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Research Article

Using soy protein isolate/glucose edible films to protect fish oil from lipid oxidation

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Abstract

Edible soy protein isolate (SPI)-based films were prepared using hot film-forming solutions containing SPI, glycerol and either glucose or sucrose. As a result of the Maillard reaction SPI/glucose films were darker, higher in tensile strength, lower in water vapour transmission rate, oxygen gas transmission rate, solubility in disruptive solvents (sodium dodecyl sulphate plus β-mercaptoethanol) and swelling index than SPI/sucrose films. Both films were tested for potential protective effects on fish oil oxidation during storage. Compared to oils covered with SPI/sucrose films, results indicated a higher oxidative stability of oil covered with SPI/glucose films. This protection was attributed mainly to additional protein cross-linking within SPI/glucose films that had affected their physical properties, and to a lesser extent on the oxygen scavenging activities of Maillard products within the films.

Keywords: Soy protein isolate/glucose, edible films, lipid oxidation, fish oil, Malaysia

Introduction

Marine lipids have received increasing attention for the past decade due to their beneficial health effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on diseases such as cardiovascular diseases [1], rheumatoid arthritis [2] and Crohns disease [3]. The most obvious way to secure an increased intake of these healthy fatty acids in the population is via increased dietary fish consumption or partaking of fish oil capsules. Due to their unsaturated nature, n-3 polyunsaturated fatty acids (PUFA) are highly prone to accelerated oxidative rancidity. Lipid oxidation gives rise to the formation of undesirable off-flavours (e.g. fishy and rancid off-flavour)

and unhealthy compounds such as free radicals and reactive aldehydes [4]. Therefore, a prerequisite for successful development of food enriched with these PUFA is to prevent the occurrence of the lipid oxidation.

The Maillard reaction or non-enzymic browning is known to occur in thermally processed food containing reducing sugars and protein. This reaction may contribute to desirable flavour and colour development of food as well as anti-oxidative activity [5, 6]. There has also been interest in the use of the Maillard reaction to enhance the functionality of proteins [7]. Several studies have reported the influence of the Maillard reaction on texture modification of food containing a high level of protein [8, 9, 10, 11]. During the Maillard reaction, the Maillard cross-linking is formed and this has enhanced the functional properties of various protein-based products [8, 12, 13].

It was thus considered that it may be possible to employ the Maillard reaction and crosslink to protect against lipid oxidation. Drush, *et al* [14] demonstrated the use of glycated casein to stabilize lipid oxidation in fish oil. In fact, glycated proteins have already been used for microencapsulation purposes [5, 6, 15]. Another approach to stabilize lipid oxidation in fish oil was demonstrated by Gan, *et al* [16] who attempted to use microbial transglutaminase, ribose or a combination of both as crosslinking agents in microcapsules containing fish oil. During storage, microcapsules prepared with ribose had longer shelf life as compared to other microcapsules. This may be due to the release of anti-oxidative Maillard reaction products during heating and storage and a slower rate of gas permeability through the capsules [16]. Ribose however is very expensive and produces excessive levels of Maillard browning to the microcapsules. One alternative is to use less active and cheaper reducing sugars, such as glucose, to modify physical properties of protein films [17]. Since Maillard crosslink could be formed within soy protein isolate films [17], the modified films could then be used to protect fish oil during storage. This approach is different from those that used microcapsule systems [14, 17] since the oil is physically separated from the Maillard system. There is no published work on the use of the Maillard-modified SPI films to retard lipid oxidation of oil.

The objective of this study was to test the applicability of SPI/glucose films as a potential edible packaging material to protect high omega three fish oil against lipid oxidation.

Materials and Methods

Materials

Soy protein isolate (SPI) powder (PP_{TM} 500E) with 90% protein content was obtained from Protein Technologies International (St.Louis, USA). High omega 3 fish oil capsules were purchased from a local pharmacy. Glucose, sucrose, glycerol and Folins reagents were purchased from Fluka Chemical Corporation (Busch, Switzerland). Bovine serum albumin (A-7030) was purchased from Sigma Chemical Co. Ltd. (St Louis, USA). Other chemicals were obtained from BDH Chemical Co. Ltd. All chemicals used were of analytical grade.

Preparation of SPI film

SPI films were prepared by a wet casting method, using a film-forming solution [18] prepared by dispersing 10 g SPI, and 1.0 g glycerol in 100 ml distilled water containing 3.2 g sugar (glucose

or sucrose). Drop-wise addition of 1 N NaOH was used to adjust the pH of the solution to 11.0 ± 0.1. After pH adjustment, the mixture was homogenized using a Labor Technic Ultraturrax homogenizer spinning at 3000 rpm (2330 *g*) for 1 min before conditioning for 30 min at 90 ± 2 ºC in a water bath with shaking. The conditioned solution was then kept in a water bath at 90 ± 2 ºC for 8 hrs. It was then strained through a grade 40 cheese cloth (Fisher Scientific, U.K.) and left to dry for 48 hrs on casting plate (dimension: 26 cm inner frame, 30 cm outer frame) in an air-conditioned room (~ 20 ºC). After drying, the films were cured at 95ºC for 24 hrs in a drying oven (Memmert, USA).

The films were conditioned in a relative humidity environment of ~ 50% (room temperature ~ 25 ºC) using a saturated solution of calcium nitrate salt for 48 hrs, then kept in polyethylene bags until used. Testing of the films was carried out within 72 hrs of production.

Physical analysis of films

Film thickness was measured using a micrometer (No. 7326, Mitutoyo Manufacturing Co. Ltd. Tokyo, Japan) to the nearest 0.01 mm.

The linear swelling test was performed according to the methods developed by Murray [19], that measured the change in diameter of a disc-shaped film during immersion in water. This provides an indirect assessment of cross-linking density of films made from polymeric materials.

L value of films was measured using a Tristimulus Colorimeter based on the Hunter Lab colour scale (Model: D25-PC2 Hunter Associate Lab, VA, USA) standardized with a standard white tile with reflectance values of X=83.24, Y=85.23 and Z=100.92.

Tensile strength was measured using a texture analyzer model TA.XT2 multi Taskin-10X with a 250 N load cell (Stable Micro System Ltd). A rectangular sample measuring 1.50 cm x 10 cm was used. A grip separation of 50 mm and a crosshead speed of 500 mm/min were used throughout [20].

Water vapor transmission rate (WVTR) values were determined gravimetrically according to the methods of Butler, *et al* [21].

Oxygen gas transmission rate (OGTR) was determined with a MOCON unit (Ox-Tran 100, Modern Control Inc., Minneapolis, MN) according to the standard method [22]. Testing was performed at 0% relative humidity and 23ºC with controlled temperature recirculating bath connected to the OX-Tran 100. Testing was monitored as described by Gennadios, *et al* [23].

Protein solubility in disruptive solvents

Film samples were homogenized in a warring blender running at high speed for 30 – 40 s. 0.5 g of the homogenized particulate was extracted with 10 ml of 1.0 % sodium dodecyl sulphate plus 1% β-mercaptoethanol with shaking for 14 hrs. The mixture was then centrifuged at 3500 rpm (2330 *g*) using a Kubota 5100 centrifuge (Kubota Corporation, Tokyo, Japan) for 20 min to remove undissolved materials and filtered through a Whatman filter paper No. 4. An aliquot of the supernatant was sampled for protein determination using the method of Lowry, *et al* [24].

Storage studies

Each capsule of high marine lipid concentrate VitaEPA or MaxEPA fish oil contains 1 g (1000mg) of long chain (20-22 C) polyunsaturated natural marine triglycerides which is equivalent to 300 mg of Omega 3 marine triglycerides, of which 180 mg is Eicosapentaenoic Acid (20:5 ω 3 EPA) and 120mg is Docosahexaenoic Acid (22:6 ω 3 DHA).

The applicability of the films in model systems were tested using high omega three fish oil during a storage study performed at 40°C for 30 days. 4 ml of fish oil was injected into a weighing boat (Length = 4.3 cm, width = 3.3 cm, depth = 1.2 cm) using hypodermic micro-syringe. The oil samples were covered with either SPI/glucose or SPI/sucrose films and the four sides of the frame of the weighing boat were sealed by using double sided masking tape and aluminum foil. Accelerated storage was performed in a Carbolite oven equipped with air blower ventilation set at 40^0C. Every three day interval three weighing boats containing oil samples covered with SPI/glucose and SPI/sucrose films were withdrawn and analyzed for thiobarbituric acid (TBA) value. Every seven day interval three weighing boats containing oil samples covered with SPI/glucose and SPI/sucrose films were withdrawn and analyzed for docosa-hexaenoic (DHA) and eicosa-pentaenoic (EPA) acids content.

Thiobarbituric acid values were determined according to AOCS Official Method Cd 19-90 [25]. Determination of Eicosapentaenoic Acid (20:5 ω 3 EPA) and Docosahexaenoic Acid (22:6 ω 3 DHA) content of the fish oil was performed as per AOCS Official Method Ce 1b-89 using gas chromatography [26]. Methyl ester was prepared according to transesterification method by [27].

Data analysis

All measurements except for determination of OGTR were carried out using triplicate samples. Results were expressed as mean ± standard deviation.

Results and Discussion

The barrier properties of soy protein isolate (SPI) may be improved by introducing additional covalent bonds that enhance protein cross-linking. The enhancement of SPI films by using the Maillard reaction and curing treatment has been shown by Goh, et al [17], however there has been no study to demonstrate the usability of the enhanced films for protection of sensitive materials such as fish oil. In this study, a cheap reducing sugar (glucose) or non-reducing sugar (sucrose, as control) was incorporated into soy protein isolate solutions and heated at 90°C. SPI films were formed using the heated solutions by wet casting methods and cured at 95°C as previously described [17].

Physical properties of the films such as thickness, colour (lightness), tensile strength, water vapour transmission rate, oxygen gas transmission rate, solubility in disruptive solvents and swelling index were assessed (Table 1). Both types of film had thickness of ~ 0.18 mm. Colour measurement was carried out in order to determine the colour formation that is known to be caused by Maillard reaction. SPI/glucose films were brown in colour with L value of ~ 25. On the other hand, the L value of SPI/sucrose films was ~ 66, and the films were yellowish in colour. The formation of brown colouration within the SPI/glucose films suggests the occurrence of the Maillard reaction in the film-forming solutions and continued to occur during curing [17]. Cured

protein films formed using heated solutions containing glucose and soy protein isolate (SPI/glucose film) showed higher tensile strength, lower water vapor transmission rate and lower oxygen permeability as compared to those produced using SPI and sucrose (SPI/sucrose film) (Table 1). The assessments of protein solubility in disruptive solvents (1% SDS + 1% β-mercaptoethanol) found lower solubility values of SPI/glucose films, thus indicating the occurrence of Maillard crosslink [7, 17] within SPI/glucose films. These are additional covalent bondings that could not be completely solubilized in the disruptive solvents [7]. The swelling index of the SPI/glucose films was also lower than SPI/sucrose films, thus further supporting the different properties of these films. The higher tensile strength, lower solubility and swelling index of SPI/glucose films were attributed to the occurrence of Maillard network that increased the density of the protein film and slowed down the permeability of gases and water vapour (Table 1).

Table 1: Comparison of physical properties of soy protein isolate films.

Parameters	SPI/glucose film	SPI /sucrose film
[a]mean oxygen gas transmission rate ($cm^3\ m^2\ d^{-1}$)	0.05 ± 0.00	0.82 ± 0.00
[b]mean water vapour transmission rate (g/m^2s)	$1.60 \times 10^{-3} \pm 0.01 \times 10^{-3}$	$3.60 \times 10^{-3} \pm 0.01 \times 10^{-3}$
[b]mean tensile strength, (MPa)	11.9 ± 0.3	5.4 ± 0.1
[b]mean swelling index (%)	140 ± 2	240 ± 1
[b]mean protein solubility in 1% SDS + 1% β-mercaptoethanol, (%)	15.5 ± 2.3	79.5 ± 1.6

[a] ± Standard Deviation of replicate samples, [b] ± Standard Deviation of triplicate samples

In the storage study, the TBA value of uncovered oil and oil covered with SPI/sucrose films increased sharply during the first 9 and 12 days of storage respectively (Fig. 1). After days 9 and 12, TBA value decreased sharply for uncovered oil or oil covered with SPI/sucrose films respectively, indicating occurrence of lipid oxidation in these samples. The changes in TBA value of oil covered with SPI/glucose films occurred more slowly with storage times, with a lower maximum TBA value which decreased sharply after day 12. A progressive decline in docosa-hexaenoic (DHA) and eicosa-pentaenoic (EPA) acids with storage times of oils covered with SPI/sucrose films were noted during storage (Fig. 2). Similar decline occurred in oil covered with SPI/glucose films however, the extent of the decrease was smaller as compared to oils covered with SPI/sucrose films. Overall, fish oil covered with SPI/glucose films exhibited a smaller increase in thiobarbituric acid (TBA) value and a higher retention of docosa-hexaenoic (DHA) and eicosa-pentaenoic (EPA) acids as compared to those covered with SPI/sucrose films (Figs. 1 and 2).

The formation of antioxidant compounds of heat-treated protein/reducing sugar mixtures has been well-documented [6]. It is possible that some antioxidant compounds may have existed within the SPI /glucose films. Since the films were physically separated from the oil, it is more likely that the low TBA value of fish oil and the high content of DHA and EPA retained in fish oil covered with SPI/glucose films were due to improved physical properties of the films as a

result of the Maillard reaction occurring during heating of film-forming solution and curing treatments [17].

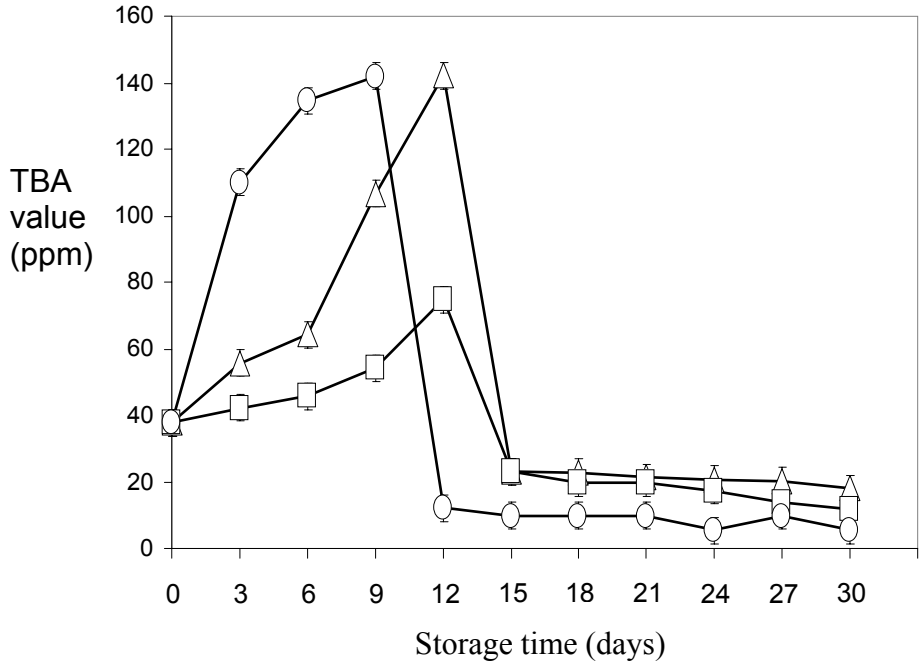

Figure 1. TBA values of fish oil covered with SPI/glucose film (□), SPI/sucrose film (Δ), or uncovered (O) as a function of storage time at 40°C. Error bar indicates standard deviation of triplicate samples.

Even though it is possible for the Maillard compounds formed within the films acted as oxygen scavengers, it is more likely that the enhanced protection of the fish oil to be attributed to the improved physical properties of the modified films. The protection exerted to the fish oil could have been due partly to the different level of oxygen permeability through the film as lipid oxidation is highly dependent on the partial pressure of oxygen, and partly to reduction in light transmission across the darkened SPI/glucose films (Table 1). The fish oil exhibits an oxygen sorption at about 6 times higher than water and oil system, and the rate of oxidation is governed by the rate at which oxygen gas diffuses into oil phase [28]. Approximately 16 times of improvement in oxygen barrier properties was achieved in SPI/glucose films (Table 1), and this should be one of important contributing factors that slowed down lipid oxidation in the fish oil. By slowing down the onset of lipid oxidation in the fish oil system, a better oxidative stability was achieved.

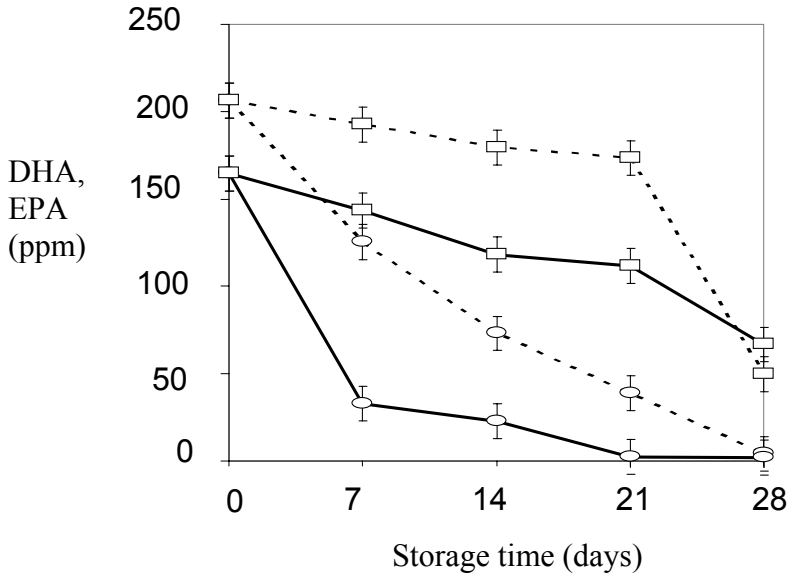

Figure 2. EPA (dotted lines) and DHA (continuous lines) content of fish oil covered with SPI/glucose film (□) or SPI/sucrose film (O) as a function of storage time at 40°C. Error bar indicates standard deviation of triplicate samples.

Conclusion

This paper indicates the possibility of using SPI/glucose film to slow down lipid oxidation of fish oil. Other than browning that could retard light penetration and the potential of oxygen scavenging activities of Maillard compounds, formation of Maillard crosslink within SPI films helped reduce oxygen gas transmission to slow down lipid oxidation.

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References

1. Yaqoob, P. (2004). Fatty acids and the immune system: from basic science to clinical applications. **Proceedings of Nutrition Society**, 63, 89-104.

2. Kremer, J.M. (2000). n-3 Fatty acids supplement in rheumatoid arthritis. **American Journal of Clinical Nutrition**, 71, 349S-351S.

3. Belluzzi, A., Boschi, S., Brignola, C., Munarini, A., Cariani, G., & Miglio, F. (2000). Polyunsaturated fatty acids and inflammatory bowel disease. **American Journal of Clinical Nutrition**, 71, 339S-342S.

4. Let, M.B., Jacobsen, C., Frankel, E.N., & Meyer, A.S. (2003). Oxidative flavour of fish oil enriched milk. **European Journal of Lipid Science & Technology**, 105, 518-528.

5. McGookin, B.J. & Augustin, M.A. (1991). Antioxidant activity of casein and Maillard reaction products from casein-sugar mixtures, **Journal of Dairy Research**, 58, 313-320.

6. McGookin, B.J. & Augustin, M.A. (1997). Antioxidant activity of a heated casein-glucose mixture in full cream milk powder, **Australian Journal of Dairy Technology**, 52, 15-19.

7. Hill, S.E., Mitchell, J.R. and Armstrong, H.J. (1992. The production of heat stable gels at low protein concentration by the use of the Maillard reaction. In: Gums and stabilizers for the food industry (edited by G.O. Phillips, D.J. Wedlock and P.A. Williams), Oxford University Press, 79-482.

8. Hill, S.E. and Easa, A.M. (1998). Linking proteins using Maillard reaction and the implications for food processors. In: The Maillard reaction in food and medicine (edited by J. O'Brien, H.E. Nursten, M.J.C. Crabbe and J.M. Ames), London Royal Society of Chemistry, 133-138.

9. Peng, L.K., Ismail, N. and Easa, A.M. (2000). Effects of reducing sugars on texture of thermally processed soy protein isolate-glucono-δ-lactone gels. **Journal of Food Science and Technology**, 37 (2), 188-190.

10. Kaye, Y.K., Easa, A.M. and Ismail, N. (2001). Reducing weight loss of retorted soy protein tofu by using glucose-and microwave-pre-heating treatment. **International Journal of Food Science and Technology**, 36, 387-392.

11. Kwan, S.W. and Easa, A.M. (2003). Comparing physical properties of retort resistant glucono-δ-lactone tofu treated with commercial transglutaminase enzyme or low levels of glucose. **LWT Food Technology**, 36, 643-646.

12. Gerrard, J.A. & Brown, P.K. (2002). Protein cross-linking in food: mechanisms, consequences, applications, International Congress series, volume 1245, Nov., 343-349.

13. Md Yasir, S.B., Sutton, K.H., Newberry, M.P., Andrews, N.R. and Gerrard, J.A. (2007). The impact of Maillard cross-linking on soy proteins and tofu texture, **Food Chemistry**, 104 (4), 1502-1508.

14. Drush, S., Berg, S., Scampicchio, N., Serfert, Y., Somoza, V., Mannino, S. and Schwarz, K. (2008). Role of glycated caseinate in stabilisation of microencapsulated lipophilic functional ingredients. **Food Hydrocolloids**, doi:10.1016/j.foodhyd.2008.07.004

15. Augustin, M.A., Sanguansri, L. and Bode, O. (2006). Maillard reaction products as encapsulants for fish oil powders, **Journal of Food Science**, 71(2), E25-E32.

16. Gan, C.Y., Cheng, L.H. and Easa, A.M. (2008). Evaluation of microbial transglutaminase and ribose cross-linked soy protein isolate-based microcapsules containing fish oil. **Innovative Food Science and Emerging Technology**, doi: 10.1016/j.ifset.2008.04.004.

17. Goh, T.B., Cheah, P.B. and Easa, A.M. (2003). Enhancement of soy protein isolate films properties by using the Maillard reactions and curing treatments. **ASEAN Food Journal**, 12(3), 149-158.

18. Gennadios, A., Ghorpade, V.M., Weller, C.L. and Hanna, M.A. (1996). Heat curing of soy protein films. **Transactions of American Society for Agricultural Engineers**, 39, 575-579.

19. Murray, P.J. (1953). Determination of crosslinking for pre-vulcanized latex. In Natural Rubber Technology (Murray, P.J. ed.), 2nd Edition, Oxford Press, London, 283-415.

20. Rhim, J.W., Gennadios, A., Weller, C.L. and Hanna, M.A. (1998). Modification of soy protein isolate film with dialdehyde starch, **Indian Crops Products**, 8, 195-203.

21. Butler, B.L., Vergano, P.J. and Wiles, J.L. (1996). Mechanical and barrier properties of edible chitosan films as affected by storage and composition. **Journal of Food Science**, 61(5), 952-955

22. ASTM (1995). Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor: D3985-95, In Annual Books of STM Standards, ASTM, Philadelphia, PA.

23. Gennadios, A., Weller, C.L. and Testin, R.F. (1993). Temperature effect of oxygen permeability of edible protein-based films, **Journal of Food Science**, 58, 212-219..

24. Lowry, O.H., Rosebrough, N.J., Farr, A. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagen, **Journal of Biological Chemistry**, 193, 265-275.

25. AOCS (1993). Official and tentative methods of the American Oil Chemists' Society, Official method Cd 19-90, The American oil Chemists' Society, Champaign, Illinois.

26. AOCS (1993). Official and tentative methods of the American Oil Chemists' Society, Official method Ce 1b-89, The American oil Chemists' Society, Champaign, Illinois.

27. Christie, W.W. (1989). Gas chromatography and lipids: A practical guide, Oily Press Ayr, New York.

28. Mathlouthi, M. (1986). Food packaging and preservation: Theory and practice, 3rd Edition, Elsevier Applied Science, London.