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Protective Edible Polymeric Coatings on Butter

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ABSTRACT

The contribution deals with employing starch-protein hydrolysate of amaranth flour, as by-product in manufacture of protein concentrate, to produce protective edible coats on butter. Tests were run on 2 coating solutions. The first was made in such manner that to hydrolysate containing 35 % (w/v) dry matter, 30 % (w/w) plasticiser (glycerol) was added and also 3 % (w/w) emulsifying agent (lecithin). The second furthermore contained 1 % (w/w) antioxidant (tocopherol). Protective layers on butter were produced by coating with coat solutions. Butters were stored for a total of 126 days in refrigerator at 6±1.5 ^{o}C and relative humidity 43±3 ^{o}C. Tests examined the oxidation course of butter by determining its peroxide value (PV) and the difference between PV of butters with coatings, without coatings, of butter wrapped in original wrapper, butters wrapped in paper wrapper and of butters in paper wrappers impregnated with coating solutions. Lower PV levels were found with butter treated with our coats than with butters wrapped in original wrapper. Coating solutions were applied to prepare films by casting and to investigate their properties – water solubility, water vapour permeability, moisture absorption and thermal properties (DSC and TGA).

Keywords: butter, coating, films, peroxide value, starch-protein hydrolysate.

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INTRODUCTION

Oxidation (rancidity) of fats is a process in which double bonds of unsaturated fatty acids are oxidised through action of atmospheric oxygen. It is the most usual type of oxidation showing in the processing and storage of foodstuffs. The process results in undesirable products, particularly aldehydes and ketones, which negatively alter both health effects as well as taste and smell of foodstuffs containing unsaturated fatty acids [1]. Conditions during storage of fats thus exert significant influence on the course of oxidation. Oxidation thus causes partial or complete depreciation of a food.

Oxidation proceeds as a three-stage radical chain reaction [2]:

a) Initiation – through action of heat or radiation, reaction rate is at first low

R–H (lipid) → R• (lipid free radical) (1)

b) Propagation – formation of peroxide radicals, hydro-peroxides, reaction rate increases with growing concentration of peroxides

Formation of peroxide radicals: $R• + O_2 \rightarrow R–O–O•$ (2)

Formation of hydro-peroxide: $R–O–O• + R–H \rightarrow R–O–OH + R•$ (3)

c) Termination reaction of formed radicals

$2R• \rightarrow R–R$ (4)

$R• + R–O–O• \rightarrow R–O–O–R$ (5)

$2R–O–O• \rightarrow R–O–O–R + O_2$ (6)

Oxidation inhibitors are the designation of compounds reducing reaction rate of oxidation by reacting with free radicals of an auto-oxidation chain and limiting activity of oxygen radicals. In particular, natural antioxidants include various vitamins and herbal extracts – for example, phenolic acids and their derivatives. Antioxidising effects are also exhibited by some vitamins (in particular, C and E) and other numerous nitrogenous and sulphuric compounds [3, 4]. Compounds used from the synthetic antioxidants group are butylhydroxyanisol (BHA), butylhydroxytoluene (BHT) or tertiary butylhydroquinone (TBHQ). Their application is governed by strict legislation, for example, TBHG is not permitted in the EU, Canada and other countries for lack of information on toxicity. Gallates, which are more polar than BHA, BHT and TBHQ, demonstrate higher efficiency in anhydrous fats; propyl gallate is suitable for stabilising animal fats [5].

In food packaging technique, required materials are light wrappers adapted to specific demands of wrapped products and made, if possible, from environmentally convenient material (for example, biopolymers). At present, active packagings (films, foils, coatings) are being increasingly used and these directly influence quality and storage life of products by limiting or regulating permeability of gases (O_2, CO_2) and water vapour, and regulate atmosphere inside the wrapping [6-8]. Such packagings may contain antioxidants or antimicrobics in the film or coat and thus prolong life of the product [9].

Wrapping of foodstuffs mostly uses packagings from synthetic polymers (PE, PP, PS, PET, PVC), which are generally marked by outstanding mechanical, barrier and chemical properties, low price, ready processability, and pose no health hazards. Disadvantage of these packagings is a long breakdown time stressing the environment. Reduced biodegradability is displayed by packagings based on semi-synthetic polymers that are made from a synthetic polymer with added natural polymer, for example, protein or saccharide. [10].

Edible films and coatings are used for improving appearance of foods and also for prolonging their durability. Films and coatings are prepared from compounds based on proteins, saccharides and lipids. The animal protein most applied is gelatine, which has excellent film-forming properties [11]. Casein films and coatings exhibit high water vapour permeability and are thus convenient for wrapping fruit and vegetables. Films and coatings of milk whey are suitable for foods with a high content of unsaturated fatty acids because they slow down lipid oxidation [12]. Most frequently applied vegetable proteins for preparing films and coats are maize zein and wheat gluten. Zein films and coats are glossy, water-insoluble, with good anti-microbe resistance and low fat permeability. Added polysaccharides (for example, methyl cellulose) and plasticisers (for example, glycerol, sorbitol, polyethylene glycols) permit to set the mechanical properties of film (tensile strength, elongation at break, Young's modulus) in accordance with intended application. Wax (beeswax, carnauba, shellac) is added for improving barrier properties and surface hydrophobicity. Coatings from soya protein are suitable for foodstuffs of high fats content because they slow down oxidation and also production of moulds [13-15]. Saccharide-based films are mostly prepared from starch, cellulose, methyl cellulose and chitosan. Chitosan films and coats possess excellent anti-microbial effects and are thus particularly suitable as packaging for meat and fish. Foods with coats based on methyl cellulose have lower absorption of fats during frying. Biopolymers may also serve to prepare twin-layer and multi-component films which can equal packagings from synthetic polymers through their mechanical and barrier properties [16-19].

In our previous publication we dealt with bio-chemical separation of proteins from amaranth [20, 21]. Separated amaranth components important for health (proteins, liquid starches and sugars, oil, fibre) can serve to prepare quality foodstuff complements and functional foods, for cosmetic purposes, as a component of animal feed or source of biological fertilisers. Hydrolysate of amaranth protein is suitable for preparing edible films, and was successfully applied as a protective coat on strawberries [22-24]. This contribution describes utilising hydrolysate of amaranth flour to produce protective edible coats on butter. The objective is to investigate peroxide value (PV) of butters provided with coatings when stored at 6 ^{o}C, and compare its levels with PV of butters without coating, of butter wrapped in original wrapper, of butters wrapped in paper wrapping as well as in paper wrapping impregnated with coating solutions. Also investigated properties of films prepared from coating solutions are water solubility, water vapour permeability, moisture absorption and thermal analysis (DSC and TGA).

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MATERIALS AND METHODS

Amaranth flour

Amaranth flour was supplied by AMR Amaranth (Hradec Kralove, the Czech Republic). Glycerol (CAS No. 56-81-5), lecithin (CAS No. 8002-43-5), α-tocopherol (CAS No. 10191-41-0) and dialdehyde starch (CAS No. 9047-50-1) were supplied by Sigma-Aldrich (St. Louis, USA). Fresh butters, quarters of 250-g weight, of 82-% fat content and same lot (Sachsenmilch AG, Germany), were purchased in a retail chain. Quarters of butter were unwrapped and cut into pieces measuring approx. 3.5x2.5x1.2 cm. Samples thus prepared were placed side by side about 1.5 cm apart on metal plates. A part of the samples was intended for coating, a part was without wrapping/coat. Three quarters of butter were kept in original wrapping, another three quarters of butter were unwrapped and wrapped in wrapping paper Testliner (white, paper density 130 g m^{-2}, Papierfabrik Hamburger-Spremberg, Germany); another 3 + 3 cakes of butter were wrapped in same wrapping papers impregnated with coatings 1 and 2.

Preparing coating solutions

Amaranth flour, with use of commercially available enzymes (supplied by Novozymes Co, Denmark) BAN 480 L, AMG 300 L and CELLUCLAST 1.5 L (mixed in volume ratio 4:3:3), was employed to prepare starch-protein hydrolysate Preparation conditions are given in Fig. 1 and were also described in detail in our previous publications [22, 23]. After filtration, the given enzyme breakdown produced a solid protein concentrate (containing 68 % protein) and liquid hydrolysate (83-% conversion of starch into glucose solution). Starch-protein hydrolysate was thickened on a vacuum evaporator (at 80 ^{o}C) to 35-% (w/v) dry matter content. Two coating solutions were thus prepared. The first solution contained a 30-% (w/v) addition of plasticiser (glycerol) and 3% (w/w) emulsifying agent (lecithin); this solution is designated “Coating 1”. Glycerine and lecithin were added to the thickened hydrolysate solution, and its pH was adapted to 10±0.1 at 70 ^{o}C with an addition of 5 mol L^{-1} NaOH; stirring was continued until all lecithin dissolved. The added plasticiser is necessary for improving flexibility of produced protective film. The second coating solution contained an additional 1-% (w/w) antioxidant (tocopherol); this solution is designated “Coating 2”. Tocopherol was added to the warm (40 ^{o}C) coating solution and under stirring (10 min) dissolved.

Formation and testing of coatings

Coatings on butter were produced with a brush on all sides of butter samples; coating solution was tempered to room temperature (23±1 ^{o}C). Two coats were made, with a time interval of 10 min in between. Thickness of the coating on butter samples was 0.26±0.07 mm. Impregnation of wrapping paper Testliner was carried out by applying coating solution with a brush in one layer on the back side of a paper sheet 25x18 cm large, and drying it in horizontal position in drier at 40±0.3 ^{o}C for 20 min. Samples of butter with coatings, without coats, and cakes of butter wrapped in original wrapping, in wrapping paper and in wrapping papers impregnated with tested coats were placed in refrigerator and stored at 6±1.5 ^{o}C and relative

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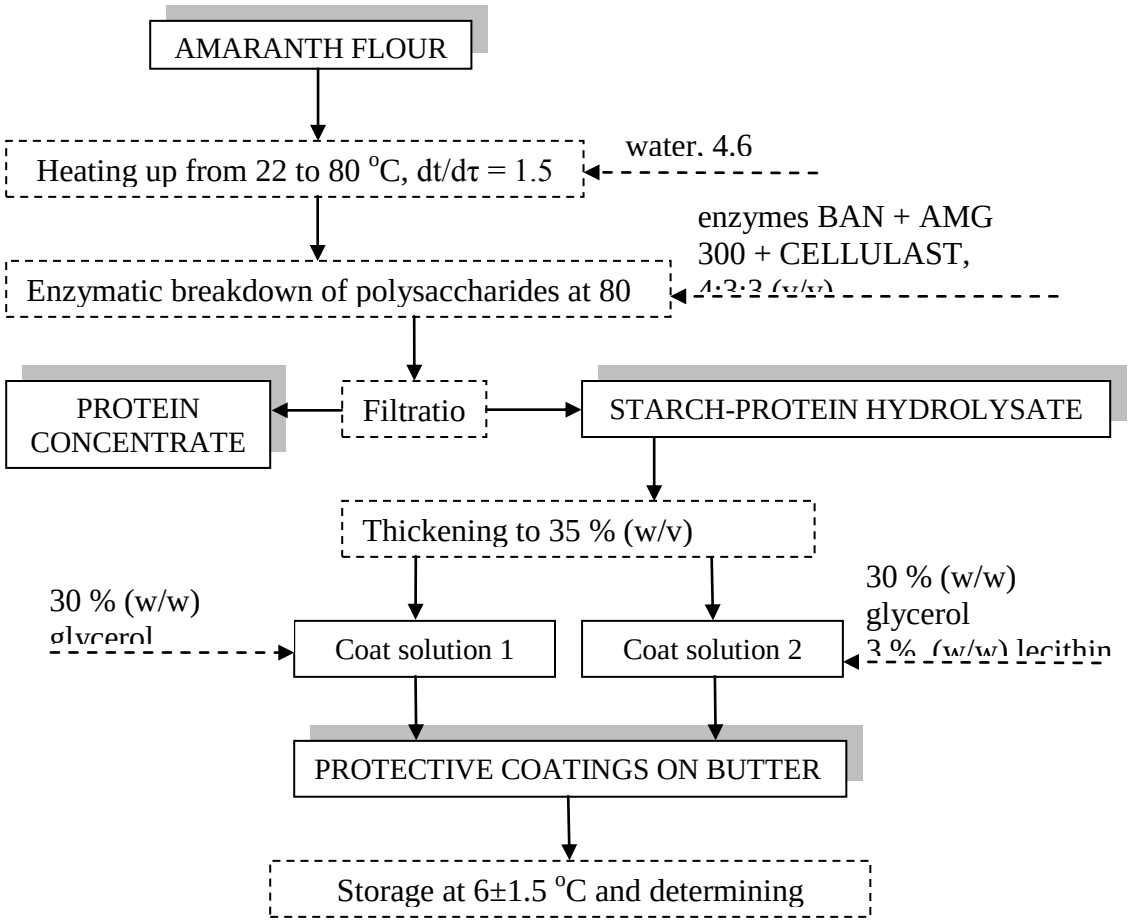
humidity 43±3 %. The refrigerator was opened five times a day for 5-minute periods in order to simulate conditions of current refrigerator daily usage. The determination performed on butter after 14, 21, 28, 42, 73, 91, 105, 119 and 126 days was peroxide value (PV) [25]. Each test was executed with three samples and calculation gave the arithmetic mean; standard deviation was ±5 %.

Figure 1: Flow chart of preparing coat solutions from amaranth flour and their application as coatings on butter



Preparation of films

Two films were prepared, the first contained 30 % (w/w) glycerol and 3 % (w/w) lecithin (the same as coating solution), and the second contained an extra 3 % (w/w) cross-linking agent – dialdehyde starch (DAS). Dialdehyde starch exerts a good cross-linking effect and is suitable cross-linking agent for foodstuff and pharmaceutical applications [26, 27]. Starch-protein hydrolysate was first thickened on vacuum evaporator (at 80 °C) to a 14-% (w/v) dry matter content. When preparing the first film, 30 % (w/w) glycerol and 3 % (w/w) lecithin were added to the thickened solution (300 mL). After adapting pH to 11 with an addition of 5 mol L^{-1} NaOH, lecithin dissolved and solution was stirred for another 10 min. Hot solution was cast onto a silicone plate measuring 27x21 cm. Drying at 35±1 °C for 72 hours produced a semitransparent

film 0.86±0.05 mm thick of light brown colour and glossy surface. Test specimens measuring 2x1.5 cm, of 4-cm diameter, were cut out of the film for further testing. A second film was prepared by similar procedure with a difference – after adapting pH of the mixture to 11, DAS was added to solution in small doses under constant stirring after which the solution was stirred another 10 min. Film containing added DAS had a brown colour.

Testing films

Solubility tests of film samples measuring 2x1.5 cm were conducted at a temperature of 25±0.1 ^{o}C. A sample of film was placed in glass weighing vessel, weighed and covered with 35 mL distilled water preheated to 25±0.1 ^{o}C. The glass weighing vessel was then placed in incubator. After the prescribed dissolution time (1, 3, 5, 10, 20, 40, 60, 80 and 100 min), non-dissolved fraction of film sample was separated by filtration, dried at 103±1 ^{o}C to constant weight and weighed. Determination was performed on three samples, results are their arithmetic mean. Water vapour permeability of films was investigated in accordance with ASTM E 96/ E96M – 10 [28]. The essence of this test is finding the loss in weight of water from a crucible enclosed by test specimen in a desiccator of determined relative humidity at constant temperature. Samples of film of 4-cm diameter are put on the rubber gasket of a crucible filled with distilled water 25±0.1 ^{o}C warm up to 1 cm under the edge. Another rubber gasket is then placed on the film and the crucible is enclosed. Immediately on closing, the crucible is covered with a ground cover glass and weighed on analytic balance (this weight is recorded as start of measurement). The ground cover glass is then removed and crucible with sample is placed in an environment of defined relative humidity (its levels for testing were 21 %, 50 % and 60 %). In 30-min intervals, the crucible is withdrawn from desiccator, covered with glass and weighed. The procedure is repeated until film sample is damaged or dissolved. Observing the moisture absorption of films (ability to take in water vapour) was conducted on same apparatus and at same temperature as observing water vapour permeability of films. The principle consists in exposing test specimen to an environment of saturated water vapour at constant temperature and constant humidity, and finding the increment in weight of film. For that reason, the crucible is closed with impermeable foil. The procedure is repeated until film sample is damaged. Temperature co-ordinates of characteristic peaks and mass loss of films were determined by differential scanning calorimetry (on instrument DSC 2010, TA Instruments, USA) in open Al crucibles and by thermogravimetric analysis (on instrument TGA Q500, TA Instruments, USA) in open Pt crucibles. In both cases a quantity of approx 5 mg of film 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 ^{o}C, dt/dτ 10 ^{o}C min^{-1}.

RESULTS AND DISCUSSION

Peroxide values

Dependency of peroxide value (PV) of butters on storage duration is indicated in Fig. 2. Initial PV level of newly purchased butters was 2.61 μg O_2 g^{-1} fat. From the course of PV curves

it is obvious that in the first 21 days since start of testing, differences between PV levels of investigated samples were not so marked (5.84-7.42 µg O_2 g^{-1} fat) except for butter with Coating 1, with which the lowest level of PV=4.70 µg O_2 g^{-1} fat was found. Rise in PV levels during investigated storage time was highest with butter samples without wrapper and with samples of butter in paper wrapper Testliner; differences between PV levels of these butter samples were not so marked. Rise in PV levels of butters kept in original wrapping during 50 to 100-days' storage was always higher than with butters having Coatings 1 and 2 and with butters wrapped in Testliner impregnated with Coatings 1 and 2. With storage duration up to 50 days, smallest rise in PV was found with butter sample with Coating 1. Between storage of 42 to 91-days' duration, let us note the very slight increase in PV of butters with Coating 2 and butters wrapped in Testliner paper impregnated with Coating 2 – the rise from level 6.98 µg O_2 g^{-1} fat or 6.76 µg O_2 g^{-1} fat after 42 days' storage to level 7.47 µg O_2 g^{-1} fat or 7.18 µg O_2 g^{-1} fat respectively after 91 days of storage. With butters treated in this manner, lowest PV levels of all investigated samples were found between 55 to 95-hours' storage. When comparing PV levels of butters around 75th day of storage, butter without wrapping is found to have approx. 2.2-2.4 times greater PV than butter with Coating 2 or butter wrapped in Testliner paper impregnated with Coating 2 (15.99 µg O_2 g^{-1} fat versus 7.2 µg O_2 g^{-1} fat or 6.77 µg O_2 g^{-1} fat). When focusing on the end of investigated storage duration (from 105 hours on), we find butters with Coatings 1 and 2 exhibit lowest levels of peroxide values. Butter wrapped in Testliner paper impregnated with Coating 2 displays somewhat lower levels of PV than butter kept in original wrapping; on the opposite, butter wrapped in Testliner paper impregnated with Coating 1 has slightly higher PV levels.

Solubility of films

Figure 3 indicates solubility of films prepared from coating solution in water at 25 ^{o}C. Difference in dissolution rate of films without added DAS and films containing a 3-% addition of DAS is particularly obvious between 5- and 80-mins' dissolution. While films without added cross-linking agent exhibited almost 87-% dissolution after 10 minutes of dissolving, with films containing 3 % DAS it was merely 50 %. After 20 min, 95 % film without added cross-linking agent was dissolved but with film containing 3 % DAS the level was only 61 %. After 100-min dissolution, both films were almost dissolved.

Water vapour permeability of films

Figure 4 indicates water vapour permeability of films at 25 ^{o}C. Films without added DAS (Fig. 4a) exhibited greatest water vapour permeability at relative humidity 21 % – after 2.5 hours testing

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Figure 2: Dependency of butter peroxide value on storage time

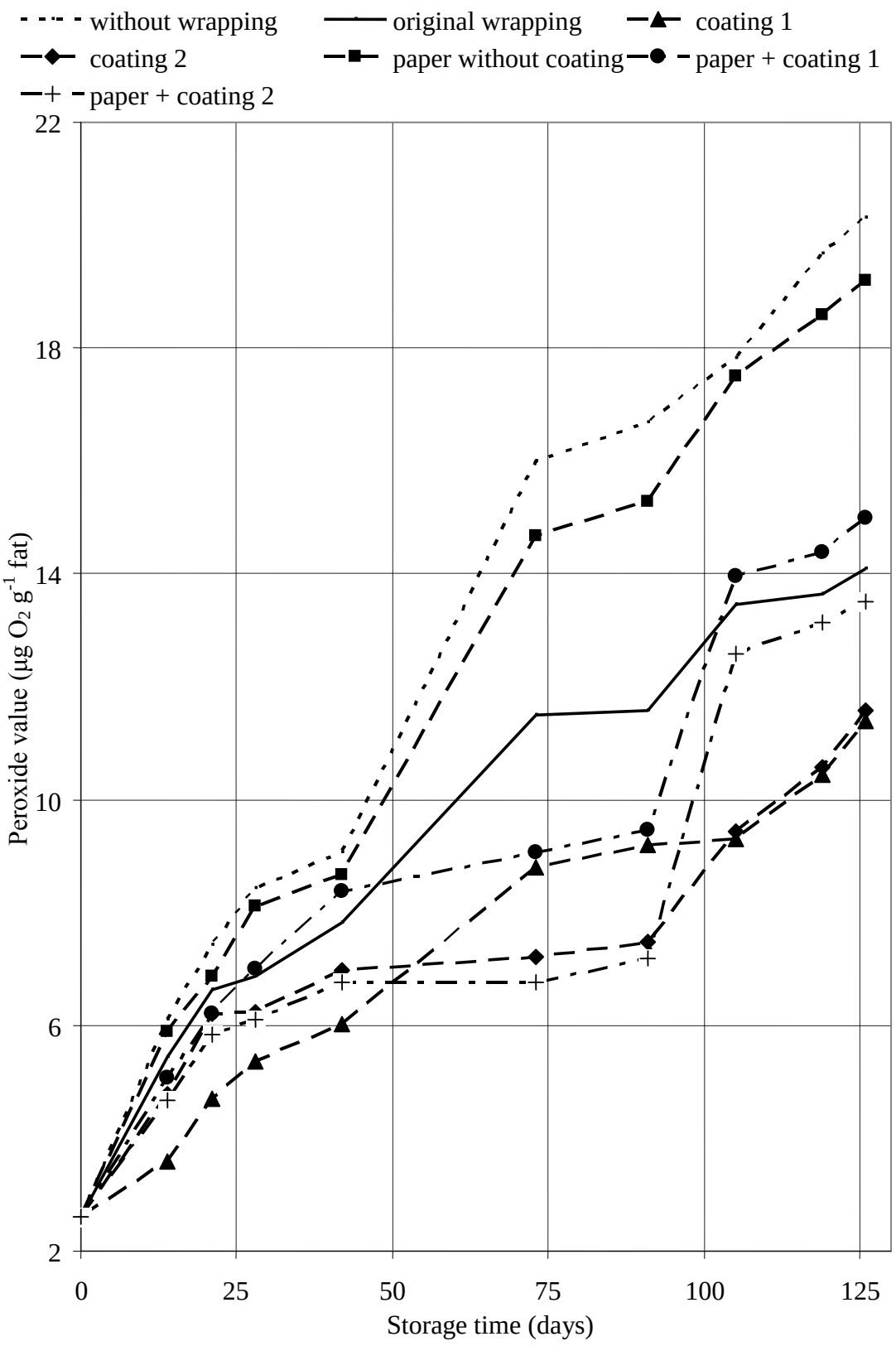

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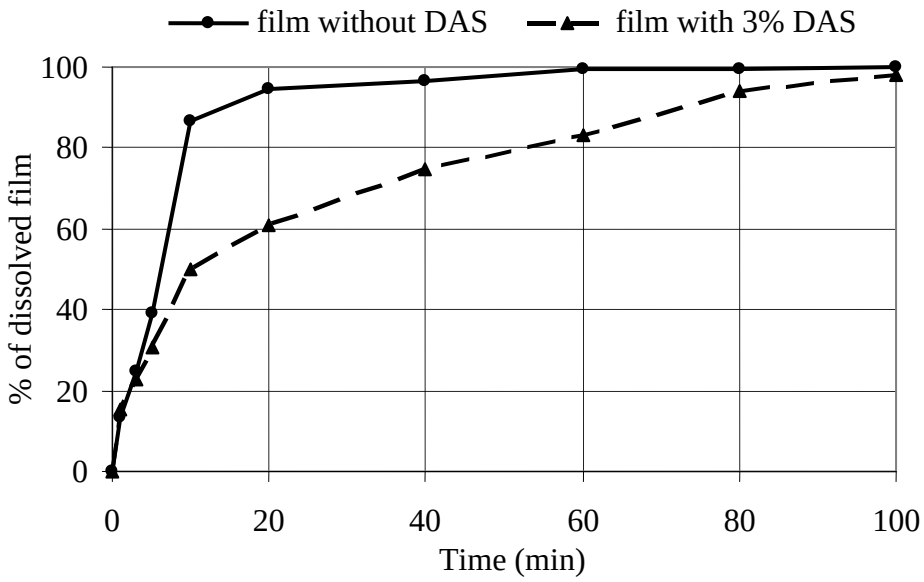
Figure 3: Solubility of films in water at 25°C



the level was 3.7 mg h^{-1} cm^{-2}. Film showed lowest water vapour permeability (max.1.0 mg h^{-1} cm^{-2}) at relative humidity 60 % and was damaged after 1.5 hours of testing. Similar tendencies were found with film containing 3 % added DAS (Fig. 4b), where highest water vapour permeability was recorded at relative humidity 21 % – after 2 hours of testing it was 4.2 mg h^{-1} cm^{-2}. Lowest water vapour permeability (1.3 mg h^{-1} cm^{-2}) was recorded at relative humidity 60 % after 3.5 hours of testing.

Moisture absorption

Figure 5 indicates moisture absorption of films at 25 °C. From graphs it is obvious that during first 7 hours the moisture absorption of films without added DAS and with 3-% addition of DAS is essentially the same. Highest moisture absorption (21.7 mg cm^{-2}) were found with film containing 3 % DAS after 6 hours; film was subsequently damaged. With film containing no DAS, moisture absorption increased to maximum level in up to 22 hours, and that was almost twice greater (38.6 mg cm^{-2}) than with film containing 3 % added DAS; film was subsequently damaged.

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Figure 4: Water vapour permeability of films at 25 °C; a) film without DAS, b) film with 3 % DAS

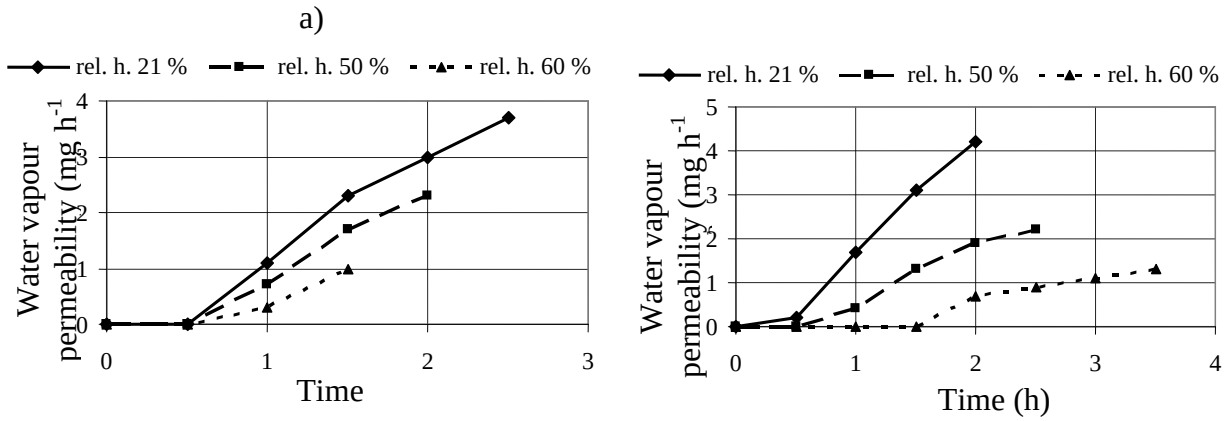
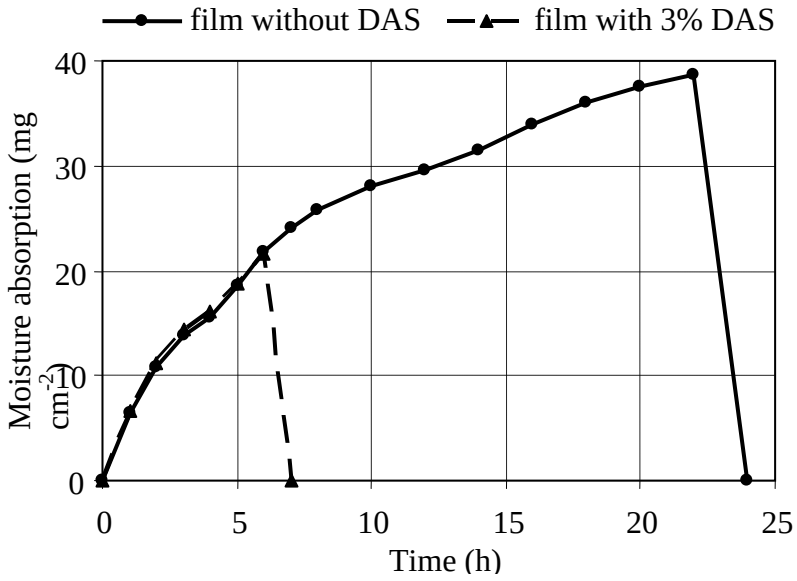


Figure 5: Moisture absorption of films at 25 °C

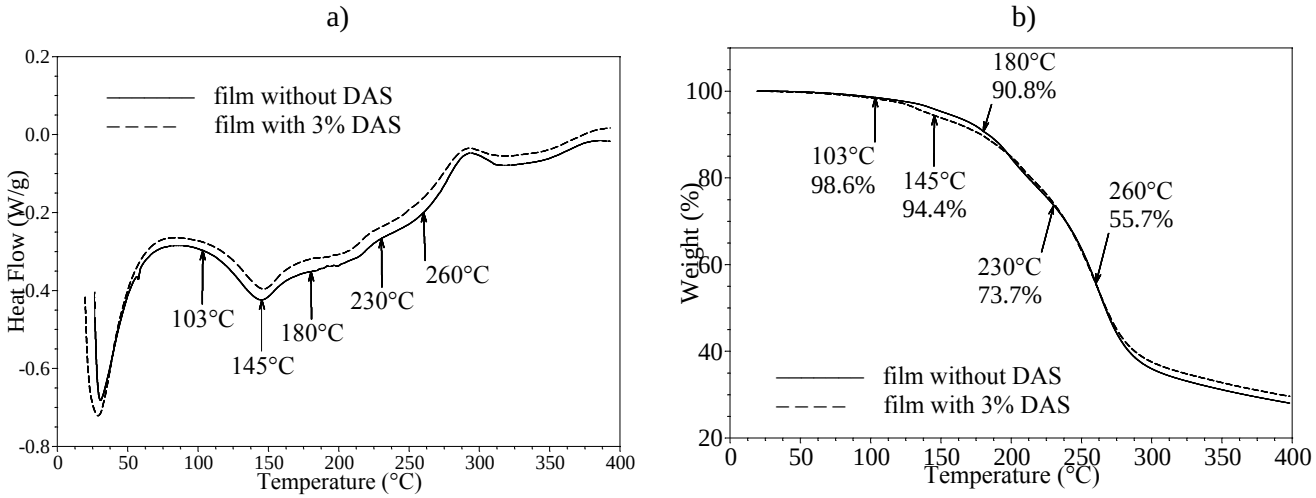


Thermal properties of films

Figure 6 indicates thermal properties of films with no addition and with 3-% DAS addition; the course of DSC curves (Fig. 6a) and TG curves (Fig. 6b) is essentially identical. When heating films in interval 20-103 °C, freely bound water is at first released, which is apparent on prominent endothermal peaks on DSC curves; this temperature interval on TG curves relates to a films weight decrease of 1.4 %. When heated above 103 °C, structurally bound water is gradually released from films, and temperatures around 145 °C relate to film melting (T_m) which is distinctly noticeable on the small endothermal peak on DSC curve. Gradual releasing of structurally bound water proceeds up to about 180 °C, which shows on TG curves by an approx. 9-% decrease in weight of films. At temperatures above 180 °C, evaporation of plasticiser (glycerol) from films already occurs. Thermal degradation of the film protein component sets in

at temperatures around 230 °C, which shows on TG curves by an approximately 26-% drop in weight of films. We arrived at a similar conclusion when studying thermal properties of films based on collagen hydrolysate with added dialdehyde starch, where the start of film thermal breakdown was found at temperatures between 230-240 °C [29]. At temperatures about 260 °C there is an approx. 44-% decrease in weight of films on TG curves, which is related to the already proceeding breakdown of film saccharide component. This find is in good agreement with literary data on breakdown of starch [30-32].

Figure 6: Thermal properties of films; a) DSC curves, b) TG curves



CONCLUSION

Protective coats on butter were produced by coating with a 35-% (w/v) solution of hydrolysate of amaranth flour. Two coating solutions were tested. Each contained a 30-% (w/w, per hydrolysate dry matter) addition of plasticiser (glycerol) and 3-% (w/w) addition of emulsifying agent (lecithin). Second solution contained an additional 1-% (w/w) antioxidant (tocopherol). Packing material also tested was wrapping paper of paper density 130 g m^{-2} and the same paper impregnated with tested coating solutions. Butter samples were stored at 6±1.5 °C and 43±3 % relative humidity, the parameter under study was peroxide value (PV). Butters processed with our coats were found with lower PV levels during cold storage than butter wrapped in standard wrappings. Between hours 73 and 91 hours of storage, butter with Coating 2 exhibited approx. 1.6 times lower level of PV than butter in standard wrapper. Positive influence of added antioxidant in Coating 2 was also recorded in the same time interval. Wrapping paper impregnated with Coating 2 also surpassed standard butter wrapping throughout storage duration. Coatings of starch-protein hydrolysate of amaranth flour possess very good barrier properties against oxygen. Coating solutions were also employed to prepare films – without added cross-linking agent and with 3-% addition of cross-linking agent (dialdehyde starch, DAS). Films with added DAS dissolve in water more slowly and display even twice higher moisture absorption. Tested films have T_m levels around 145 °C and are thermally stable to about 180 °C, when gradual evaporation of plasticiser sets in. Thermal breakdown of films occurs at temperatures above 230 °C.

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