

## Decelerating weight loss of harvested strawberries by applying edible coatings based on starch-protein hydrolysate of Amaranth flour

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(Received: August 10, 2009; Accepted: October 01, 2009)

### ABSTRACT

The work studied weight losses of harvested strawberries provided with protective edible coating. Coatings were produced by dipping strawberries in 50 % (w/w) starch-protein hydrolysate of Amaranth flour. Tests investigated influence of added glycerol (10 or 30 %) and dialdehyde starch (1 or 4 %) on barrier properties. A protective film was produced on the strawberries after drying 5 min. at 23, 30 or 40 °C. Strawberries were stored at 7±1.5 °C or 23±1.5 °C. The lowest losses in mass caused by humidity evaporating were provided by a protective coating produced with 30 % added glycerol at both storage temperatures.

**Key words:** Amaranth, coating, starch-protein hydrolysate, storage, strawberry, weight loss.

### INTRODUCTION

Fruit and vegetables are biologically active even after harvesting. Their principal biological procedure is respiration and evaporation of moisture, which leads to losses in mass, surface drying and altered appearance. If fruit and vegetables are to be kept fresh for a longer period of time, such changes have to be minimized. Retarded drying of fruit and vegetables is particularly important with products imported from distant countries. One mode of treating products is applying a suitable covering of biopolymer, mostly in the form of thin coating. These coatings possess the advantage of being edible and biodegradable and also improve product appearance, above all gloss<sup>1</sup>.

The chief component of fruit and vegetables is water, comprising 75-90 %wt of fruit and 25-95 %wt of vegetables and together with soluble substances makes up fruit juice. Saccharides, particularly glucose and fructose, are primary constituents in fruit. Sucrose is more sparsely represented. The building material of fruit cellular walls is cellulose and hemicelluloses. In fruit, nitrogenous substances are present in minute quantities, whereas in vegetables they may attain as high as 4 %. Minority components are mineral substances, vitamins, pectins, aromatic and gustatory substances, pigments, organic acids and tannins. In order to keep raw materials in an optimal condition, vital processes should be moderated as quickly as possible and micro-organism activity suppressed both by adapting the environment as

well as by taking potential disinfectant measures<sup>2</sup>. Influences acting on food in storage are climatic, biological, hygienic and mechanical. Climatic influences involve the effect produced by air temperature and humidity which is dominant for preserving food nutritive value. Temperature conditions the rate of proceeding chemical and biological processes as well as micro-organism activity – elevated temperature considerably accelerates their course.

Apart from paper and cardboard, traditional packaging materials for food were made from non-renewable sources<sup>3</sup>. Nevertheless, since the nineties of the last century, interest in employing renewable sources for producing packaging materials started increasing<sup>4,5</sup>. Animal proteins employed most are collagen, gelatin, casein, whey protein isolate, keratin; vegetable proteins are then maize zein, wheat gluten, soya protein isolate and others<sup>6,7</sup>. Most significant of polysaccharides are starch, cellulose and their derivatives; also used are waxes<sup>8</sup>. Packaging materials for food based on natural polymers have to meet same criteria as those required for conventional packing materials made from synthetic polymers.

With a view to the already mentioned chemical and biological procedures under way in fruit and vegetables after harvesting, it is essential that their packings have certain permeability for oxygen, CO<sub>2</sub> and water vapor so that "fruit suffocation" and deterioration of quality do not occur. On the contrary, high water vapor permeability is undesirable and leads to losses in mass<sup>9</sup>. Sensory properties of films and coatings are of no lesser importance – they should be without smell or foreign flavor and, if possible, transparent. Some studies confirm that apart from reducing mass losses and improving fruit and vegetable appearance, a suitable coating may reduce rate at which fruits spoil and go soft by 25-80 %<sup>10,11</sup>. The important point is choosing a suitable mode of producing the coating (film) on food product, which may be achieved by coating the food with a solution, spraying solution on the food or dipping food in solution. In some cases, painted coatings exhibit better mechanical properties and barrier properties against water vapors than films applied by spraying<sup>12</sup>. Frozen strawberries treated with coating based on starch

containing a high amylose content exhibited lower losses in humidity and better preserved their original shape on defrosting<sup>13</sup>. Chilled fruit may also be treated in similar manner. For example, coatings made from lactic protein and vegetable oil derivatives markedly reduce loss in humidity and prevent oxidative browning of apple slices. Coatings of sodium caseinate and stearic acid increase storage stability and reduce loss of water in peeled carrot<sup>14,15</sup>.

### **The objective of the study**

In our previous research we have been dealing with enzyme technology for separating starch and protein from amaranth grain. Separated amaranth protein concentrate may be effectively applied in the production of functional food and quality supplements. The utilisation of a starch-protein hydrolysate (i.e. by-product of preparation of amaranth protein concentrate) remains an open issue. *The aim of this paper was to evaluate the efficiency of a starch-protein edible protective coating based on amaranth flour on decelerating water loss of strawberries stored at 7 and 23 °C.*

### **MATERIAL AND METHODS**

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

#### **Stock solution of enzymes**

Liquefaction of starch 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 (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 liters 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 liters enzymes per 1,000 kg flour dry matter).

### 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 Sigma Aldrich Co, U.S.A. (Product No G9012) and NaOH p.a. grade was supplied by Petr Lukes (The Czech Republic).

### Apparatus and equipment comprised

Water bath GFL 1003 (Germany), drier Memmert UPL 400 (Germany), vacuum evaporator Laborota 4000 (Heidolph Instruments, Germany), magnetic stirrer IKA RCT basic (Germany), electronic balance Kern 770/GS/GJ (Germany), thermo-hygro meter Huger PTH-338 (Germany), pH-meter WTW pH 526 (Germany), refrigerator Samsung Calex C 180 (The Czech Republic), Polarimeter Krüss P1000 (Germany) with polarimetric tube 200 mm.

Strawberries of first-class quality were purchased from fresh deliveries. Strawberries were selected in such manner that their size was approximately the same for all tests. Tested systems comprised 5 types of coating solutions and 3 different modes of applying protective coating on strawberries (see below), strawberries were stored at two different temperatures (7 and 23 °C); parallel tests were always performed with 3 pieces of strawberries; 6 strawberries were comparative–references– without protective coating (3 pieces for 7 °C and 3 pieces for 23 °C). In all, 96 pieces of strawberries (5×3×2×3 + 6) were thus selected for testing. Strawberries were washed with clean water, blotted dried with paper napkins and immediately processed.

### Preparation of starch-protein hydrolysate of amaranth flour

Enzymatic breakdown of polysaccharides (starch and fibre composing cellulose) 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 a ratio of 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 a water bath and stirring of its contents with a shaft stirrer began (600 rpm), heating proceeded at a rate of 1.5 °C min<sup>-1</sup>

until a temperature of 80 °C was attained. A 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 °C for 5 min. Afterwards the mixture was cooled (under running cold water) to room temperature. Starch-protein hydrolysate was subsequently separated from solid fraction (amaranth protein concentrate) by filtering through polyamide cloth (pore diameter 150 μm) folded eightfold. Enzymatic breakdown resulted in 83 % conversion of starch and 32 % conversion of proteins into soluble fraction. Starch content was determined according to standard CSN 56 0512-16<sup>16</sup>. This determination is based on transforming starch into soluble starch by the action of diluted HCl while warm. After clarification, soluble starch was determined by polarimetry. Coarse proteins were determined by multiplying nitrogen content by conversion factor 5.70. Total Kjeldahl nitrogen was determined by mineralizing a sample of flour by boiling for 30 min (at approx. 440 °C) in sulphuric acid with added catalyst. 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<sup>17</sup>.

### Preparation of coating solutions

Starch-protein hydrolysate was thickened in a vacuum evaporator (at temperature not exceeding 80 °C) to 50 % (w/w) dry matter content and subsequently cooled to room temperature (23±2 °C). A total of 5 coating solutions were prepared. The first coating solution contained no additives – its designation is CS-50%H. The second and third coating solution contained 10 % or 30 % (per hydrolysate dry matter) added plasticiser – glycerol (GLY). Glycerol was added to cool 50 % hydrolysate solution and stirred at room temperature for 30 minutes. These coating solutions were designed as CS-50%H+10%GLY and CS-50%H+30%GLY. The fourth and fifth coating solution contained 1 % or 4 % added cross-linking agent – dialdehyde starch (DAS). The pH level of cooled 50 % hydrolysate solution was adjusted to 11±0.1 by adding 5N NaOH (consumption approx. 2.5 ml). DAS was then added and dissolved under stirring at room temperature for 30 minutes. Designations of these coating solutions were CS-50%H+1%DAS and CS-50%H+4%DAS.

### Producing protective coatings and observing water loss of strawberries in storage

Strawberries were put in a stainless steel sieve, immersed for 30 seconds in coating solution and on withdrawal let to drip for 60 seconds. A thin protective film (thickness  $H \approx 100$  nm) was produced on sample strawberries after drying 5 minutes in forced ventilation drier at temperatures of 23, 30 or 40 ( $\pm 0.5$ ) °C. At start of test, after a protective coating on the fruit was made, sample strawberries were weighed on analytical balance; reference strawberry samples (not coated) were also weighed. Half of the strawberry samples were stored in the laboratory at room temperature ( $23 \pm 1.5$  °C) and at relative atmospheric humidity  $48 \pm 2\%$  without direct impingement of sunlight, and the second half of samples were stored in refrigerator at  $7 \pm 1.5$  °C and relative humidity  $40 \pm 3\%$ . After 18, 42, 66, 138, 162, 186, 234, 306, 330, 378 and 402 hours (storage at

7 °C), and/or after 6, 21, 29, 46, 53, 70, 78, 140 and 164 hours (storage at 23 °C), strawberry samples were weighed on analytical balance; prior to weighing, samples stored in refrigerator were left to temper at room temperature for 30 minutes. Calculation determined the percent decrease in strawberry sample mass (related to mass of strawberry samples at start of test). Each test was conducted with three pieces of strawberries and the arithmetic mean was calculated; standard deviation was  $\pm 4\%$ .

### RESULTS AND DISCUSSION

Decreases in mass of strawberries (non-coated as well as with tested coatings) dependently on storage time at storage temperature 23 °C are indicated in Fig. 1. From graphical dependencies it is obvious that strawberries treated with all five

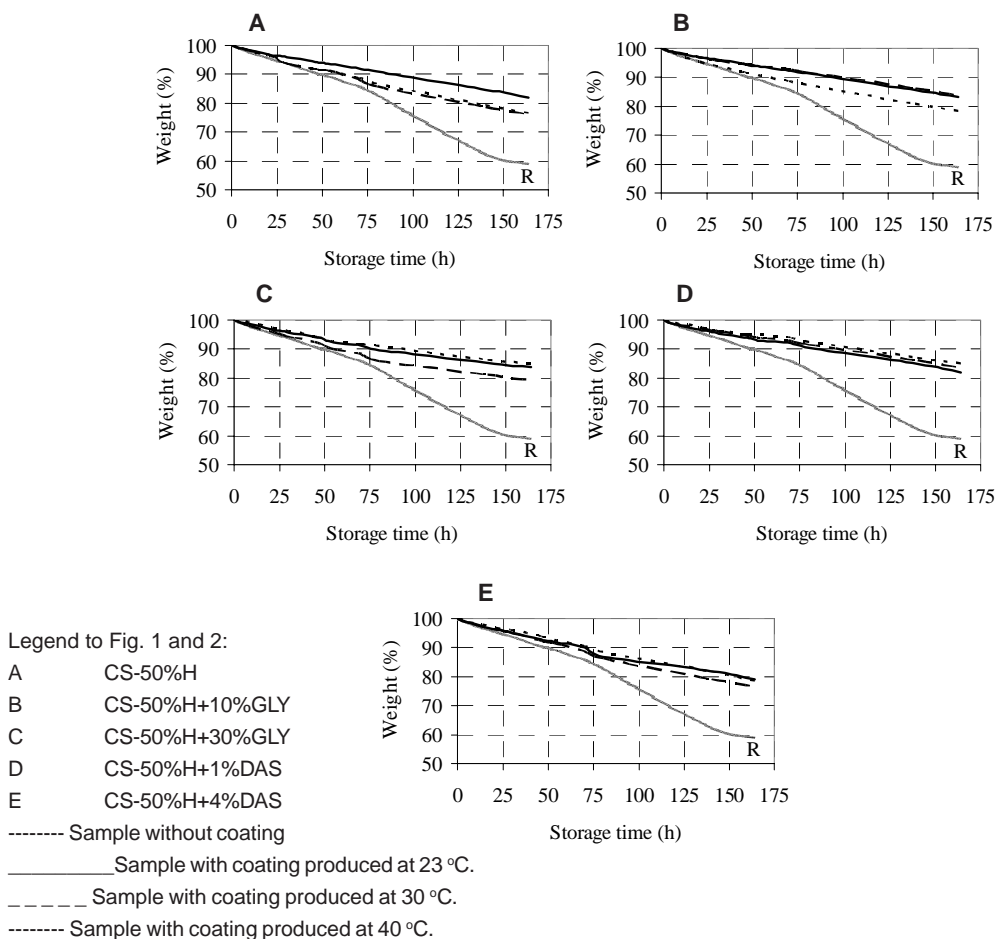
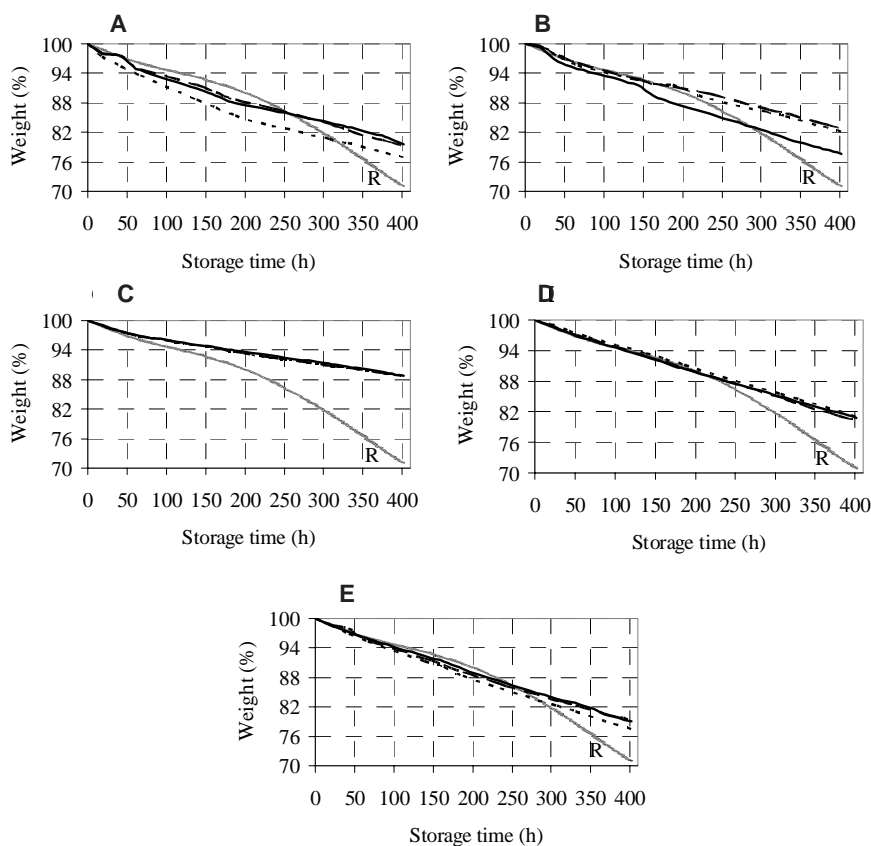


Fig. 1: Losses in mass of strawberry samples stored at 23 °C

tested coatings including 3 different modes of producing coat (5-minute drying at 23, 30 or 40 °C) displayed lower mass losses than strawberries with no coating. With coating CS-50%H (Fig. 1-A), let us first note the striking difference in mass loss rate of non-coated (reference) and coated strawberries. During the first approx. 50 hours of storage, difference between tested samples is not very noticeable, but after 100 hours of storage more distinct differences may already be discerned – while mass loss of strawberries without coat was approx. 25 %, with coated strawberries it attained 11-17 % (depending on mode of producing coat). After 150-hour storage, difference in mass loss is much more obvious – strawberries without coating lost 40 % mass while strawberries with a coat fixed at 23 °C lost approx. 17 % mass. Let us also note the difference in mass loss rate of strawberries in dependence on mode of producing coat. Strawberries with coat fixed at 23 °C showed lower mass losses during the storage time under study than strawberries with coatings fixed at 30 or 40 °C.

Similar positive results were obtained with coats having added glycerol – CS-50%H+10%GLY (Fig. 1-B) and CS-50%H+30%GLY (Fig. 1-C). In the case of a 10 % GLY addition, smallest mass losses were found with strawberries having coatings fixed at 23 or 30 °C (moreover being almost identical) – after 100-hour storage an approx. 10 % mass loss was recorded (strawberries without coat showed an approx. 25 % loss) and after 150-hour storage it was an approx. 15 % mass loss (strawberries without coat showed an approx. 40 % loss). Very similar results are achieved with coat containing 30 % glycerol – CS-50%H+30%GLY (Fig. 1-C). The best coating here appeared to be that fixed at 40 °C: it showed an approx. 10 % loss in moisture content (reference displayed a 25 % loss) after 100-hour storage, and an approx. 14 % loss in moisture content (reference displayed 40 %) after 150-hour storage. Excellent results were also achieved with coats containing 1 % DAS additions – CS-50%H+1%DAS (Fig. 1-D). Regardless of mode of



**Fig. 2: Losses in mass of strawberry samples stored at 7 °C**

**Table 1: Composition of Amaranth flour**

Parameter	Value (%)
Dry matter	86.9
Starch in dry matter	65.8
Total Kjeldahl nitrogen in dry matter	2.8
Coarse proteins (nitrogen × 5.70) in dry matter	16.1
Fat in dry matter	9.8
Fiber in dry matter	4.9
Inorganic solids in dry matter	3.6

fixing coat, mass losses of strawberries treated with this coating belonged to the lowest. Despite that, the coat fixed at 40 °C appeared as optimal: the loss recorded after 100-hour storage was slightly below 10 % moisture content of strawberries (reference displayed a 25 % loss), and loss recorded after 150-hour storage was slightly less than 14 % moisture content (reference displayed 40 %). Strawberries treated with a coat containing 4 % added DAS (CS-50%H+4%DAS, Fig. 1-E) did not show such low mass losses as in the case of a coat containing 1 % added DAS.

Losses in mass of strawberries without coating and with tested coatings, dependently on storage time and storage temperature of 7 °C, are indicated in Fig. 2. Strawberries coated with CS-50%H (Fig. 2-A), during 2/3 of their storage time (coatings fixed at 23 or 30 °C) and 3/4 of their storage time (coatings fixed at 40 °C), displayed higher mass losses than strawberries without coating. It was similar with strawberries coated with CS-50%H+10%GLY (Fig. 2-B) which, when their coating was fixed at 23 °C, exhibited during 3/4 of their storage time mass losses higher than strawberries without coating. Strawberries having coats fixed at 23 or 30 °C recorded lower mass losses only after approx. 175-hour storage. Strawberries provided with coats containing 1 % (Fig. 2-D) and 4 % added DAS (Fig. 2-E) show losses in mass almost identical to mass losses of strawberries without coating. Only at end of storage time (approx. from 330 hours on), coated strawberries recorded lower mass losses, in an interval of 3-8 %. Best barrier properties against moisture were found with coat CS-50%H+30%GLY (Fig. 2-C). In addition, this type of coating did not

present almost any differences relative to mode of coat preparation – strawberries treated with coating fixed at three tested temperatures (23, 30 or 40 °C) demonstrated almost identical mass losses during entire time of storage. After 200-hour storage, differences in strawberry mass losses are still not too significant – the difference between strawberries without coating and with coating attain approx. 4 %. After 300-hour storage, non-coated strawberries revealed an 18 % loss in mass, but coated strawberries displayed only approx. 9 %; after 400-hour storage, loss in mass was 29 % with strawberries having no coat and merely 12 % with coated strawberries. It may be generally said that strawberries during storage longer preserved their natural texture, softening and first symptoms of fruit drying out were postponed, and gloss of strawberries was enhanced.

## CONCLUSIONS

Enzyme starch-protein hydrolysate of amaranth flour, containing in dry organic matter approximately 8.6 % of protein and 91.4 % of hydrolysed starch provides systems with low viscosity allowing its usage for producing coatings. Tests confirmed that applying a protective and edible polymeric coating produced from a 50 % solution of amaranth flour starch-protein hydrolysate containing plasticiser (glycerol) or cross-linking agent (dialdehyde starch) enables to achieve lower mass losses of strawberries occurring through evaporation of humidity. The lowest mass losses recorded with strawberries stored at room temperature (23 °C) was found with samples treated with a coating containing 30 % glycerol (GLY) or added 1 % dialdehyde starch (DAS). For as much as mass losses of both coats were almost the same, we recommend to employ in practice the coating with 30 % added GLY as glycerol is economically much more convenient than dialdehyde starch. Strawberries treated with a coating having 30 % added glycerol exhibited 4 % lower mass loss after 50 hours of storage than non-coated strawberries, while this difference already attained 15 % after 100-hour storage and even 26 % after 150-hour storage. Lowest mass losses of strawberries stored at cold storage temperature (7 °C) were achieved after their treatment with a coat containing 30 % added glycerol. After 200-hour storage, strawberries with

this coating displayed a mass loss 4 % lower than strawberries without coating, and the difference reached 9 % after 300-hour storage and 17 % after 400-hour storage. Data on the effect of edible biopolymer coating on microbiological quality of strawberries are also essential for a reliable assessment of the effectiveness of the treatments applied and need to be studied in the ongoing research.

#### ACKNOWLEDGMENTS

The authors would like to thank to Ministry of Education of The Czech Republic for financial support to this work executed under MSM Grant N<sup>o</sup>. 7088352102.

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