5	1.1	–	
E 3	200.2	6.4	215.4	8.2	218.4	7.4	209.0	3.6	232.7	6.2
E 4	219.4	12.5	253.3	21.1	254.0	20.0	247.9	15.4	256.2	17.3
Film containing 1% dialdehyde starch										
E 1	126.3	1.5	135.4	2.4	115.7	1.0	121.7	3.0	113.0	2.1
E 2	147.9	5.1	152.1	4.2	152.8	4.3	136.1	4.0	144.5	4.4
E 3	185.8	8.9	205.5	8.9	189.6	7.6	191.1	8.5	187.7	7.8
E 4	222.9	23.4	233.6	18.9	225.2	19.8	226.0	23.7	226.7	24.2
Film containing 5% dialdehyde starch										
E 1	127.8	1.8	113.4	1.9	119.1	1.0	118.7	1.5	–	
E 2	143.7	4.1	137.3	5.0	144.9	3.0	146.0	3.8	150.4	2.1
E 3	188.8	9.3	183.9	11.4	180.9	6.6	183.5	7.3	186.9	5.2
E 4	226.7	25.2	218.0	20.1	218.0	20.2	221.1	22.7	222.9	21.5

Temperature co-ordinates of detected endothermal DSC peaks (T,°C) and related average cumulated mass loss on TGA curves (–Δm, mass %)

for the transitions (E 1 and E 2) obviously shift to lower temperatures. Slant towards a further endothermal peak (co-ordinate associate with start of releasing of plasticizer—E 3) of film samples containing 1 and 5% DAS moved to lower temperatures in comparison with film samples with no added DAS (see Fig. 1a, c, e). Nevertheless, E 3 of films containing 1% DAS, due to thermal exposures, moved to slightly higher temperatures (see Table 2). Contrarily, E 3 of films with added 5% DAS, due to thermal exposures, shifted in all cases to slightly lower temperatures (Table 2). Index for the transition corresponding to the onset of film decomposition (E 4) with films containing 1 and 5% added DAS without thermal exposure was recorded at temperatures slightly higher than with film samples with no added DAS. Films that were subjected to thermal exposures displayed co-ordinate E 4 ranging from 225.2 to 233.6 °C (1% added DAS) and from 218 to 222.9 °C (5% added DAS); with films having no added DAS it was 247.9–256.2 °C. Sample mass loss at 325 °C ranged within limits 62–64%.

Graphical presentations of film solubility in an aqueous environment at 25 °C are shown in Figs. 2, 3 and 4. It is obvious from curves that solubility of films is strongly affected by added cross-linking agent (dialdehyde starch) in film preparation as well as by additional thermal exposure of prepared films.

It was found with films having no added DAS (see Fig. 2) that their additional thermal treatment produced a slowed dissolution rate. Solubility curves allow tracing a trend of decelerating film dissolution rate with increasing temperature of additional thermal treatment (65 and 95 °C) and with its prolonging time (6 and 48 h). For example, after dissolution lasting 20 min, 73.1% film without thermal exposure was dissolved, 63.4% film thermally exposed for 6 h at 65 °C and 56.7% film exposed 48 h, and 54.8% film after thermal exposure of 6 h at 95 °C and even merely 36.5% film after exposure of 48 h. After dissolution lasting 50 min, film with no thermal exposure dissolved completely, 96.5% film dissolved after thermal exposure at 65 °C for 48 h, and merely 69.2% film thermally exposed at 95 °C for 48 h.

There are somewhat different results with film containing 1% added DAS (see Fig. 3). Additional thermal treatment of this film at 65 °C caused decelerated film dissolution rate after treatment of both 6 h as well as 48 h. On the opposite, thermal exposure at 95 °C produced a reverse effect—films after exposure of 6 and 48 h dissolved more quickly than films after no thermal exposure. For example, after dissolution lasting 20 min, 62.1% film without thermal exposure was dissolved; with film thermally exposed at 65 °C, dissolution dropped to 41% (in the case of 6-h exposure) or to 30.1% (in the case of 48-h exposure), but 74.7% film was dissolved after thermal exposure at 95 °C (in the case of 6-h exposure) and even 91.3% film (in the case of 48-h exposure). Film with no thermal exposure completely dissolved after 70 min, film

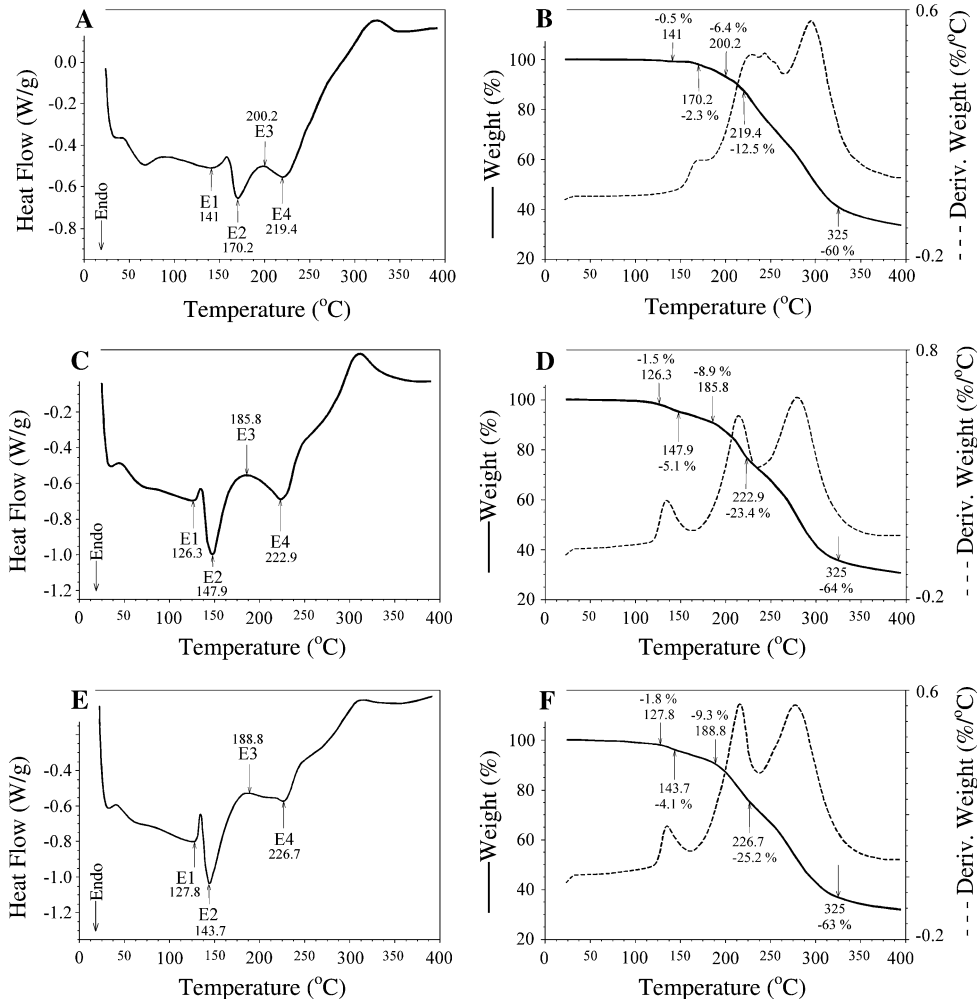

Fig. 1 DSC, TGA and DTGA curves of films based on amaranth flour starch–protein hydrolysate without thermal exposure. **a** DSC curve of film without added DAS. **b** TGA and DTGA curves of film without added DAS. **c** DSC curve of film containing 1% (w/w) DAS. **d** TGA and DTGA curves of film containing 1% (w/w) DAS. **e** DSC curve of film containing 5% (w/w) DAS. **f** TGA and DTGA curves of film containing 5% (w/w) DAS. *E 1* Index for the transition associated with release of remaining sorbed water, *E 2* Index for the transition corresponding to the minimum of endothermal peak associated with release of structurally bound water, *E 3* Index for the transition associated with release of plasticizer, *E 4* Index for the transition corresponding to the onset of film decomposition. DAS dialdehyde starch

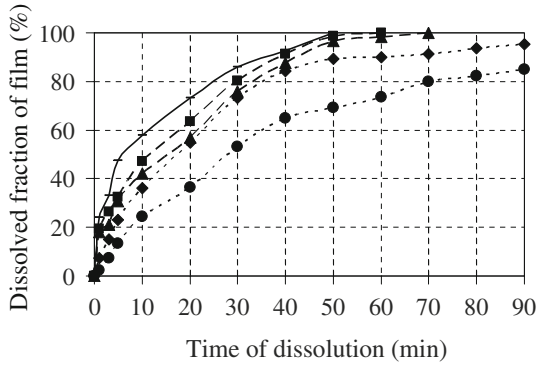

Fig. 2 Solubility of films without added dialdehyde starch in an aqueous environment at 25 °C. *Solid line* film without thermal exposure. *Filled square* film thermally exposed for 6 h at 65 °C. *Filled triangle* film thermally exposed for 48 h at 65 °C. *Filled diamond* film thermally exposed for 6 h at 95 °C. *Filled circle* film thermally exposed for 48 h at 95 °C

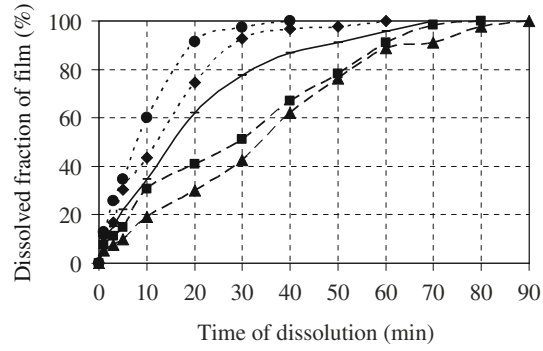

Fig. 3 Solubility of films containing 1% dialdehyde starch in an aqueous environment at 25 °C. *Solid line* film without thermal exposure. *Filled square* film thermally exposed for 6 h at 65 °C. *Filled triangle* film thermally exposed for 48 h at 65 °C. *Filled diamond* film thermally exposed for 6 hr at 95°C. *Filled circle* film thermally exposed for 48 h at 95 °C

after thermal exposure of 48 h at 65 °C exhibited dissolution 20 min longer; contrarily, film thermally exposed 48 h at 95 °C already completely dissolved in 40 min.

Similar changes in film dissolution rate were recorded with film containing 5% added DAS (see Fig. 4) as well as with film containing 1% DAS addition, however, exhibiting much greater differences. Thus, for example, 41.1%

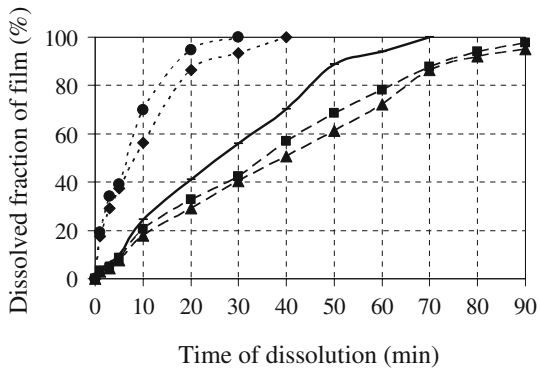

Fig. 4 Solubility of films containing 5% dialdehyde starch in an aqueous environment at 25 °C. *Solid line* film without thermal exposure. *Filled square* film thermally exposed for 6 h at 65 °C. *Filled triangle* film thermally exposed for 48 h at 65 °C. *Filled diamond* film thermally exposed for 6 h at 95 °C. *Filled circle* film thermally exposed for 48 h at 95 °C

film without thermal exposure was dissolved in 20 min, solubility of film thermally exposed at 65 °C was again lower—32.9% (in the case of 6 h exposure) or 29.3% (in the case of 48 h exposure); on the opposite, film thermally exposed at 95 °C dissolved much more quickly—86.3% film (in the case of 6 h exposure) and 94.6% film (in the case of 48 h exposure). Film without thermal exposure completely dissolved in 70 min, film thermally exposed 48 h at 65 °C dissolved by 95.1% in 90 min; on the contrary, film thermally exposed for 48 h at 95 °C already completely dissolved in 30 min.

In order to mutually compare in greater detail dissolution rate of films without added DAS, films containing 1 and 5% DAS, without thermal exposure as well as with examined thermal exposures (65 and 95 °C for 6 and 48 h) we refer to Fig. 5. With films without additional thermal exposure (Fig. 5a) and with films thermally exposed for

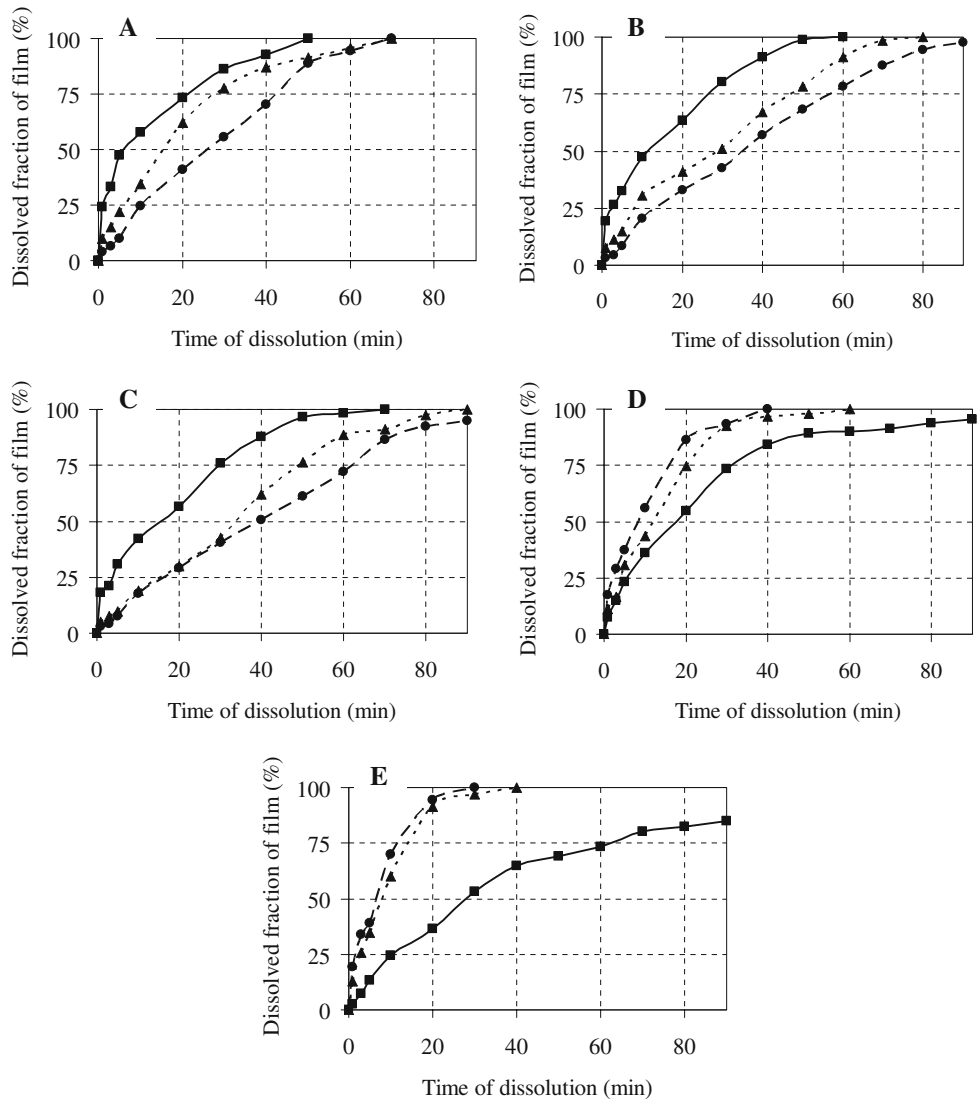

Fig. 5 Solubility of films containing various additions of dialdehyde starch compared. *Filled square* film without added DAS. *Filled triangle* film containing 1% DAS. *Filled circle* film containing 5% DAS. **a** Film without thermal exposure. **b** Film thermally exposed for 6 h at 65 °C. **c** Film thermally exposed for 48 h at 65 °C. **d** Film thermally exposed for 6 h at 95 °C. **e** Film thermally exposed for 48 h at 95 °C

6 h at 65 °C (Fig. 5b) as well as for 48 h (Fig. 5c), dissolution rate decelerates with increasing addition of DAS. In the case of films without thermal exposure (Fig. 5a), film without DAS completely dissolved in 50 min, but film containing 1% DAS dissolved in the same time by 91.2% and film with 5% added DAS dissolved by merely 88.8%. With films thermally exposed for 6 h at 65 °C (see Fig. 5b), film without added DAS completely dissolved only after 60 min, which is 10 min longer than film without thermal exposure; film containing 1% DAS displayed 91.2% dissolved film in the same time, and with film containing 5% DAS dissolution was merely 78.2%. With films thermally exposed for 48 h at 65 °C (see Fig. 5c) film with no added DAS completely dissolved only after 70 min, which is 20 min longer than film without thermal exposure or 10 min longer than film thermally exposed for 6 h at 65 °C; film containing 1% added DAS had 91.1% dissolved film and film with 5% added DAS had 86.5% dissolved film in the same time. In the case of films thermally exposed at 95 °C for 6 h (Fig. 5d) and 48 h (Fig. 5e), film dissolution rate contrarily accelerates with increasing DAS addition. With 6-h thermal exposure (Fig. 5d), film containing 5% added DAS already completely dissolved in 40 min, while film with 1% added DAS only dissolved in 60 min and film without DAS did not quite dissolve even after 90 min. The difference in film dissolution rate is much more prominent after 48-h thermal exposure (Fig. 5e): film with 5% added DAS already fully dissolved in 30 min, film with 1% added DAS already in 40 min, while percentage of dissolved film containing no DAS found after 90 min was 85.1%.

Conclusion

Starch–protein hydrolysate of amaranth flour possesses film-forming properties, which may be used for preparing biodegradable and edible films. We produced films by casting a 25% (w/w) solution of hydrolysate containing added 20% glycerol (per hydrolysate dry matter) and different additions of dialdehyde starch—0, 1 and 5% (per hydrolysate dry matter). After thermal exposure at 65 and 95 °C for 6 and 48 h, differential scanning calorimetry and thermogravimetric analysis were employed to study thermal properties of films, and tests were executed on their solubility in an aqueous environment at 25 °C. It was found that chemical modification (added cross-linking agent—dialdehyde starch, DAS) and physical modification (the effect of thermal exposure of films) alter thermal properties of films and their solubility. Film with no added DAS which was not subjected to thermal exposure had its onset of thermal breakdown recorded at a temperature of 219 °C. Due to the effect of heating at 65 and 95 °C the onset of thermal breakdown of films moved to higher temperatures. Film containing 1% added DAS (per hydrolysate dry matter) which was not subjected to thermal exposure recorded its start of thermal breakdown at 223 °C and due to the effect of heating similar tendency of the onset of thermal breakdown of films was recorded. Thermal stability of films with 5% added DAS is somewhat different. While the onset of thermal degradation with film without thermal exposure was recorded at 227 °C, film thermal exposure made the start of thermal degradation shift to somewhat lower temperatures. Solubility tests of films brought conclusions as follow. With films without added DAS, a lower film dissolution rate occurs with elevated temperature (65 and 95 °C) of additional thermal treatment and prolonged time (6 and 48 h) of this treatment. Films containing 1% added DAS exhibited slower film dissolution rate after additional thermal treatment at 65 °C; on the contrary, films thermally exposed at 95 °C dissolved faster than films lacking thermal exposure. Films containing 5% added DAS recorded changes in film dissolution rate analogous to those with film containing 1% added DAS, but with much greater differences. Results of thermal studies and solubility tests of biodegradable and edible films prepared from amaranth flour starch–protein hydrolysate provide a survey of their thermal stability and of their behaviour in an aqueous environment, which may be well utilised in practical application.

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