รายงานวิจัยฉบับสมบูรณ์

โครงการ
การประเมินลักษณะเฉพาะเชิงเปรียบเทียบและการใช้ฟิล์มบริโภคได้เชิงหน้าที่จากไคโตซานซึ่งเตรียมโดยวิธีการอบแห้งแบบต่าง ๆ ในการรักษาคุณภาพของอาหาร

โดย

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กรกฎาคม พ.ศ. 2554

สัญญาเลขที่ BRG5180021

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

Executive Summary

Plastic films have widely been used to improve mechanical properties or handling characteristics of foods. However, due to many adverse effects of plastics, including environmental and potential health problems, biodegradable films, which can be degraded completely by microorganisms into natural compounds, have been viewed as an alternative to plastic packagings. In addition to their usual functions of improving mechanical properties or handling characteristics of foods, edible films could also serve as carriers for antimicrobial or antioxidant compounds to maintain high concentration of preservatives on the food surfaces. Amongst the biopolymers that can be formed into films, chitosan is of interest because it has a good film forming ability and is biodegradable, biocompatible and nontoxic.

Despite its importance information on the effects of drying methods and conditions on the mechanical and functional properties of edible chitosan films is lacking. The present study first investigated the effects of drying methods and conditions as well as the concentration of galangal extract, which was incorporated into edible chitosan films as a natural antimicrobial agent, on the antimicrobial activity of the films. The antimicrobial activity and functional group interaction of the antimicrobial films were found to be affected by the drying methods and conditions as well as the concentration of the galangal extract. Then, the effects of drying methods and conditions on the release behavior of chitosan films incorporated with Indian gooseberry extract, which has proved to be a potent natural antioxidant, were investigated. The release behavior of the antioxidant from the films was also investigated. The release characteristics of the antioxidant films were again found to be affected by the drying methods and conditions as well as the concentration of the Indian gooseberry extract. The third part of the study proposed and tested an idea of using advanced drying methods, in combination with appropriate concentration of plasticizer, to improve the mechanical properties of the films. The drying methods and plasticizer concentration significantly affected the mechanical properties and glass transition temperature of the films. In some cases, there was a limiting value of plasticizer concentration beyond which the effect of the plasticizer concentration on the mechanical properties was negligible. Finally, various simple mathematical models for prediction of the release of antioxidant from edible chitosan films were compared and discussed. A simple-liquid diffusion model assuming the effective diffusion coefficient as a function of the phenolics concentration were noted to give the best agreement with the experimental results.

Acknowledgements

The Principal Investigator expresses his sincere appreciation to the Thailand Research Fund (TRF) for supporting the study financially through the Basic Research Grant no. BRG5180021. Additional supports from the National Science and Technology Development Agency (NSTDA); the Commission on Higher Education (CHE); the International Foundation for Science (IFS), Sweden and the Center for Advanced Microscopy, University of Reading, UK are also appreciated.

Abstract

Project Code: BRG5180021

Project Title: Comparative Characterization and Use of Functional Edible Chitosan Films Prepared by Different Drying Methods to Preserve Quality of Foods

Investigator: Associate Professor Dr. Sakamon Devahastin

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Project Period: 3 years (September 2008-July 2011)

The present study first investigated the effects of drying methods and conditions as well as the concentration of galangal extract, which was incorporated into edible chitosan films as a natural antimicrobial agent, on the antimicrobial activity of the films. Fourier transform infrared (FTIR) spectroscopy was performed to investigate functional group interaction between chitosan and the added active agent. The mechanism of the extract and of the antimicrobial films to inhibit bacterial cell growth was observed using transmission electron microscopy (TEM). The antimicrobial activity and functional group interaction of the antimicrobial films were found to be affected by the drying methods and conditions as well as the concentration of the galangal extract. Then, the effects of drying methods and conditions on the total phenolics content (TPC) of chitosan films incorporated with Indian gooseberry extract, which has proved to be a potent natural antioxidant, were investigated. The release behavior of the antioxidant from the films was also investigated. FTIR spectroscopy was again performed to investigate functional group interaction between chitosan and the added active agent. Swelling of the films was measured to help explain the release behavior of the films. The release characteristics, swelling and functional group interaction of the antioxidant films were found to be affected by the drying methods and conditions as well as the concentration of the Indian gooseberry extract. The third part of the study proposed and tested an idea of using advanced drying methods, in combination with appropriate concentration of plasticizer, to improve the mechanical properties of the films. The drying methods and plasticizer concentration significantly affected the mechanical properties and glass transition temperature of the films. In some cases, there was a limiting value of plasticizer concentration beyond which the effect of the plasticizer concentration on the mechanical properties was negligible. Finally, various simple mathematical models for prediction of the release of antioxidant from edible chitosan films were compared and discussed. A simple-liquid diffusion model assuming the effective diffusion coefficient as a function of the phenolics concentration were noted to give the best agreement with the experimental results.

Keywords: Active packaging; Antimicrobial activity; Antioxidant; Chitosan; Low-pressure superheated steam drying; Mathematical models; Mechanical properties; Release; Vacuum drying.

บทคัดย่อ

รหัสโครงการ: BRG5180021

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โครงการวิจัยนี้แบ่งออกเป็น 4 ส่วน โดยส่วนแรกเป็นการศึกษาผลของวิธีการและสภาวะการอบแห้ง ตลอดจนความเข้มข้นของสารสกัดจากข่าซึ่งเติมลงในฟิล์มบริโภคได้จากไคโตซานเพื่อให้เป็นสารต้านจุลชีพที่มีต่อฤทธิ์ต้านจุลชีพของฟิล์มที่ผลิตได้ นอกจากนี้ยังได้ศึกษาสหสัมพันธ์ระหว่างหมู่ฟังก์ชันของไคโตซานและสารสกัดซึ่งเติมลงในฟิล์มโดยวิธี Fourier transform infrared (FTIR) spectroscopy และศึกษากลไกการต้านจุลชีพทั้งของสารสกัดและของฟิล์มบริโภคได้ซึ่งมีสารสกัดเป็นองค์ประกอบโดยวิธี Transmission electron microscopy (TEM) จากผลการทดลองพบว่าวิธีการและสภาวะการอบแห้งตลอดจนความเข้มข้นของสารสกัดส่งผลอย่างมีนัยสำคัญต่อฤทธิ์ต้านจุลชีพและสหสัมพันธ์ระหว่างหมู่ฟังก์ชันของฟิล์มบริโภคได้ซึ่งมีสารสกัดข่าเป็นองค์ประกอบ สำหรับส่วนที่สองของโครงการเป็นการศึกษาผลของวิธีการและสภาวะการอบแห้งที่มีต่อปริมาณสารฟินอลิกทั้งหมด (Total phenolics content, TPC) ในฟิล์มบริโภคได้ซึ่งมีสารสกัดจากมะขามป้อมเป็นองค์ประกอบ ทั้งนี้ใช้สารสกัดจากมะขามป้อมเป็นตัวอย่างสารต้านอนุมูลอิสระที่เติมลงในฟิล์มบริโภคได้ นอกจากนี้ยังได้ศึกษาพฤติกรรมการปลดปล่อยและสหสัมพันธ์ระหว่างหมู่ฟังก์ชันของไคโตซานและสารสกัดตลอดจนการบวมของฟิล์มเพื่อใช้อธิบายผลพฤติกรรมการปลดปล่อยของฟิล์มอีกด้วย จากผลการทดลองพบว่าวิธีการและสภาวะการอบแห้งตลอดจนความเข้มข้นของสารสกัดส่งผลอย่างมีนัยสำคัญต่อพฤติกรรมการปลดปล่อย สหสัมพันธ์ระหว่างหมู่ฟังก์ชันและการบวมของฟิล์มบริโภคได้ซึ่งมีสารสกัดมะขามป้อมเป็นองค์ประกอบในลักษณะที่คล้ายคลึงกับผลการศึกษาในส่วนแรก ส่วนที่สามของโครงการเป็นการทดสอบแนวคิดการใช้วิธีการอบแห้งขั้นสูงร่วมกับการใช้พลาสติไซเซอร์ที่มีความเข้มข้นที่เหมาะสมในการปรับปรุงสมบัติเชิงกลของฟิล์มบริโภคได้จากไคโตซาน ผลการทดลองแสดงให้เห็นว่าวิธีการอบแห้งและความเข้มข้นของพลาสติไซเซอร์ส่งผลอย่างมีนัยสำคัญต่อสมบัติเชิงกลและอุณหภูมิการเปลี่ยนสถานะคล้ายแก้วของฟิล์ม นอกจากนี้ยังพบว่าในบางกรณีแม้เพิ่มความเข้มข้นของพลาสติไซเซอร์ขึ้นไปจากค่าวิกฤติค่าหนึ่ง ก็ไม่ทำให้สมบัติเชิงกลของฟิล์มที่ผลิตได้มีการเปลี่ยนแปลง สำหรับส่วนสุดท้ายของโครงการวิจัยเป็นการเปรียบเทียบและประเมินความสามารถของแบบจำลองทางคณิตศาสตร์แบบต่างๆ ในการทำนายพฤติกรรมการปลดปล่อยสารต้านอนุมูลอิสระจากฟิล์มบริโภคได้จากไคโตซาน จากผลการทดสอบพบว่าแบบจำลองที่ใช้ค่าสัมประสิทธิ์การแพร่ยังผลที่เป็นฟังก์ชันของความเข้มข้นของสารสกัดสามารถทำนายผลการทดลองการปลดปล่อยได้ดีที่สุด

คำสำคัญ: การปลดปล่อย การอบแห้งโดยใช้ไอน้ำร้อนยวดยิ่งที่สภาวะความดันต่ำ การอบแห้งแบบสุญญากาศ ไคโตซาน บรรจุภัณฑ์แบบแอคทีฟ แบบจำลองทางคณิตศาสตร์ ฤทธิ์ต้านจุลชีพ สมบัติเชิงกล สารต้านอนุมูลอิสระ

Contents

1. Introduction

Food packaging materials, in particular packaging films, are commonly used to inhibit migration of moisture, oxygen, carbon dioxide, aromas, etc., as well as to improve mechanical properties or handling characteristics of foods. Plastic films have widely been used for these purposes. However, due to many adverse effects of plastics, including environmental and potential health problems, biodegradable films, which can be degraded completely by microorganisms into natural compounds, have been viewed as an alternative to plastic packagings. To further alleviate the problem, edible films may be an even better alternative for food applications because it does not have to be eliminated as solid wastes. Edible films may be formed from edible biomaterials such as polysaccharides, proteins or lipids. Amongst these biopolymers, chitosan is of interest because it has a good film forming ability and is biodegradable, biocompatible and nontoxic.

In most solid foods, contamination and microbial growth occur on the surface of foods, leading to a reduction in the product shelf life. In addition, many oxidative reactions could occur, leading to quality deterioration of foods. In such situations, edible films, in addition to their usual functions of improving mechanical properties or handling characteristics of foods, could also serve as carriers for antimicrobial or antioxidant compounds to maintain high concentration of preservatives on the food surfaces. To enhance the antimicrobial/antioxidant activity of edible films, herbs and spices or plant extracts, which are amongst the most promising natural antimicrobial/antioxidant agents, could be incorporated into the films.

Among the steps involved in the production of edible films, film formation is considered one of the most important. Edible films can be formed by a casting/solvent evaporation technique and different drying methods, e.g., ambient drying, hot air drying or even infrared-assisted drying, have been employed for this purpose. Recently, an alternative drying technique viz. low-pressure superheated steam drying (LPSSD), which has been studied extensively by the Principal Investigator during the past decade through the support of the Thailand Research Fund (TRF), has been utilized to produce edible chitosan films (Mayachiew and Devahastin, 2008). The LPSSD films were compared with the films obtained from other drying processes, i.e., ambient air drying, hot air drying and vacuum drying. It was found that the films (not yet incorporated with antimicrobial/antioxidant agents) produced by LPSSD had superior mechanical and physical properties to those films produced by other tested drying processes.

The present study extended the original work of Mayachiew and Devahastin (2008) by first studying the effects of drying methods and conditions as well as the concentration of galangal extract, which was incorporated into edible chitosan films as a natural antimicrobial agent, on the antimicrobial activity of the films by both the disc diffusion and viable cell count methods. Fourier transform infrared (FTIR) spectroscopy was performed to investigate functional group interaction between chitosan and the added active agent. The mechanism of the extract and of the antimicrobial films to inhibit bacterial cell growth was observed using transmission electron microscopy (TEM). Overall, the antimicrobial activity and functional group interaction of the antimicrobial films were found to be affected by the drying methods and conditions as well as the concentration of the galangal extract. An increase in the galangal extract concentration in chitosan films led to a higher antimicrobial activity against *Staphylococcus aureus* and stronger functional group interaction. The transmission electron microscopic observations revealed that cell walls and cell membranes of *S. aureus* treated either by the extract or the antimicrobial films were significantly damaged (Mayachiew et al., 2010).

In the second part of the study, the effects of drying methods and conditions on the total phenolics content (TPC) of chitosan films incorporated with Indian gooseberry extract, which has proved to be a potent natural antioxidant, were investigated. A study of the release behavior of the antioxidant compound from the films was also conducted. Fourier transform infrared (FTIR) spectroscopy was again performed to investigate functional group interaction between chitosan and the added active agent. Swelling of the films was measured to help explain the release behavior of the films. Overall, it was found that the drying methods and conditions have significant effects on the percentage of residual TPC. The release characteristics, swelling and functional group interaction of the antioxidant films were found to be affected by the drying methods and conditions as well as the concentration of the Indian gooseberry extract (Mayachiew and Devahastin, 2010).

Due to the nature of chitosan films, which is rigid and brittle, use of these films in food applications is still rather limited. For this reason the third part of the study proposed and tested an idea of using advanced drying methods, in combination with appropriate concentration of plasticizer, to improve the mechanical properties of the films. Physical and mechanical properties of chitosan films plasticized at various plasticizer concentrations and prepared by different drying methods were investigated. It

was found that the drying methods and plasticizer concentration significantly affected the tensile strength, percent elongation and glass transition temperature of the films. On the other hand, the drying methods and plasticizer concentration did not affect the thickness and final moisture content of the film samples at lower glycerol concentrations. Interestingly, in the cases of vacuum drying and LPSSD, there was a limiting value of plasticizer concentration beyond which the effect of the plasticizer concentration on the mechanical properties was negligible (Thakhiew et al., 2010).

In the final part of the study various simple mathematical models for prediction of the release of antioxidant from edible chitosan films were compared and discussed. It was found that a simple-liquid diffusion model assuming the effective diffusion coefficient as a function of the phenolics concentration gave the best agreement with the experimental results (Thakhiew et al., 2011). The model should be proved useful for an effective design of antioxidant-added films in the future.

2. Materials and Methods

Please refer to the Appendix for detailed Materials and Methods used in the study.

3. Results and Discussion

Please refer to the Appendix for detailed Results and Discussion of the study.

4. Conclusion

Please refer to the Appendix for detailed Conclusion of the study.

5. References

Mayachiew, P., Devahastin, S., Mackey, B.M., Niranjan, K. 2010. Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract. Food Research International, 43: 125-132.

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Thakhiew, W., Waisayawan, P., Devahastin, S. 2011. Comparative evaluation of mathematical models for release of antioxidant from chitosan films prepared by different drying methods. Drying Technology, DOI: 10.1080/07373937.2011.588816.

Outputs of the Project

I. Refereed papers in international journals (4 papers)

1. Mayachiew, P., Devahastin, S., Mackey, B.M., Niranjan, K. 2010. Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract. Food Research International, 43: 125-132 (2010 IF = 2.416).
2. Mayachiew, P., Devahastin, S. 2010. Effects of drying methods and conditions on release characteristics of edible chitosan films enriched with Indian gooseberry extract. Food Chemistry, 118: 594-601 (2010 IF = 3.458).
3. Thakhiew, W., Devahastin, S., Soponronnarit, S. 2010. Effects of drying methods and plasticizer concentration on some physical and mechanical properties of edible chitosan films. Journal of Food Engineering, 99: 216-224 (2010 IF = 2.168).
4. Thakhiew, W., Waisayawan, P., Devahastin, S. 2011. Comparative evaluation of mathematical models for release of antioxidant from chitosan films prepared by different drying methods. Drying Technology, DOI: 10.1080/07373937.2011.588816 (2010 IF = 1.662).

II. Papers presented at international conferences (2 papers)

1. Mayachiew, P., Devahastin, S. 2009. A comparative evaluation of drying methods and conditions for enhancing the performance of antimicrobial edible films. *Proceedings of the 6th Asia-Pacific Drying Conference*, Bangkok, Thailand, pp. 311-315.
2. Thakhiew, W., Devahastin, S., Soponronnarit, S. 2009. Effects of drying methods and plasticizer concentration on physical and mechanical properties of edible chitosan film. *Proceedings of the 6th Asia-Pacific Drying Conference*, Bangkok, Thailand, pp. 95-101.

Appendix

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Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract

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ABSTRACT

The aim of this work was to study the effects of drying methods and conditions (i.e., ambient drying, hot air drying at 40 °C, vacuum drying and low-pressure superheated steam drying within the temperature range of 70–90 °C at an absolute pressure of 10 kPa) as well as the concentration of galangal extract on the antimicrobial activity of edible chitosan films against *Staphylococcus aureus*. Galangal extract was added to the film forming solution as a natural antimicrobial agent in the concentration range of 0.3–0.9 g/100 g. Fourier transform infrared (FTIR) spectra and swelling of the films were also evaluated to investigate interaction between chitosan and the galangal extract. The antimicrobial activity of the films was evaluated by the disc diffusion and viable cell count method, while the morphology of bacteria treated with the antimicrobial films was observed via transmission electron microscopy (TEM). The antimicrobial activity, swelling and functional group interaction of the antimicrobial films were found to be affected by the drying methods and conditions as well as the concentration of the galangal extract. The electron microscopic observations revealed that cell wall and cell membrane of *S. aureus* treated by the antimicrobial films were significantly damaged.

1. Introduction

Microbial deteriorations are responsible for enormous losses of foods. Various chemical and physical means have been developed to help alleviate these undesirable phenomena. Nowadays, there is a considerable interest in the possibility of using edible films to delay or to prevent growth of microorganisms since these films can serve as carriers for a wide range of food additives, including antimicrobial agents, which can extend the shelf-life of foods. Among many materials that can be used to form edible films, chitosan (β-(1,4)-2-amino-2-deoxy-D-glucose) is one of the most promising as it has a good ability to form film and due to its biodegradability, biocompatibility and non-toxicity (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004).

Among many possible natural antimicrobial agents that can be incorporated into edible films, galangal (*Alpinia galanga* Linn.) or "khaa" in Thai is one of the most promising. Galangal is a traditional spice used extensively for flavoring and medicinal purposes. Galangal extract has also proved to be an effective natural antimicrobial agent against some food poisoning bacteria, e.g., *Staphylococcus aureus* (Mayachiew & Devahastin, 2008a). The main compounds of galangal extract are the terpenes, which have potential antimicrobial activity (Burt, 2004; Cowan, 1999; Holley & Patel, 2005; Mohammed & Al-Bayati, 2009).

Recently, many types of antimicrobial edible films have been developed and used to inhibit growth of microorganisms, resulting in an ability to prolong the shelf-life of foods. For example, Seydim and Sarikus (2006) investigated the antimicrobial properties of whey protein isolate (WPI) films containing oregano, rosemary and garlic essential oils against *Escherichia coli* O157:H7, *S. aureus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Lactobacillus plantarum*. The results suggested that spice extracts exhibited antimicrobial activity in WPI based edible films. Maizura, Fazilah, Norziah, and Karim (2007) assessed antimicrobial films prepared from partially hydrolyzed sago starch and alginate and incorporated with lemongrass oil. The films containing lemongrass oil were effective in inhibiting the growth of *E. coli* based on the zone of inhibition assay. Sivarooban, Hettiarachchy, and Johnson (2008) found that soy protein edible films incorporated with grape seed extract, nisin and EDTA were effective against *L. monocytogenes*, *E. coli* and *Salmonella typhimurium*.

Drying is one of the most challenging steps in the production of edible films. It is well known that different drying methods and conditions affect the properties and functionalities of edible films differently (Mayachiew & Devahastin, 2008b; Srinivasa, Ramesh,

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Kumar, & Tharanathan, 2004). Recently, Mayachiew and Devahastin (2008b) investigated the effects of drying methods and conditions, namely, ambient drying (~30 °C), hot air drying at 40 °C, vacuum drying and low-pressure superheated steam drying (LPSSD) within the temperature range of 70–90 °C at an absolute pressure of 10 kPa, on the physical properties of selected chitosan film. It was found that LPSSD at 70 °C led to films with higher tensile strength and percent elongation than the films dried by other drying methods and at other drying conditions. Ambient dried and LPSSD films had higher crystallinity than the films dried by vacuum drying. However, no information is so far available on the effects of these (or any other) different drying methods and conditions on the antimicrobial activity and other physico-chemical properties of chitosan films incorporated with galangal (or any other) extract.

The aim of this study was therefore to assess the effects of drying methods (i.e., ambient drying, hot air drying, vacuum drying and LPSSD) and conditions (drying temperature of 70, 80 and 90 °C) on the antimicrobial activity of chitosan films incorporated with galangal extract by both the disc diffusion and viable cell count methods. Fourier transform infrared (FTIR) spectroscopy was also performed to investigate functional group interaction between chitosan and the added active agent. Degree of swelling of the films was measured to help explain the release behavior of the films. The mechanism of the extract and of the antimicrobial films to inhibit bacterial cell growth was observed using transmission electron microscopy (TEM).

2. Materials and methods

2.1. Materials

Chitosan (molecular weight of 900,000 Da and degree of deacetylation of 90.2%) was obtained from S.K. Profishery Co., Ltd. (Bangkok, Thailand). Glycerol was purchased from Carlo Erba (Val de Reuil, Italy) while acetic acid was obtained from Merck (Darmstadt, Germany). For antimicrobial tests, Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB), Mueller Hinton agar (MHA) and buffer peptone water were purchased from Difco (Detroit, USA). Galangal rhizomes were purchased from a local market.

2.2. Preparation of chitosan solution

1.5% (w/v) chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid under constant stirring at 300 rpm using a magnetic stirrer (Framo®-Gerätechnik, model M21/1, Eisenbach, Germany) at room temperature for 24 h. 25% glycerol (w/w chitosan) was then added into the chitosan solution; stirring was continued at room temperature for 1 h. After mixing the solution was centrifuged for 15 min at 12,400 rpm by a refrigerated centrifuge (Hitachi, model Himac CR21, Ibaragi, Japan) to remove undissolved impurities and bubbles in the solution.

2.3. Preparation of galangal extract

Galangal powder (10 g dry basis), dried by a tray dryer at 40 °C with particle size between 125–425 μm, was extracted with 100 mL of 95% (v/v) ethanol (Oonmetta-aree, Suzuki, Gasaluck, & Eumkeb, 2006). The extract was filtered through a filter paper (Ø110 mm, Cat. No. 1001 110, Schleicher and Schuell GmbH, Dassel, Germany); the filtrate was collected and concentrated by a rotary evaporator (Resona Technics, model Labo Rota 300, Gossau, Switzerland) at 40 °C for 10 min and kept at 4 °C in a dark bottle until its use (Mayachiew & Devahastin, 2008a).

2.4. Preparation of antimicrobial chitosan films

Galangal extract was added to the chitosan solution at concentrations of 0.3, 0.6 and 0.9 g/100 g. These concentrations were selected based on a minimum inhibitory concentration (MIC) of the extract against *S. aureus* (Mayachiew & Devahastin, 2008a). The final concentrations of galangal extract in the films were 126, 252 and 378 mg/g film, respectively. The mixture was homogenized by a bench top homogenizer (Ika® Works (Asia), model T 25 basic, Selangor, Malaysia) at 9500 rpm for 2 min. The film solution (21 g) was poured on an acrylic plate with dimensions of 13 × 10 cm to cast an antimicrobial film. Drying of the film was performed by four methods, which are ambient air drying (~30 °C), hot air drying at 40 °C, vacuum drying and LPSSD at 70, 80 and 90 °C at 10 kPa, following the methods of Mayachiew and Devahastin (2008b). After drying the films were conditioned for at least 48 h in a desiccator at a relative humidity (RH) of 53% containing saturated salt solution of magnesium nitrate (Ajax Finechem, Seven Hills, Australia).

2.5. Antimicrobial activity evaluation

2.5.1. Microorganism and cultural methods

S. aureus (ATCC 25923) was obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. The stock culture was maintained by regular subculture on TSA slants at 4 °C and transferred monthly. A loopful of bacteria was inoculated to 10 mL of TSB and incubated at 37 °C for 18 h. This culture served as an inoculum for an antimicrobial test.

2.5.2. Agar diffusion method

Qualitative antimicrobial activity of the films was evaluated by the agar diffusion method following the procedure recommended by Maizura et al. (2007). The inoculum (100 μL) of *S. aureus* containing approximately 10^6 CFU/mL was spread on the surface of Mueller Hinton agar plate. An edible film sample was cut into a 6-mm diameter disc and then placed on the agar plate. The plate was incubated at 37 °C for 24 h. The plate was then examined for a zone of inhibition of the film disc. The total diameter of the inhibition zone including the film disc was measured.

2.5.3. Viable cell count method

The biocide property of the antimicrobial films was evaluated by employing the macrodilution method recommended by the National Committee of Clinical Laboratory Standards (NCCLS, 1999). About 0.3 g of each film specimen was placed in a sterilized flask into which 10 mL of *S. aureus* culture containing approximately 10^7 CFU/mL was added. The suspension was incubated at 37 °C. 100 μL of the sample was taken at 0, 6, 12, 18 and 24 h and spread on a TSA agar plate, which was incubated at 37 °C for 24 h. The number of colonies was counted. The inhibition of bacteria growth was expressed as the reduction of cell number by $\log N/N_0$. The test was performed in triplicate.

2.6. Transmission electron microscopy (TEM)

S. aureus was grown in TSB at 37 °C for 18 h. One millilitre of the cell culture was centrifuged using a Biofuge 28 RS (Heraeus Sepatech GmbH, model 3654, Osterode, Germany) at 11,000 rpm for 10 min. The cell pellets were resuspended in 10 mL of TSB, which contained 0.3 g of antimicrobial film or 120 μL of a filter paper disc (6 mm in diameter) soaked with the galangal extract. After incubation at 37 °C for 6 h, the suspension was centrifuged at 11,000 rpm for 10 min. The cell pellets were washed twice with 0.1 M sodium phosphate buffer (pH 7.3) and were then fixed with 2.0% (w/v) paraformaldehyde, 2.5% (v/v) glutaraldehyde in 25 mM buffer.

The sample was washed twice in buffer, each time for 20 min. After that the sample was postfixed with 1% (w/v) osmium tetroxide for 2 h at room temperature, washed twice with the same buffer, dehydrated with series of acetone solution, then embedded in low-viscosity embedding medium (Agar 100, Agar Scientific, Essex, UK). Fifty nm ultra thin section of the specimen was cut with a diamond knife on an Ultracut Ultramicrotome (Reichert-Jung GmbH, model Ultracut E, Heidelberg, Germany). The section was stained with 1.5% (w/v) uranyl acetate for 15 min. The ultrastructural thin section was examined with a transmission electron microscope (Philips, model CM 20, Eindhoven, The Netherlands) at an operating voltage of 80 kV.

2.7. FTIR analysis

Attenuated total reflection–Fourier transform infrared (ATR–FTIR) spectra were collected at 25 °C by coupling the ATR accessory to an FTIR spectrometer (Perkin-Elmer, model 1760X, Norfolk, CT) available at Chulalongkorn University, Bangkok. Time resolved experiments were collected by averaging, depending on the experiment, 10 or two scans at 4 cm^{-1} spectral resolution at time intervals.

2.8. Swelling analysis

A film was cut into sizes of 2 × 2 cm and dried in a vacuum oven (Sanyo, Model Gallenkamp/OM-09980, Loughborough, UK) at 70 °C at an absolute pressure of 10 kPa for 24 h. The film was weighed and left at ambient temperature in 30 mL of distilled water for 24 h. The film was then blotted with tissue paper and the mass of the film was measured periodically with a microbalance (Sartorius, model RC 250S, Göttingen, Germany) until the equilibrium was reached. The degree of swelling was calculated using:

$$\text{Degree of swelling} = \frac{\text{Mass of film(g)} - \text{Mass of dehydrated film(g)}}{\text{Mass of dehydrated film(g)}} \times 100 \quad (1)$$

2.9. Statistical analysis

All data were subjected to the analysis of variance (ANOVA) using SPSS® software (Chicago, IL) and were presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95%. All experiments were performed in duplicate except when stated otherwise.

3. Results and discussion

3.1. Antimicrobial activity of films

3.1.1. Clear zone of disc diffusion

Inhibitory activity of antimicrobial films was evaluated based on the clear zone surrounding a circular film disc. If there is no clear zone, it is assumed that there is no inhibition. Table 1 shows the antimicrobial activity of chitosan films incorporated with galangal extract as tested by the agar diffusion method. The results showed that the chitosan films containing galangal extract at 0.6% and 0.9% (w/w) were effective in inhibiting the growth of *S. aureus*. The diameters of the inhibition zones varied from 19.5 mm to 29.5 mm for chitosan films incorporated with 0.9% (w/w) galangal extract and from 15.7 mm to 23.8 mm for the films with 0.6% (w/w) galangal extract. No inhibition zone was observed when the extract concentration of 0.3% (w/w) was used.

Table 1
Antimicrobial activity (in terms of inhibition zone diameter, mm) of antimicrobial films prepared by different drying methods and conditions against *S. aureus*.

Drying method	Galangal extract concentration		
	0.3%	0.6%	0.9%
Ambient drying	–	23.8 ± 0.6^{a}	29.5 ± 0.7^{a}
Hot air drying	–	23.4 ± 0.6^{a}	29.2 ± 0.4^{a}
Vacuum drying			
70 °C	–	20.5 ± 1.0^{b}	24.9 ± 0.9^{c}
80 °C	–	15.7 ± 0.5^{c}	19.5 ± 1.1^{d}
90 °C	–	$-^{d}$	$-^{e}$
LPSSD			
70 °C	–	22.3 ± 0.8^{a}	27.1 ± 1.0^{b}
80 °C	–	20.4 ± 0.9^{b}	23.6 ± 0.7^{c}
90 °C	–	$-^{d}$	$-^{e}$

– Indicates no inhibition.
Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

This could be ascribed to a limited galangal extract release probably due to interaction between the extract and chitosan as indicated by a new absorption peak at 1717 cm^{-1} and the shift of absorption band at 1236 and 1542 cm^{-1} (see Fig. 4). Another possible reason could be the limit of detection of antimicrobial activity when using the disc diffusion method (Coma et al., 2002; Pranoto, Rakshit, & Salokhe, 2005).

Regarding the effects of drying methods and conditions it was noted that drying methods and conditions had significant effects on the antimicrobial activity of chitosan films incorporated with galangal extract. The results showed that ambient dried film had the highest antimicrobial activity; this was followed by LPSSD films and vacuum dried films. This may be due to the fact that the film temperature increased more rapidly and stayed at higher levels in the case of vacuum drying than in the case of LPSSD, thus inducing more thermal degradation of the antimicrobial compound (Burt, 2004; Mayachiew & Devahastin, 2008b). In addition, different intermolecular interactions also contributed to the observed results. The decrease in bacteria inhibition might be due to lower diffusion of the active agent into the agar medium as a result of higher interaction between chitosan and galangal extract (shown later in FTIR results).

The antimicrobial films prepared at higher drying temperatures had lower antimicrobial activity, both in the cases of vacuum drying and LPSSD. The antimicrobial films prepared by LPSSD at 70 °C had the highest antimicrobial activity compared with films prepared at other conditions of vacuum drying and LPSSD.

3.1.2. Viable cell count

The rapidity of bactericidal effect of antimicrobial films was determined by a viability test. The viability test was also chosen to study the reduction of cell population by the antimicrobial films.

The test results indicated that the concentration of the galangal extract significantly affected the cell viability of the tested microorganism (Figs. 1–3). The results showed that the antimicrobial activity of the films increased with an increase in the extract concentration, as expected. Chitosan film incorporated with 0.9% (w/w) galangal extract and prepared by ambient drying could reduce the number of *S. aureus* by about 3.6 log cycle within the contact time of 24 h. On the other hand, ambient dried film incorporated with 0.3% (w/w) galangal extract exhibited lower cell reduction number of around 2.0 log cycle.

Considering the effects of the drying methods and conditions, the final bacterial counts when applying a film dried at control condition (ambient drying and hot air drying at 40 °C) and a film dried by LPSSD at 70 °C were lower than those when using films prepared at other conditions of LPSSD and vacuum drying. In addi-

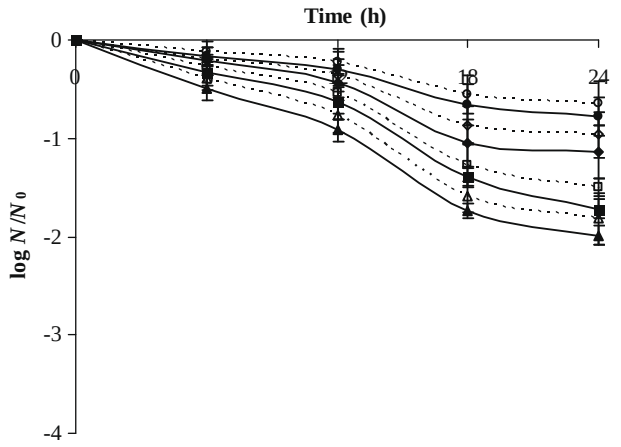

Fig. 1. Reduction of cell number in contact with chitosan films enriched with 0.3% (w/w) galangal extract and prepared by ambient drying (—▲—); hot air drying at 40 °C (- - -△- - -); vacuum drying at 70 °C (- - -□- - -), 80 °C (- - -◇- - -), 90 °C (- - -○- - -); LPSSD at 70 °C (—■—), 80 °C (—♦—), 90 °C (—●—).

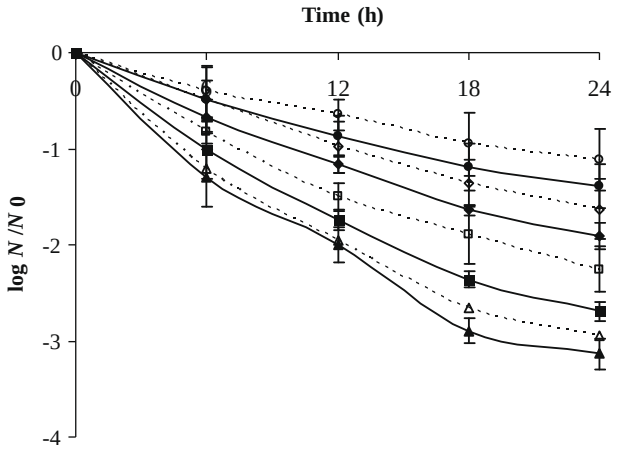

Fig. 2. Reduction of cell number in contact with chitosan films enriched with 0.6% (w/w) galangal extract and prepared by ambient drying (—▲—); hot air drying at 40 °C (- - -△- - -); vacuum drying at 70 °C (- - -□- - -), 80 °C (- - -◇- - -), 90 °C (- - -○- - -); LPSSD at 70 °C (—■—), 80 °C (—♦—), 90 °C (—●—).

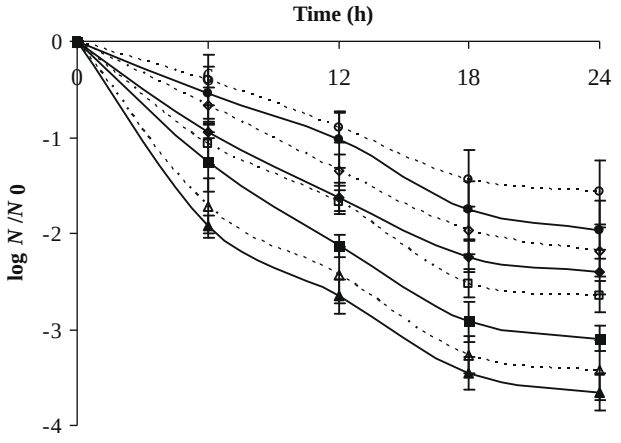

Fig. 3. Reduction of cell number in contact with chitosan films enriched with 0.9% (w/w) galangal extract and prepared by ambient drying (—▲—); hot air drying at 40 °C (- - -△- - -); vacuum drying at 70 °C (- - -□- - -), 80 °C (- - -◇- - -), 90 °C (- - -○- - -); LPSSD at 70 °C (—■—), 80 °C (—♦—), 90 °C (—●—).

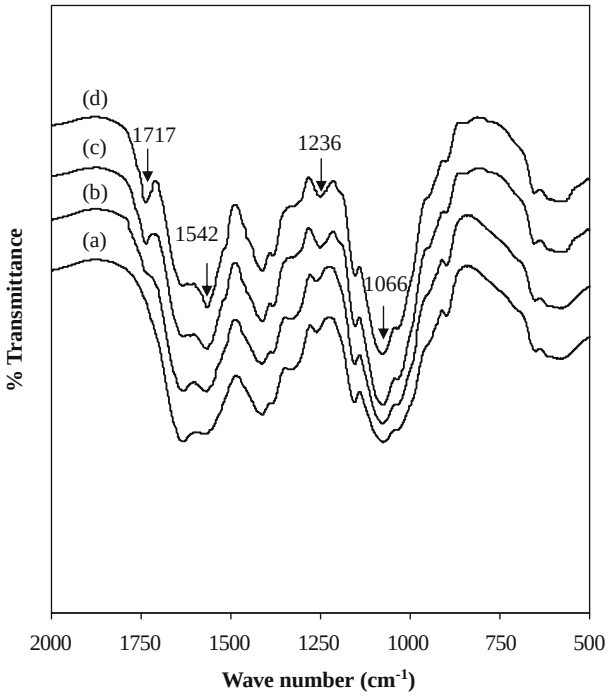

Fig. 4. FTIR spectra of ambient dried films with different concentrations of galangal extract. (a) With no extract; (b) with 0.3% (w/w) galangal extract; (c) with 0.6% (w/w) galangal extract; (d) with 0.9% (w/w) galangal extract.

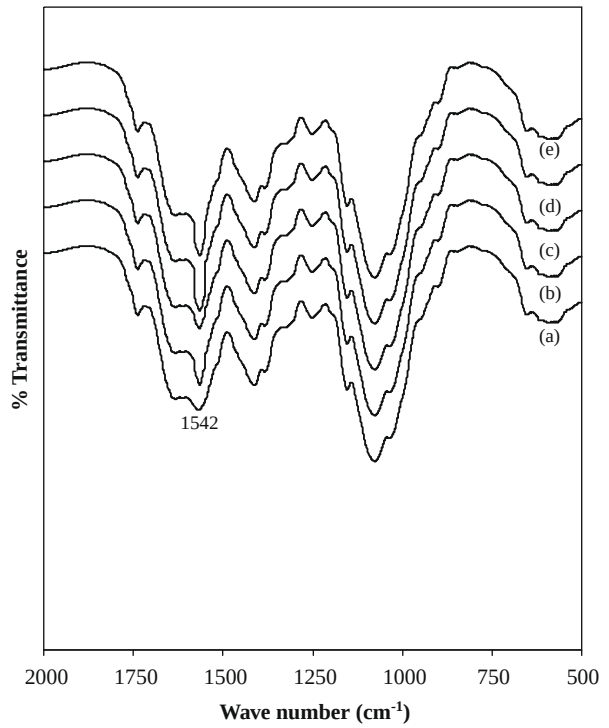

Fig. 5. FTIR spectra of chitosan films enriched with 0.6% (w/w) galangal extract and prepared by different drying methods and conditions. (a) Ambient drying; (b) vacuum drying at 70 °C; (c) LPSSD at 70 °C; (d) vacuum drying at 90 °C and (e) LPSSD at 90 °C.

tion, the LPSSD films could decrease the viable cell number faster than the vacuum dried films prepared at the same drying temperature. This might be due to different intermolecular interactions between the extract and chitosan films (as shown by the results of FTIR spectra in Fig. 5), different microstructure of the films as

well as different degrees of film swelling. The results on film swelling will be discussed later.

Antimicrobial films incorporated with 0.9% (w/w) galangal extract and prepared by ambient drying, hot air drying at 40 °C and LPSSD at 70, 80 and 90 °C resulted in an inhibition of *S. aureus* by up to 3.66, 3.42, 3.10, 2.41 and 1.97 log cycles, respectively (Table 2). On the other hand, the decrease in the number of microorganisms in contact with vacuum dried films prepared at 70, 80 and 90 °C was up to 2.66, 2.19 and 1.57 log cycles, respectively, during the same contact period of 24 h. The evaluation of the efficiency of chitosan films incorporated with galangal extract and prepared by different drying methods on *S. aureus* verified that the film incorporated with 0.9% (w/w) galangal extract and prepared by ambient drying, hot air drying at 40 °C and LPSSD at 70 °C had the highest antimicrobial activity and could reduce the cell count by 3.66, 3.42 and 3.10 log cycles, respectively, after 24 h.

From the higher rate of cell number reduction during an initial contact period of 6 h and the slower rate of reduction afterward, it is reasonable to suggest that some parts of the added antimicrobial agent was chemically bonded to chitosan; this is shown by the results of the FTIR spectra in Figs. 4 and 5. Chitosan might interact with terpenes and phenolic compounds of galangal extract, mainly by weak interactions such as hydrogen bonding. Hydroxyl groups of phenolics could form H-bond with NH_3^+ of chitosan (Kanatt, Chander, & Sharma, 2008). Moreover, the amine groups probably also contributed to ionic interaction with carboxylic groups of acid phenols (Spagna et al., 1996). These bound fractions could affect the release of the added antimicrobial agent.

3.2. Transmission electron microscopy (TEM)

A TEM study could provide information on the antimicrobial action of the galangal extract and of the antimicrobial films against the tested microorganism. TEM was therefore used to compare the morphological changes of bacterial cells, which were treated with galangal extract and antimicrobial films.

The electron micrographs of *S. aureus*, both untreated cells and cells treated with galangal extract and with chitosan film incorporated with 0.9% (w/w) galangal extract and prepared by ambient drying, are presented in Fig. 6. For the control cells, it is seen that the cells had a typical structure showing a regular shape including clear and smooth cell walls. The untreated cells clearly exhibited a dark outer peptidoglycan layer and a densely-stained cytoplasmic membrane bilayer. The electron micrographs of *S. aureus* treated with galangal extract showed important morphological changes of cell wall. In galangal-treated samples the peptidoglycan layer appeared to be much more diffuse and lighter in appearance than in control cells and, in some cases, it almost disappeared. Galangal extract thus seems to lead to cell wall deterioration in *S. aureus*.

Table 2
Antimicrobial activity (in terms of $\log N/N_0$) of antimicrobial films prepared by different drying methods and conditions against *S. aureus*.

Drying method	Galangal extract concentration		
	0.3%	0.6%	0.9%
Ambient drying	1.99 ± 0.10^{a}	3.14 ± 0.16^{a}	3.66 ± 0.19^{a}
Hot air drying	1.82 ± 0.27^{ab}	2.95 ± 0.08^{a}	3.42 ± 0.31^{a}
Vacuum drying			
70 °C	1.50 ± 0.09^{bc}	2.26 ± 0.23^{bc}	2.66 ± 0.16^{bc}
80 °C	0.97 ± 0.23^{de}	1.63 ± 0.31^{de}	2.19 ± 0.25^{cd}
90 °C	0.64 ± 0.23^{e}	1.12 ± 0.32^{f}	1.57 ± 0.34^{e}
LPSSD			
70 °C	1.72 ± 0.10^{ab}	2.69 ± 0.10^{ab}	3.10 ± 0.13^{ab}
80 °C	1.14 ± 0.27^{cd}	1.91 ± 0.13^{cd}	2.41 ± 0.23^{cd}
90 °C	0.78 ± 0.20^{de}	1.39 ± 0.23^{ef}	1.97 ± 0.30^{de}

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

Galangal extract used in this study contained a mixture of lipophilic compounds including 1,8-cineole (20.95%), β-caryophyllene (13.16%), β-bisabolene (17.95%) and β-selinene (10.56%), with α-selinene, farnesene and 1, 2-benzenedicarboxylic acid as minor components (Mayachiew & Devahastin, 2008a). These compounds might attack the phospholipid cell membrane, causing increased permeability and leakage of cytoplasm (Burt, 2004; Cowan, 1999; Mohammed & Al-Bayati, 2009; Tassou, Koutsoumanis, & Nychas, 2000). Other membrane-active agents such as triton X-100 and nisin are known to trigger cell wall lysis in staphylococci by activating endogenous murein hydrolases (Bierbaum & Sahl 1987; Boyle-Vara, Challapalli, & Daum, 2003) and this may provide an explanation for the action of galangal seen here.

Considering the cells treated with the antimicrobial film, the electron micrographs showed that the antimicrobial film led to damaged cell wall and cell membrane of *S. aureus*. TEM results showed the same characteristics of cell damage, whether the cells were treated with galangal extract or the antimicrobial film. This implies that the antimicrobial agent could travel from the film into the aqueous system. The cell damage results were correlated with the decrease of the cell number of bacteria based on the viability test and the clear zone of disc diffusion test.

3.3. FTIR spectra

3.3.1. Effect of antimicrobial agent

FTIR spectroscopy was used to examine an interaction between chitosan and galangal extract. The FTIR spectra of pure chitosan film and chitosan films incorporated with galangal extract at different concentrations and prepared by ambient drying are shown in Fig. 4. The FTIR spectrum of pure chitosan film showed characteristic absorption bands at 1631 cm^{-1} and 1566 cm^{-1}, which represent the presence of amide I band (C═O) and amide II vibration (N—H), respectively. The chitosan spectra are similar to those previously reported (Pranoto et al., 2005; Ritthidej, Phaechamud, & Koizumi, 2002; Wang, Dong, Du, & Kennedy, 2007).

Considering the FTIR spectra of chitosan films incorporated with galangal extract, it was found that the absorption bands exhibited some modifications within the range of 1000–1275 cm^{-1}. The peak at 1066 cm^{-1}, which is attributed to C—H bending, was stronger and sharper with an increase in the galangal extract concentration. In addition, the absorption band at 1249 cm^{-1}, concerning with C—O stretching, was shifted to a lower wave number at 1236 cm^{-1}. The changes of these absorption bands might correspond to the antimicrobial agent spectra of the films (Mohammed & Al-Bayati, 2009).

Another important change was observed as the shift of amide II band from 1566 cm^{-1} to 1542 cm^{-1}. At the same time, these bands showed a stronger peak intensity compared with that of the pure chitosan film. The increase of intensity of amide band was attributed to the increase of amide formation, which could decrease the degree of swelling with an increase in the extract concentration. Besides, the new peak appeared at 1717 cm^{-1}, corresponding to the C═O stretching band, which indicates ester linkage between the extract and chitosan. The absorption intensity increased with increasing extract concentration. All these changes could be attributed to the intermolecular interaction and molecular compatibility between chitosan and galangal extract (Xu, Kim, Hanna, & Nag, 2005).

As mentioned earlier, the antimicrobial activity of galangal extract has been attributed to terpene compounds such as 1,8-cineole and β-bisabolene (Mayachiew & Devahastin, 2008a). Terpenes consist predominantly of hydrocarbons, alcohols, aldyhydes, ketones, carboxylic acids and esters. Chitosan could bind with these

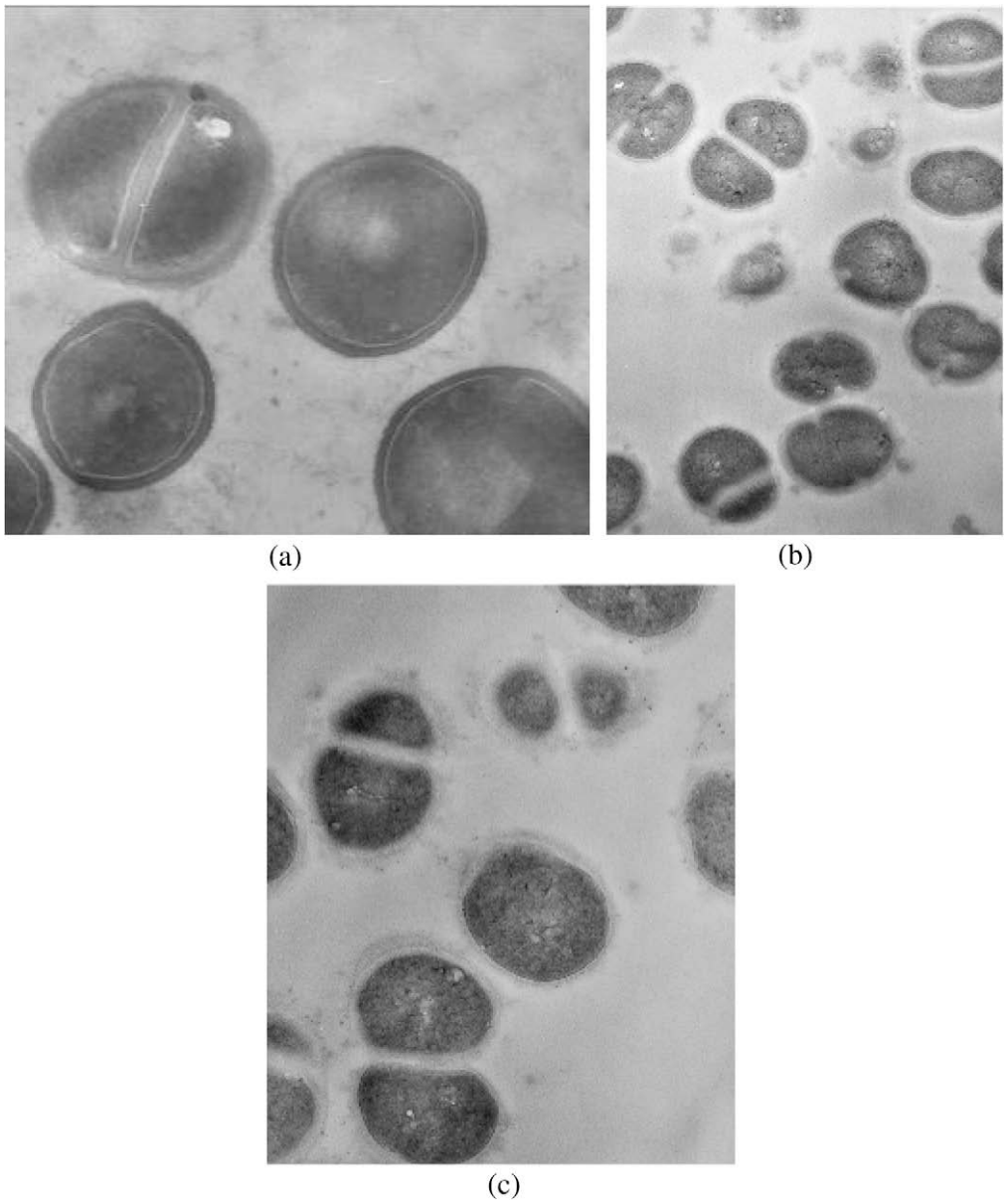

Fig. 6. Transmission electron micrographs of *S. aureus* (a) control cells (at 56,000× magnification); (b) cells treated with galangal extract (at 25,500× magnification); (c) cells treated with antimicrobial film (at 28,500× magnification).

compounds when the galangal extract was incorporated into films (Ravi Kumar et al., 2004).

3.3.2. Effects of drying methods and conditions

The FTIR spectra of chitosan films prepared by LPSSD and vacuum drying at 70 and 90 °C and incorporated with 0.6% (w/w) galangal extract were compared with those of the film prepared at ambient condition to investigate the effects of drying methods and conditions on functional group interaction. It is shown in Fig. 5 that drying methods and conditions had some effects on the FTIR spectra. Antimicrobial films prepared by LPSSD and vacuum drying showed higher peak intensity of amide linkage at 1542 cm^{-1} than ambient dried film. However, LPSSD led to films of lower intermolecular interaction, which was shown by the lower peak intensity, than vacuum drying.

The bacteria treated with the film prepared by vacuum drying at 90 °C exhibited the highest viable cell number; the rate of cell reduction was also significantly different from the cells treated with the film prepared by ambient drying. This may be due to a higher intermolecular interaction between chitosan and the antimicrobial agent, which is shown by an absorption peak at 1542 cm^{-1}. The viable cell number increased probably due to an increase in the intermolecular interaction, which limited the release of the antimicrobial agent from the film. Muzzarelli and Muzzarelli (2005) also reported that amidation, which is shown by an absorption peak at 1542 cm^{-1}, led to a decrease in swelling of the heat-treated chitosan films.

Regarding the effect of the drying temperature on intermolecular interaction, it was found that the peaks at 1542 cm^{-1} of the films dried at 90 °C were slightly sharper than those of the films dried at 70 °C. This might be due to the fact that higher temperature induced more interchain crosslinkage, which involves NH_2 group with amide formation (Lim, Khor, & Ling, 1999). Ritthidej et al. (2002) reported that longer time of heat treatment led to the stronger peak intensity of amide I band but in this study drying affected amide II vibration. Higher intermolecular interaction due

Table 3
Percentage of swelling of antimicrobial films.

Drying method	Galangal extract concentration		
	0.3%	0.6%	0.9%
Ambient drying	110.5 ± 4.4[a]	80.5 ± 3.1[a]	46.0 ± 4.9[a]
Hot air drying	105.4 ± 3.9[a]	74.5 ± 2.6[ab]	42.1 ± 3.6[a]
Vacuum drying			
70 °C	94.0 ± 3.9[bc]	62.5 ± 2.3[cd]	29.7 ± 2.3[bc]
80 °C	91.1 ± 2.1[bc]	58.8 ± 2.8[de]	23.3 ± 2.0[cd]
90 °C	87.2 ± 1.8[c]	54.1 ± 5.5[e]	21.7 ± 1.6[d]
LPSSD			
70 °C	96.3 ± 2.5[b]	70.5 ± 3.0[bc]	39.4 ± 2.5[a]
80 °C	92.5 ± 3.1[bc]	65.3 ± 4.2[cd]	32.4 ± 2.6[bc]
90 °C	90.4 ± 4.0[bc]	63.5 ± 2.7[cd]	29.9 ± 3.4[bc]

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

to the use of a higher drying temperature thus led to less release of the antimicrobial agent.

3.4. Swelling

The water resistance of films was characterized by the degree of swelling. Table 3 shows the effect of galangal extract concentration on the degree of film swelling. The results showed that the percentage of swelling for ambient dried chitosan film in distilled water was about 173%, which is quite comparable to the value observed by Yao et al. (1996). From our observation, the films still retained its integrity even after the swelling study of more than 24 h.

In comparison with the films made only with chitosan, the films prepared with added galangal extract exhibited lower degrees of swelling; swelling also decreased with an increase in the extract concentration. Swelling of the films was influenced by the hydrophobic nature of galangal extract (Qu, Wirsén, & Albertsson, 2000); incorporation of the extract into the chitosan matrix helped develop intermolecular interaction between chitosan and the extract, as shown by the FTIR results, and this led to a decrease in the film swelling.

Considering the effects of drying methods on the degree of swelling, it was found that swelling of LPSSD and vacuum dried films was significantly less than that of films prepared at ambient condition and at 40 °C (Table 3). The lower degrees of swelling could be due to an increase in the degree of crosslinkage. The higher extents of thermal crosslinkage in LPSSD and vacuum dried films resulted in the lesser extents of swelling due to more rigid chains (Cowan, 1999; Lim et al., 1999). In addition, thermal treatment of chitosan led to amide band formation at 1542 cm^{-1}, which reduced the number of hydrophilic groups, thus decreasing swelling of the films (Muzzarelli & Muzzarelli, 2005; Ritthidej et al., 2002).

The antimicrobial results showed that vacuum dried films, which had lower degrees of swelling compared with the films prepared by LPSSD and drying at control conditions (ambient drying and hot air drying at 40 °C), led to higher viable cell number. The degree of swelling indeed possessed a direct correlation with the release of the active agent from chitosan films; the release of active agent from chitosan films normally increased with the degree of swelling (Risbud, Hardikar, Bhat, & Bhonde, 2000; Wang, Du, Luo, Lin, & Kennedy, 2007).

In addition, it was found that the degree of swelling slightly decreased with an increase in the drying temperature. This is again because of thermal crosslinkage, which is accelerated by temperature (Lim & Wan, 1995). The decrease of the degree swelling could again be seen to affect the release of active agent and hence the antimicrobial activity of the films. From the viability test it was found that higher drying temperatures of both LPSSD and vacuum drying led to higher final viable cell number and slower decrease of the cell population.

The decrease in the cell number might also be related to the film microstructure (Berger et al., 2004; Risbud et al., 2000). High-porosity film enhanced swelling, which was responsible for more release of the antimicrobial agent. It was found that at the same drying temperature LPSSD films led to a higher reduction of the viable cell number than did vacuum dried films. This might be due to the higher porosity of the LPSSD films. It has been shown earlier that biomaterials undergoing LPSSD had higher porosity than the products undergoing vacuum drying (Léonard, Blacher, Nimmol, & Devahastin, 2008). However, optimum drying method and condition as well as concentration of galangal extract that should be incorporated into a film forming solution should be determined to increase the diffusion of the galangal extract from the film to the aqueous environment and to bacterial cells.

4. Conclusion

The present work illustrated that an increase in the galangal extract concentration in chitosan films leads to a higher antimicrobial activity against *S. aureus* and stronger functional group interaction. On the other hand, the degree of film swelling decreased. The antimicrobial activity, degree of swelling and intermolecular interaction were all affected by the tested drying methods and conditions. Ambient drying, low-temperature hot air drying and LPSSD at 70 °C led to films with higher antimicrobial activity and higher degrees of swelling due to lower intermolecular interaction, as shown by the results of the FTIR analysis. The mechanism of cell destruction of both antimicrobial films and galangal extract, as observed by TEM, involved cell wall and cell membrane disruption. Optimization of antimicrobial characteristics of chitosan films incorporated with galangal extract will be useful for the design of active packaging films, which could help prevent surface growth of microorganisms.

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Effects of drying methods and conditions on release characteristics of edible chitosan films enriched with Indian gooseberry extract

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ABSTRACT

The present work was aimed at studying the effects of drying methods and conditions (i.e., ambient drying, hot air drying at 40 °C, vacuum drying and low-pressure superheated steam drying within the temperature range of 70–90 °C at an absolute pressure of 10 kPa), as well as the concentration of Indian gooseberry extract, (added to edible chitosan film-forming solution as a natural antioxidant, at concentrations of 1, 2 and 3/100 g), on the residual total phenolic content (TPC) of the films. The swelling and release behaviour of TPC from the films were also studied. Drying methods and conditions were found to have significant effects on the percentage of residual TPC. The release characteristics, swelling and functional group interaction of the antioxidant films, as assessed by Fourier-transform infrared (FTIR) spectroscopy, were found to be affected by the drying methods and conditions, as well as the concentration of the Indian gooseberry extract.

1. Introduction

Edible films have recently received increased attention due to their various advantages including biodegradability and edibility. Among many materials that can be used to form edible films, chitosan (β-(1,4)-2-amino-2-deoxy-D-glucose) is one of the most promising as it has a good film-forming ability, which makes it suitable for use as a food packaging. Many investigators have evaluated chitosan films for many food-related applications (Han, Zhao, Leonard, & Traber, 2004; Sebti, Martial-Gros, Carnet-Pantiez, Grelier, & Coma, 2005). To make edible films even more useful, functional edible films that contain active agents have been developed, to enhance food quality and product shelf-life (Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Chitosan can be easily formed into films by a casting/solvent evaporation technique. Different drying methods and conditions have been used to prepare chitosan films. Numerous researchers prepared chitosan films by drying them at ambient temperature (Caner, Vergano, & Wiles, 1998; Hwang, Kim, Jung, Cho, & Park, 2003; Wiles, Vergano, Barron, Bunn, & Testin, 2000); other researchers prepared chitosan films by oven drying (Butler, Vergano, Testin, Bunn, & Wiles, 1996) or infrared drying (Srinivasa, Ramesh, Kumar, & Tharanathan, 2004). Recently, Mayachiew and Devahastin (2008a) investigated the influences of different drying methods and conditions on the drying kinetics and various properties of chitosan films. Drying at control conditions (ambient air drying and hot air drying at 40 °C) were compared with vacuum drying and low-pressure superheated steam drying (LPSSD) at an absolute pressure of 10 kPa and different drying temperatures (70, 80, and 90 °C). The properties of chitosan films, in terms of colour, tensile strength, percent elongation, water vapour permeability (WVP), glass transition temperature (T_g) and degree of crystallinity were determined. In terms of the drying kinetics, the drying methods were found to have a significant effect on the rates of moisture reduction of the samples. Vacuum drying and LPSSD required much shorter drying time than did ambient and hot air drying at 40 °C. In terms of properties it was found that LPSSD at 70 °C led to films with higher tensile strength and percent elongation than the films prepared by other drying methods and under other drying conditions. Ambient dried and LPSSD films had more crystallinity than the films dried by vacuum drying.

In terms of active agents that can be incorporated into films, plant extracts have recently received much attention and have a tendency of replacing synthetic agents. Generally, plant extracts which contain high concentrations of phenolic compounds possess strong antioxidant properties. Indian gooseberry (*Phyllanthus emblica* Linn.), or "Ma-khaam Pom" in Thai, is one of the most

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often-used herbs and is widely available in most tropical and subtropical countries. Indian gooseberry has been shown to possess high antioxidant activity *in vitro* (Mayachiew & Devahastin, 2008b).

Recently, many antioxidant edible films have been developed to reduce oxidation in packed foods (Gómez-Estaca, Bravo, Gómez-Guillén, Alemán, & Montero, 2009; Han & Krochta, 2007; Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004). For example, Oussalah et al. (2004) studied the use of milk protein-based films containing essential oils for the preservation of whole beef muscle. Films incorporated with oregano stabilised lipid oxidation in beef muscle samples, while films incorporated with pimento possessed the highest antioxidant activity. Gómez-Estaca et al. (2009) investigated the antioxidant properties of gelatin-based edible films containing oregano or rosemary aqueous extracts. It was found that the films supplemented with antioxidant extracts exhibited higher reducing ability and free radical-scavenging capacity than the control films; the degree of antioxidant power was generally proportional to the amount of the added extract.

For functional edible films, controlled release of active agents from the films to food is important, since there is a need to maintain the concentration of active compounds in packed food (Buonocore, Del Nobile, Panizza, Corbo, & Nicolais, 2003). Although many studies have been made on the effects of many parameters on the release of active agents from many types of films (Jeon, Park, Kwak, Lee, & Park, 2007; Tovar, Salafranca, Sánchez, & Nerín, 2005), the effects of drying methods and conditions used to prepare functional edible films, in particular chitosan films, on the retention and release characteristics of added antioxidant compounds have not been well established. The objectives of this study were therefore to investigate the effects of drying methods (i.e., ambient-temperature drying, hot air drying, vacuum drying, low-pressure superheated steam drying) and conditions on the total phenolic content (TPC) of chitosan films incorporated with Indian gooseberry extract. A study of the release behaviour of the antioxidant compound from the films was also conducted. Fourier-transform infrared (FTIR) spectroscopy was performed to investigate functional group interaction between chitosan and added active agent. Swelling of the films was also measured, to help explain the release behaviour of the films.

2. Materials and methods

2.1. Materials

Chitosan (molecular weight of 900,000 Da and degree of deacetylation of 90.2%) was obtained from S.K. Profishery Co., Ltd. (Bangkok, Thailand). Glycerol was purchased from Carlo Erba (Val de Reuil, Italy) while acetic acid was obtained from Merck (Darmstadt, Germany). Folin–Ciocalteu reagent, sodium carbonate and absolute ethyl alcohol were purchased from Carlo Erba (Vigevano, Italy).

2.2. Preparation of chitosan solution

Chitosan solution (1.5% w/v) was prepared by dissolving chitosan in 1% (v/v) acetic acid under constant stirring at 300 rpm using a magnetic stirrer (Framo®-Gerätechnik, model M21/1, Eisenbach, Germany) at room temperature for 24 h. Glycerol 25% (w/w chitosan) was then added into the chitosan solution; stirring was continued at room temperature for 1 h. After mixing the solution was centrifuged for 15 min at 12,400 rpm using a refrigerated centrifuge (Hitachi, model Himac CR21, Ibaragi, Japan) to remove undissolved impurities and bubbles in the solution.

2.3. Preparation of Indian gooseberry extract

To prepare Indian gooseberry extract, Indian gooseberry powder (10 g dry basis), dried by a tray dryer at 40 °C with particle size between 125–425 μm, was extracted with 50 ml of 95% (v/v) ethanol (Ahmad, Mehmood, & Mohammad, 1998). The extract was filtered through a filter paper (Ø110 mm, Cat. no. 1001 110, Schleicher and Schuell GmbH, Dassel, Germany); the filtrate was collected and concentrated using a rotary evaporator (Resona Technics, Labo Rota 300, Gossau, Switzerland) at 40 °C for 10 min and kept at 4 °C in a dark bottle until use. For a detailed preparation method the reader is referred to Mayachiew and Devahastin (2008b).

2.4. Preparation of antioxidant chitosan films

Indian gooseberry extract was added to the chitosan solution at concentrations of 1, 2 and 3/100 g. The concentration of the extract was varied since it was hypothesised that different concentrations of the extract might lead to different polymer structure modification, which would in turn affect the release behaviour of the active compound. All mixtures were homogenised by a bench top homogeniser (Ika® Works (Asia), Model T 25 basic, Selangor, Malaysia) at 9500 rpm for 2 min. The film solution (21 g) was poured onto an acrylic plate with dimensions of 13 × 10 cm to cast an antioxidant film. Drying of the film was performed by four methods, which were ambient air drying, hot air drying at 40 °C, and vacuum drying and low-pressure superheated steam drying (LPSSD) at 10 kPa, both at 70, 80 and 90 °C following the procedures of Mayachiew and Devahastin (2008a). After drying the film was conditioned for at least 48 h in a desiccator containing saturated salt solution of magnesium nitrate (Ajax Finechem, Seven Hills, Australia), which produced a relative humidity (RH) of 53%.

2.5. Determination of total phenolic content of films

The total phenolic content (TPC) of the antioxidant film was evaluated by an elution technique described by Zhang and Kosaraju (2007) with some modification. A portion of the film (0.5 g; 2 × 2 cm) was placed in 50 ml of 0.1 M NaOH and shaken with an environmental incubator shaker (New Brunswick Scientific, Model G-24, Edison, NJ) at room temperature for 48 h. The sample was then taken to determine the TPC, using Folin–Ciocalteu reagent (Zhou & Yu, 2006). The reaction mixture contained 50 μl of the eluted sample, 250 μl of the Folin–Ciocalteu reagent, 0.75 ml of 20 g/100 ml sodium carbonate and 3 ml of pure water. After 2 h of reaction at ambient temperature the absorbance at 765 nm was measured using a Shimadzu UV-2101 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan); the absorbance was used to calculate the TPC, using gallic acid as a standard. The results were presented in terms of the percentage of residual TPC. The percentage of residual TPC was calculated according to the following equation:

$$\%\ \text{Residual TPC} = 100 \times \left(\frac{\text{Mass of dried sample} \times (\text{conc. of TPC in dried sample})}{\text{Mass of film solution} \times (\text{conc. of TPC in film solution})}\right) \quad (1)$$

where the mass of dried sample is the mass of the sample after a drying procedure has been applied.

2.6. Release of antioxidant agent from films

A release test was conducted in an environmental incubator shaker at room temperature. A film (2 × 2 cm) was placed in a bea-

ker containing 100 ml of distilled water. The solution sample was taken out at 15 min intervals until 1 h and every 1 h afterward, to determine the TPC of the solution in order to follow the release kinetics of the antioxidant from the film. The release result was reported in terms of the percentage of release of TPC:

$$\%\ \text{Released TPC}_t = 100 \times \frac{\text{TPC}_t}{\text{TPC}_0}. \quad (2)$$

where TPC_0 is the amount of TPC after drying, TPC_t is the amount of release of TPC at any time.

2.7. FTIR analysis

Attenuated total reflection/Fourier-transform infrared spectroscopic (ATR/FTIR) spectra were collected at 25 °C by coupling the ATR accessory to an FTIR spectrometer (Perkin–Elmer Inc., Model 1760X, Norfolk, CT) available at Chulalongkorn University, Bangkok. Time-resolved experiments were collected by averaging, depending on the experiment, 10 or 2 scans at 4 cm^{-1} spectral resolution at various time intervals.

2.8. Swelling of antioxidant films

A film was cut into sizes of 2 × 2 cm and dried in a vacuum oven (Sanyo, Model Gallenkamp/OM-09980, Loughborough, UK) at 70 °C at an absolute pressure of 100 mbar for 24 h. The film was weighed and left at ambient temperature in 30 ml of distilled water for 24 h. The film was then blotted with a tissue paper and the mass of the film was measured periodically with a microbalance (Sartorius, Model RC 250S, Göttingen, Germany) until equilibrium was reached. The degree of swelling was evaluated using Eq. (3):

$$\text{Degree of swelling} = 100 \times \left(\frac{\text{Mass of film (g)} - \text{Mass of dried film (g)}}{\text{Mass of dried film (g)}}\right) \quad (3)$$

2.9. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SPSS® software (Chicago, IL) and were presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95%. All experiments were performed in duplicate except where stated otherwise.

3. Results and discussion

3.1. Total phenolic content of antioxidant films

Phenolic compounds in plants have high antioxidant activities, mainly due to redox properties, which include free radical-scavenging, hydrogen-donating and singlet oxygen-quenching. Mayachiew and Devahastin (2008b) reported that Indian gooseberry extract had high antioxidant activity *in vitro* and that phenolics were the main compounds of the extract.

Table 1 shows the percentage of residual TPC of chitosan films incorporated with various concentrations of Indian gooseberry extract and prepared by different drying methods and conditions; the drying time to reach the desired final film moisture content of 14% (d.b.) is listed in Table 2. Indian gooseberry extract concentration did not seem to have any significant effect on the percentage of residual TPC. However, antioxidant films incorporated with higher concentrations of the extract showed higher intensity of absorption peaks at 1250, 1620 and 1720 cm^{-1} and lower intensity of absorption peak at 1566 cm^{-1} (Fig. 4), indicating more intermolecular interaction between chitosan film and Indian gooseberry extract when the concentration of the added extract increased.

Table 1
Percentage of residual total phenolic content of antioxidant films prepared by different drying methods and conditions.

Drying method	Indian gooseberry extract concentration		
	1%	2%	3%
Ambient drying	97.90 ± 1.88[a]	98.18 ± 1.32[a]	98.67 ± 0.49[a]
Hot air drying	96.54 ± 2.20[a]	96.50 ± 1.08[a]	96.68 ± 1.07[a]
Vacuum drying			
70 °C	84.78 ± 1.59[c]	85.65 ± 0.60[bc]	85.36 ± 0.49[c]
80 °C	81.65 ± 1.13[d]	81.83 ± 1.20[de]	82.69 ± 1.07[d]
90 °C	76.58 ± 1.24[e]	77.18 ± 1.08[f]	77.47 ± 0.66[f]
LPSSD			
70 °C	87.82 ± 1.81[b]	86.42 ± 0.72[b]	87.76 ± 0.33[b]
80 °C	83.81 ± 1.59[c]	84.72 ± 0.48[cd]	84.44 ± 1.40[c]
90 °C	79.65 ± 1.59[de]	80.15 ± 0.96[e]	80.39 ± 0.74[e]

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

Table 2
Drying time to reach final moisture content of 14% (d.b.).

Drying method	Indian gooseberry extract concentration		
	1%	2%	3%
Ambient drying	52 h	50 h	47 h
Hot air drying	17 h	16 h	15 h
Vacuum drying			
70 °C	85 min	84 min	84 min
80 °C	65 min	64 min	64 min
90 °C	60 min	59 min	59 min
LPSSD			
70 °C	130 min	129 min	129 min
80 °C	95 min	94 min	94 min
90 °C	90 min	89 min	89 min

Regarding the effects of drying methods and conditions, it was noted that drying methods and conditions had significant effects on the percentage of residual TPC of the films. It was found that ambient dried films had the highest residual TPC. The higher losses of TPC were observed for films dried by vacuum drying and LPSSD; LPSSD films had slightly higher residual TPC than the vacuum-dried films, however. This may be due to the fact that the film temperature increased more rapidly and stayed at higher levels in the case of vacuum drying, thus inducing more thermal degradation of TPC compared with the LPSSD films (Mayachiew & Devahastin, 2008a). The temperature of the vacuum-dried films increased more rapidly than that of the LPSSD films, due to the fact that the electric heater was used more often during vacuum drying, since it was the only source of energy for drying. This might increase the amount of radiation absorbed by the film surfaces. The constant rate drying periods of vacuum-dried films were also shorter than those of LPSSD films. These are typical comparative characteristics of vacuum drying and LPSSD (Devahastin, Suvarnakuta, Soponronnarit, & Mujumdar, 2004).

Higher drying temperatures led to a lower percentage of residual TPC in both vacuum-dried films and LPSSD films. The antioxidant films prepared by LPSSD at 70 °C had the highest percentage of residual TPC, compared with films prepared at other conditions of LPSSD and vacuum drying. It is indeed well recognised that thermal treatment induces degradation of phenolic compounds (Chan et al., 2009; Guan, Cenkowski, & Hydamaka, 2005; Larrauri, Rupérez, & Saura-Calixto, 1997). The minimum percentage of residual TPC was found in the case of chitosan film incorporated with 1% Indian gooseberry extract and prepared by vacuum

drying at 90 °C. The presence of oxygen in the case of ambient and low-temperature hot air drying did not seem to have any significant effect on the percentage of residual TPC.

3.2. Release of antioxidant agent from films

Figs. 1–3 show the evolution of the percentage of release of TPC from antioxidant films prepared by different drying methods and conditions. At the same concentration of Indian gooseberry extract antioxidant chitosan films prepared by ambient drying, hot air drying and LPSSD at 70 °C showed the highest percentage of release of TPC; the release was lowest in the case of the films prepared by vacuum drying at 90 °C. This might be due to the different intermolecular interactions between the extract and chitosan films (as shown by the results of FTIR spectra in Section 3.3), the microstructure of the films, as well as different degrees of film swelling. The results on film swelling will be discussed in further detail in Section 3.4.

It can be seen in Fig. 1 that the percentage of TPC released from chitosan films incorporated with 1% (w/w) Indian gooseberry extract prepared by ambient drying, hot air drying and LPSSD at 70, 80 and 90 °C reached 92%, 90%, 87%, 84% and 79% within 24 h, respectively. The percentage of TPC released from vacuum-dried films at 70, 80 and 90 °C at 24 h was approximately 86%, 81% and 75%, respectively. TPC was released from LPSSD films faster than from vacuum-dried films prepared at the same temperature. The same trends were found in the cases of chitosan films incorporated with 2% and 3% (w/w) Indian gooseberry extract (see Figs. 2 and 3).

Regarding the effect of Indian gooseberry extract concentration, the percentage of antioxidant released decreased when the Indian gooseberry extract concentration increased. Chitosan films incorporated with 3% (w/w) Indian gooseberry extract had lower percentage of antioxidant release than the films incorporated with 1% and 2% extract. This might also be due to the effect of higher intermolecular interaction.

The release profiles of the antioxidant films could be separated into two parts. During an initial period (0–8 h), the release profiles were generally non-linear; the release of TPC was rapid at first and then slowed down with an increase in time. However, the release profiles of the films incorporated with 1% Indian gooseberry extract were linear within 2 h, which might be due to the fact that the films required a shorter time to become fully hydrated, as shown later by a higher degree of swelling; the rapid release of TPC from the films was thus attributed to the rapid swelling of the films in distilled water.

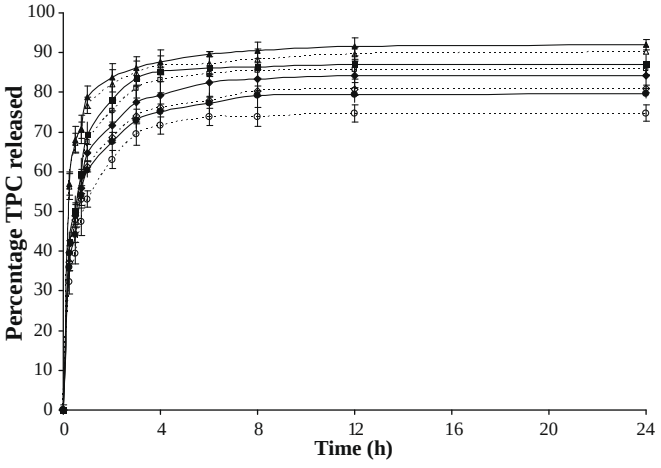

Fig. 1. Percentage of release of TPC from chitosan films enriched with 1% (w/w) Indian gooseberry extract and prepared by ambient drying (—▲—); hot air drying at 40 °C (---△---); LPSSD at 70 °C (—■—), 80 °C (—◆—), 90 °C (—●—); vacuum drying at 70 °C (---□---), 80 °C (---◇---), 90 °C (---○---).

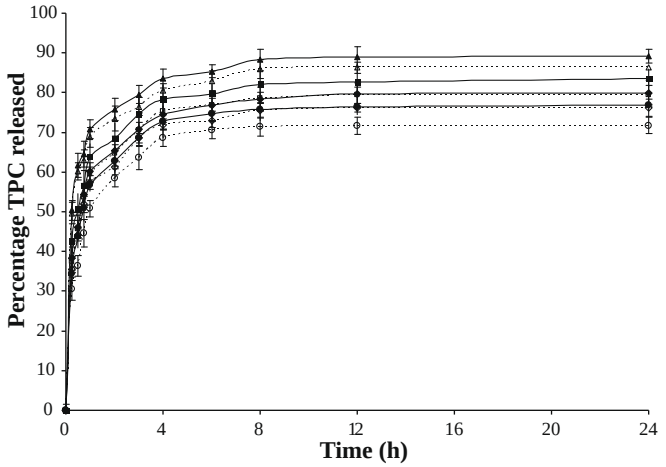

Fig. 2. Percentage of release of TPC from chitosan films enriched with 2% (w/w) Indian gooseberry extract and prepared by ambient drying (—▲—); hot air drying at 40 °C (---△---); LPSSD at 70 °C (—■—), 80 °C (—◆—), 90 °C (—●—); vacuum drying at 70 °C (---□---), 80 °C (---◇---), 90 °C (---○---).

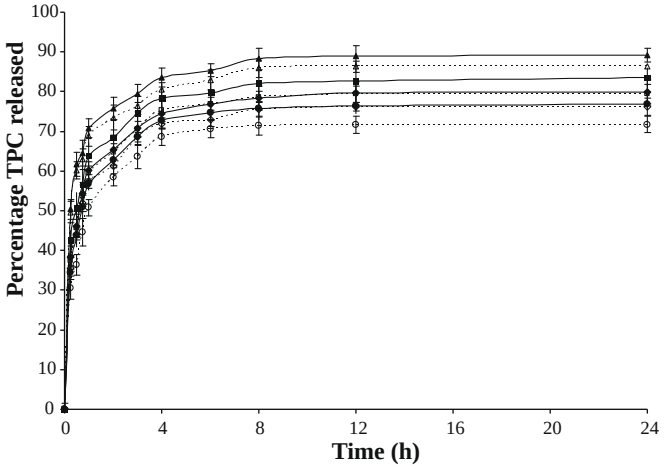

Fig. 3. Percentage of release of TPC from chitosan films enriched with 3% (w/w) Indian gooseberry extract and prepared by ambient drying (—▲—); hot air drying at 40 °C (---△---); LPSSD at 70 °C (—■—), 80 °C (—◆—), 90 °C (—●—); vacuum drying at 70 °C (---□---), 80 °C (---◇---), 90 °C (---○---).

In the second period (8–24 h) the release was rather constant. The constant rate of release might be due to the interaction of the extract with the chitosan matrix. Popa, Aelenei, Popa, and Andrei (2000) studied the interaction between chitosan and polyphenols extracted from spruce wood bark and found that chitosan and polyphenols formed a complex and that the release of polyphenols followed a two-step process as well.

Chitosan might interact with phenolic compounds of Indian gooseberry extract, mainly by weak interactions such as hydrogen bonding. Hydroxyl groups of phenolics could form H-bonds with NH_3^+ of chitosan (Kanatt, Chander, & Sharma, 2008). Moreover, the amine groups probably also contributed to ionic interactions with carboxylic groups of acid phenols (Spagna et al., 1996). These bound fractions could affect the release of phenolic compounds. On the other hand, phenolics retention might be achieved through strong covalent bonds (Popa et al. 2000).

3.3. FTIR analysis of antioxidant films

3.3.1. Effect of antioxidant agent

The interaction between chitosan and the extract was characterised by FTIR analysis. Fig. 4 shows the transmission infrared spectra of the chitosan films enriched with different concentrations of Indian gooseberry extract and prepared by ambient drying. Pure chitosan film had characteristic bands at 1631 cm^{-1} (amide I band) and 1566 cm^{-1} (amide II vibration). The present FTIR spectra are similar to the results described by many studies (Pranoto, Rakshit, & Salokhe, 2005; Ritthidej, Phaechamud, & Koizumi, 2002). Chitosan films showed a symmetric carboxylate anion stretching at 1413 cm^{-1}, indicating free chitosan films (Puttipipatkhachorn, Nunthanid, Yamamoto, & Peck, 2001).

When chitosan films were incorporated with Indian gooseberry extract, changes in FTIR spectra were noted. The spectra of antioxidant films exhibited small modifications of the positions of some bands within the range 1500–1700 cm^{-1}, which are related to amine and carbonyl groups. The new absorption peak at 1620 cm^{-1} (amide I band) was shifted from 1631 cm^{-1}. In addition, the peak was sharper with an increase in the extract concentration, indicating more interaction between chitosan and the extract. Pasanphan and Chirachanchai (2008) reported that chitosan could form a linkage with phenolic compounds, such as gallic acid, *via* an amide linkage, which is shown as the absorption peak at 1620 cm^{-1}.

Another important change was found as new peaks at 1720 cm^{-1}, indicating an ester linkage, which could be attributed to the more intensive interaction between chitosan and Indian gooseberry extract. However, the peak intensity at 1566 cm^{-1}, which is attributed to the amine group ($-NH_2$) of chitosan, decreased. The loss of this peak might be due to the interaction of the amine group with a functional group of the extract. In addition, the peak at 1250 cm^{-1}, which corresponds to C–O stretching of the phenol compounds, was stronger with an increase in the concentration of the extract.

Structurally, phenolics are compounds consisting of aromatic rings bonded to one or more hydroxyl substituents and range from simple phenolic molecules to highly polymerised compounds. Phenolic compounds are naturally present as conjugates with polysaccharides, linked to one or more of the phenolic groups and may also occur as functional derivatives such as esters (Balasundram, Sundram, & Samman, 2006). Therefore, chitosan could bind with phenolics when Indian gooseberry extract was incorporated into films.

3.3.2. Effects of drying methods and conditions

The effects of drying methods and conditions on functional group interaction were also investigated. The IR spectra of chitosan films enriched with 2% (w/w) Indian gooseberry extract and prepared by vacuum drying and LPSSD at 70 and 90 °C were compared with those of the film prepared at ambient conditions. It is seen in Fig. 5 that drying methods and conditions had some effects on the IR spectra. Antioxidant films prepared by LPSSD and vacuum drying showed stronger peak intensity of the amide linkage at 1620 cm^{-1} than did ambient dried film. However, LPSSD led to films of lower intermolecular interaction, which was shown by the lower peak intensity of the amide linkage at 1620 cm^{-1}, relative to that from vacuum drying. Drying led to intermolecular interaction in the films, which was shown by the alteration of the peak intensity at 1620 cm^{-1}.

The percentage of release of TPC decreased, due probably to an increase in the intermolecular interaction. The antioxidant film

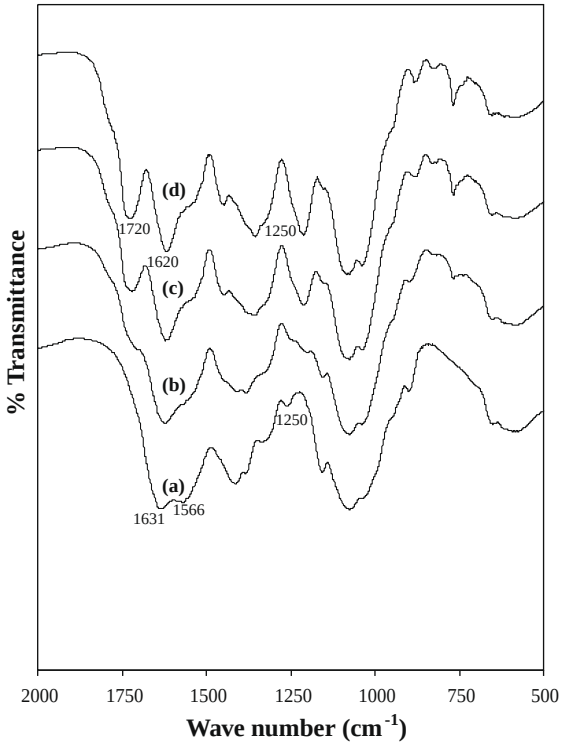

Fig. 4. FTIR spectra of ambient dried films with different concentrations of Indian gooseberry extract: (a) with no extract, (b) with 1% (w/w) Indian gooseberry extract, (c) with 2% (w/w) Indian gooseberry extract, (d) with 3% (w/w) Indian gooseberry extract.

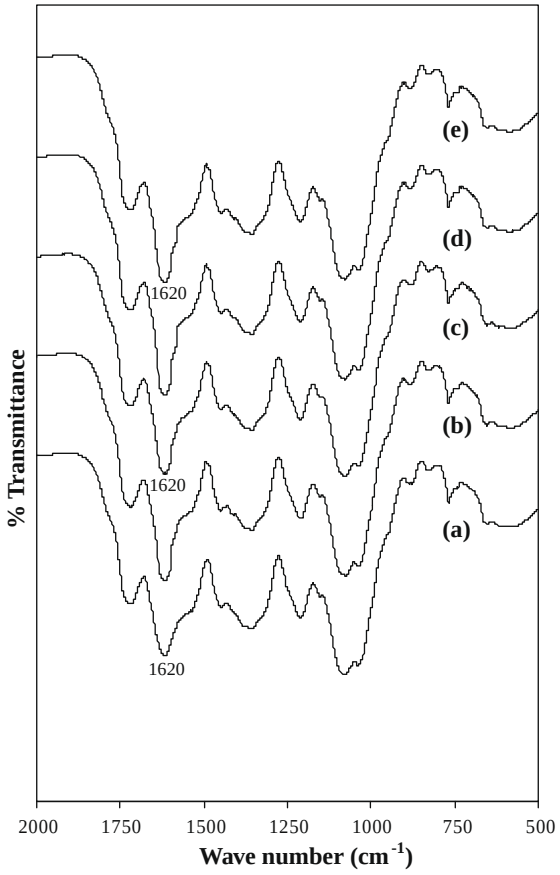

Fig. 5. FTIR spectra of chitosan films enriched with 2% (w/w) of Indian gooseberry extract prepared using different drying methods and conditions: (a) ambient drying, (b) vacuum drying at 70 °C, (c) LPSSD at 70 °C, (d) vacuum drying at 90 °C and (e) LPSSD at 90 °C.

dried by vacuum drying at 90 °C exhibited the lowest percentage of release of TPC and showed a different release profile compared with the film prepared by ambient drying. This may be due to the high intermolecular interaction between chitosan and phenolic compounds, such as gallic acid, in Indian gooseberry extract via amide linkage, which was shown by an absorption peak at 1620 cm^{-1} (Pasanphan & Chirachanchai, 2008). Muzzarelli and Muzzarelli (2005) reported that amidation, which was shown by the absorption peak at 1620 cm^{-1}, might lead to a decrease in swelling of the heat-treated films, resulting in the lower percentage of antioxidant release.

Regarding the effect of the drying temperature on intermolecular interaction, it was found that the peaks at 1620 cm^{-1} of the films dried at 90 °C were slightly sharper than those of the films dried at 70 °C. This might be due to the fact that the higher temperature induced more interchain crosslinkage, which involves NH_2 group with amide formation (Lim, Khor, & Ling, 1999). Ritthidej et al. (2002) reported that longer time of heat treatment led to a stronger peak intensity of amide I band. Higher intermolecular interaction due to the use of higher drying temperature might thus lead to the lower percentage of release of TPC mentioned earlier in Section 3.2. Lower percentage of release of TPC was observed for both LPSSD and vacuum-dried films.

Suppakul et al. (2003) indeed stated that different interactions between an active agent and chitosan had an effect on the release of the active agent from the film. Drying methods and conditions may thus be used to engineer chitosan films for controlled release applications.

3.3.3. FTIR of films after release study

FTIR spectroscopy was performed to determine the characteristics of the film matrix as well as the changes of the intermolecular interaction both before and after the release study. By considering the IR spectra of chitosan film enriched with 2% (w/w) Indian gooseberry extract prepared by ambient drying both before and after release study (Fig. 6) it was found that the polymer signal diminished when the film was in contact with water because of the sorption of moisture and subsequent film swelling (Lagaron, Fernandez-Saiz, & Ocio, 2007).

It was also found that the film after the release study exhibited a drop of the relative peak intensities at 1250, 1620 and 1720 cm^{-1}, indicating a decrease in the interaction of active agent with the film. The changes of the peak intensity could be due to the loss of active agent linkage with the chitosan matrix after being released into the liquid phase. It was indeed shown by the release profiles in Figs. 1–3 that the percentage of release of TPC from the films increased with time.

The peak remaining after release might be due to the interaction of the extract with the chitosan matrix, which was shown by the constant rate of release in the second period (8–24 h) of release study in Section 3.2. After the release study the film still retained some of the polymer signals, which indicated that part of the film still remained intact, as also observed visually.

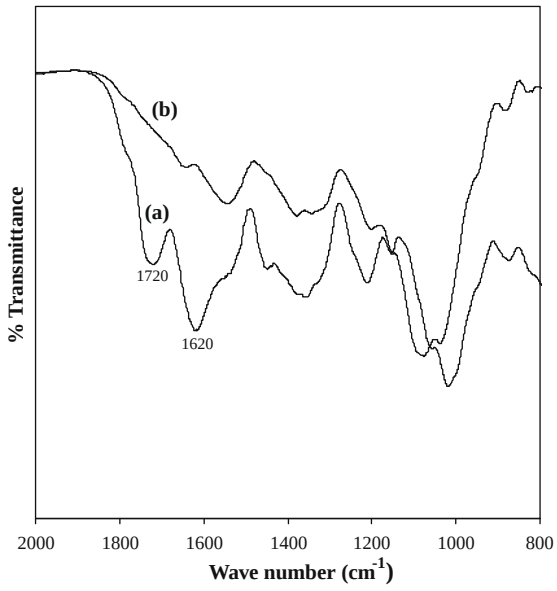

Fig. 6. FTIR spectra of chitosan films enriched with 2% (w/w) Indian gooseberry extract: (a) before release and (b) after release.

3.4. Swelling of antioxidant films

The water resistance of films was measured in terms of swelling as shown in Table 3. It was observed that the swelling ability of antioxidant films was affected by the extract concentration. Swelling of pure chitosan film prepared at ambient conditions was around 173%, a value which is similar to that of Yao et al. (1996). The high value of swelling is due to the hydrophilic characteristic of chitosan. Nevertheless, from our observation, the films did not break apart even after the swelling study.

Swelling of the films was reduced when the films were incorporated with the extract. Although chitosan has hydrophilic groups, such as carboxylic groups, in its structure and these groups could easily interact with water, resulting in swelling of the films, upon being enriched with the extract intermolecular interaction between chitosan and the extract developed and this resulted in a decrease in the film swelling. In addition, a lower level of swelling could be attributed to the hydrophobic properties of the extract. Di Pierro et al. (2006) also reported that the degree of swelling of a polymeric material strongly depends on the amount and nature of intermolecular chain interactions.

It was noted that drying methods significantly affected the degree of swelling. It is seen in Table 3 that swelling of LPSSD and vacuum-dried films was less than that of films prepared at ambient conditions and at 40 °C. Swelling of the films could be reduced through an increase in the degree of cross-linkage. The lesser extents of swelling of LPSSD and vacuum-dried films were because of the higher extents of thermal cross-linkage, thus inducing less swelling of the films due to more rigid chains (Lim et al. 1999; Mayachiew & Devahastin, 2008a). In addition, thermal treatment of chitosan led to amide formation, which reduced the number of hydrophilic groups, thus decreasing swelling of the films (Muzzarelli & Muzzarelli, 2005; Ritthidej et al. 2002).

In addition, it was found that the degree of swelling slightly decreased with an increase in the drying temperature. This is again

Table 3
Percentage of swelling of antioxidant films.

Drying method	Indian gooseberry extract concentration		
	1%	2%	3%
Ambient drying	124.8 ± 2.8^{a}	84.0 ± 1.9^{a}	56.2 ± 0.9^{a}
Hot air drying	123.5 ± 2.6^{a}	83.0 ± 2.2^{a}	55.8 ± 1.6^{a}
Vacuum drying			
70 °C	103.9 ± 2.2^{bc}	68.3 ± 3.0^{bc}	44.0 ± 2.3^{bc}
80 °C	97.7 ± 1.9^{de}	64.3 ± 3.1^{cd}	39.9 ± 1.2^{cde}
90 °C	91.6 ± 2.8^{f}	60.7 ± 3.9^{d}	36.2 ± 2.9^{e}
LPSSD			
70 °C	109.4 ± 2.6^{bc}	73.4 ± 1.2^{b}	46.0 ± 2.6^{b}
80 °C	100.0 ± 2.7^{de}	66.6 ± 1.7^{cd}	42.6 ± 1.7^{bcd}
90 °C	95.6 ± 3.0^{ef}	62.4 ± 2.5^{cd}	38.4 ± 2.1^{de}

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

because of thermal cross-linkage, which is accelerated by temperature (Lim & Wan, 1995). From release results in Figs. 1–3 it was found that higher drying temperatures of both LPSSD and vacuum drying led to lower percentage of release of TPC.

The results indicated that vacuum-dried films, which had lower degrees of swelling compared with the films prepared by LPSSD and drying at control conditions, showed the lowest percentage of release of TPC. The increased release that occurred with an increase in the degree of film swelling could be related to a change in the integrity of the film matrix (Sujja-areevath, Munday, Cox, & Khan, 1998). This is because the films might undergo relaxation during the release process, making the films become more flexible. This allowed the extract to diffuse out of the matrix more easily (Wu, Wang, Tan, Moochhala, & Yang, 2005). It was found from the release study that the films with lower degrees of swelling had lower percentage of release of TPC. Risbud, Hardikar, Bhat, and Bhonde (2000) also reported a direct correlation between the degree of swelling and release of antibiotics from chitosan films; the release of active agent from chitosan films normally increased with the degree of swelling.

In addition, release of the extract from the films might also be related to the film microstructure (Berger et al., 2004; Risbud et al., 2000). High-porosity film enhanced swelling, which was responsible for the release. It was found that at the same drying temperature LPSSD films had higher percentage of release of TPC than vacuum-dried films. This might be due to the higher porosity of the LPSSD films. It has been shown earlier that biomaterials undergoing LPSSD had higher porosity than products undergoing vacuum drying (Léonard, Blacher, Nimmol, & Devahastin, 2008).

4. Conclusion

The effects of drying methods and conditions on the percentage of residual TPC, release characteristics, intermolecular interaction and degree of swelling of chitosan films incorporated with different concentrations of Indian gooseberry extract were investigated. An increase in the extract concentration led to stronger functional group interaction, while the degree of swelling and percentage of release of TPC from antioxidant films decreased. The percentage of residual TPC, percentage of release of TPC, degree of swelling and functional group interaction were affected by drying methods and conditions. Ambient drying, low-temperature hot air drying and LPSSD at 70 °C led to antioxidant chitosan films with less intermolecular interaction, higher degree of swelling and percentage of release of TPC than did the other drying methods and conditions. Drying methods and conditions may be used to engineer chitosan films for controlled release in food packaging applications. Work is underway to develop a mathematical model that can be used to explain the release characteristics of TPC from chitosan films.

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Effects of drying methods and plasticizer concentration on some physical and mechanical properties of edible chitosan films

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ABSTRACT

In order to alleviate shortcomings of edible chitosan films, which are rigid and brittle in nature, an idea of using advanced drying methods, in combination with appropriate concentration of plasticizer, to improve the mechanical properties of the films was proposed and tested. Physical (thickness and color) and mechanical (tensile strength and percent elongation) properties of edible chitosan films plasticized at four glycerol concentrations (0%, 25%, 75% and 125% w/w chitosan) and prepared by three drying methods, namely, hot air drying (≈40 °C), vacuum drying and low-pressure superheated steam drying (LPSSD) (90 °C, 10 kPa) were investigated. Dynamic mechanical thermal analysis (DMTA) was used to determine the glass transition temperature to verify the compactness of edible chitosan films. It was found that the drying methods and plasticizer concentration significantly affected the drying time, tensile strength, percent elongation and glass transition temperature of the films. On the other hand, the drying methods and plasticizer concentration did not affect the thickness and final moisture content of the film samples at lower glycerol concentrations. In the cases of vacuum drying and LPSSD, there was a limiting value of plasticizer concentration (25% w/w) beyond which the effect of the plasticizer concentration on the mechanical properties was negligible. In all cases, the color of all tested films was not significantly different.

1. Introduction

Food manufacturer and distributor have regularly faced with problems related to product deterioration during distribution. It is obvious that the value of a food product is based on its quality, which simply decreases with time. Use of various packaging materials could help prolong the shelf life of a food product by protecting it from various deterioration factors (Beaudry, 2007). Currently, the most popular packagings in food applications are plastics, e.g., low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polyvinyl chloride (PVC), due to their non-breakable, light weight and easy handling characteristics. However, two serious problems of plastic packagings exist. First of all, these packagings have short use time and turn to waste quickly. The quantity of waste from plastic packagings, which are not biodegradable and persist in the environment for a very long period of time, is now very high (Callegarin et al., 1997; Imam et al., 2005; Mathew and Abraham, 2008). Another problem is that plastic materials are not completely inert. Chemical substances such as plasticizers and stabilizers can migrate from plastics to food and may lead to food quality changes and consumer health risk (Callegarin et al., 1997).

In recent years, more attention has been placed on edible biopolymer packagings as they are environmentally friendly and also naturally biodegradable (Imam et al., 2005). Among many natural biomaterials that can be used to produce biodegradable and/or edible packagings, chitosan is one of the most promising materials. Chitosan is a polysaccharide generally obtained by *N*-deacetylation of chitin. It is commercially available from a stable renewable source, i.e., shellfish waste (shrimp and crab shell) of the seafood industry. Comparing with other polysaccharides, chitosan has several important advantages, including biocompatibility, biodegradability and non-toxicity. Several studies on chitosan have reported this material as a potential candidate for edible films (Ziani et al., 2008).

However, the nature of edible packaging films, which is rigid and brittle, causes limitations in food applications (Sothornvit and Krochta, 2001). It is well recognized that mechanical properties, including tensile strength and percent elongation, of synthetic packaging films are significantly better than those of edible films (Mathew and Abraham, 2008). In order to improve the mechanical properties of edible biopolymer films, various types of plasticizers have been used. Among many possible plasticizers, glycerol is

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widely used to plasticize edible films (Srinivasa et al., 2007; Oses et al., 2009).

Among many steps needed to prepare edible films, drying is considered one of the most important (Arvanitoyannis et al., 1998; Kaya and Kaya, 2000; Sothornvit and Krochta, 2005). Numerous investigators prepared edible films by drying film solutions at ambient temperature. Other researchers prepared films by oven drying, microwave drying, infrared drying, vacuum drying or low-pressure superheated steam drying (Arvanitoyannis et al., 1998; Kaya and Kaya, 2000; Srinivasa et al., 2004; Mayachiew and Devahastin, 2008). However, there were hardly any reports on the combined effects of drying methods and plasticizer concentration on the properties of chitosan films. Hence, the aim of this study was to investigate the combined effects of drying methods (hot air drying (≈40 °C), vacuum drying and low-pressure superheated steam drying (LPSSD) (90 °C, 10 kPa)) and plasticizer concentration (glycerol concentrations of 0%, 25%, 75% and 125% w/w chitosan) on selected physical (thickness and color) and mechanical (tensile strength and percent elongation) properties of edible chitosan films.

2. Materials and methods

2.1. Materials

Chitosan (molecular weight of 900,000 Da and degree of deacetylation of 90.20%) was purchased from S.K. Profishery Co., Ltd. (Bangkok, Thailand). Glycerol and acetic acid was obtained from Carlo Erba (Val de Reuil, Italy) and Merck (Darmstadt, Germany), respectively.

2.2. Film preparation

The film preparation method was that of Mayachiew and Devahastin (2008) with some modifications. Chitosan solution was prepared by dissolving chitosan at 1.5% (w/v) and glycerol at either 0%, 25%, 75% or 125% w/w chitosan in 1% (v/v) acetic acid under constant stirring via the use of a magnetic stirrer (Framo®-Gerätechnik, model M21/1, Eisenbach, Germany) at 300 rpm at room temperature for 24 h. After mixing the chitosan solution was centrifuged for 15 min at 12,400 rpm by a refrigerated centrifuge (Hitachi, model Himac CR21, Ibaragi, Japan) to remove undissolved impurities in the solution. Later, the solution was degassed using a sonicator (Ultrawave, model U1350, Cardiff, UK) at 30 kHz for 1 h. The chitosan solution (16 g) was then poured on an acrylic plate with dimensions of 13 × 10 cm to cast a chitosan layer for a drying experiment.

2.3. Film drying

2.3.1. Hot air drying

The prepared film solution was dried in a hot air tray dryer (≈40 °C) at an inlet air velocity of 0.25 m/s (Mayachiew and Devahastin, 2008). This drying process was used as a control drying process.

2.3.2. Low-pressure superheated steam drying

According to Mayachiew and Devahastin (2008) who prepared chitosan films using several drying methods and conditions viz. ambient (≈30 °C), hot air drying (≈40 °C), vacuum and LPSSD (70, 80 and 90 °C at 10 kPa), vacuum dried films prepared at 90 °C had the poorest mechanical properties. This film drying condition was then selected because if it was possible to improve the mechanical properties of the poorest films, the mechanical properties of the other films would also be acceptable. Hence, it was adequate to use this "worst case" drying temperature (90 °C) to investigate the combined effects of drying methods and glycerol concentration on the physical and mechanical properties of edible chitosan films in this study. The main criteria for the selection of the optimum processing conditions are both tensile strength and percent elongation of the films.

The prepared film solution was dried at 90 °C and 10 kPa in an LPSSD (Devahastin et al., 2004; Mayachiew and Devahastin, 2008). As suggested by Mayachiew and Devahastin (2008), the operating pressure was reduced in steps from an atmospheric pressure to 10 kPa; this was performed to avoid bubble formation in the film during drying.

2.3.3. Vacuum drying

For a vacuum drying experiment, the prepared film solution was dried in the same experimental setup as that used for the LPSSD experiments but without an application of steam to the drying chamber. The same drying condition (90 °C at 10 kPa) was used.

2.4. Film properties determination

After drying to a final moisture content of approximately 14% (d.b.) (Mayachiew and Devahastin, 2008), a film sample was conditioned for at least 48 h prior to further property determination in a desiccator containing saturated salt solution of sodium chloride (Ajax Finechem, Seven Hills, NSW, Australia), which produced an RH of 75% (an average relative humidity of the environment in Thailand).

2.4.1. Film thickness determination

The film thickness was measured using a micrometer (Mitutoyo, model 102-309, Tokyo, Japan) with an accuracy of ±2 μm. Each film sample was measured at its center and four other positions along the strip; an average value was reported. The mechanical properties were calculated using the average thickness of each film sample.

2.4.2. Moisture content determination

The moisture content of a film sample was determined using the standard vacuum oven method (AOAC, 1995). The film was dried in a vacuum oven (Sanyo, model Gallenkamp/OM-09980, Loughborough, UK) at 70 °C at a pressure of −900 mbar for 24 h.

2.4.3. Mechanical properties determination

The measurement of the mechanical properties of chitosan films was carried out using a texture analyzer (Stable MicroSystem, model TA.XT.Plus, Surrey, UK). After conditioning a chitosan film sample was cut into a 10 × 2.54 cm strip and tested for tensile strength and percent elongation according to the ASTM Standard Method D882 (ASTM, 1995). Initial grip separation and cross-head speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum load for breaking the film by its cross-sectional area. Percent elongation was determined by dividing the film elongation at rupture by the initial grip separation.

2.4.4. Dynamic mechanical thermal analysis (DMTA)

The determination of the glass transition temperature of chitosan films was carried out using a dynamic mechanical thermal analyzer (Mettler Toledo, model DMA/SDTA 861^e, Schwerzenbach, Switzerland). After conditioning a chitosan film sample was cut into a 10.5 × 6 mm strip and tested in the tensile mode. The force amplitude of 0.15 N and the displacement amplitude of 4 μm were applied. The storage modulus (E') and the tan δ of each film sample were obtained as a function of temperature over the range of −120

to 240 °C at a fixed frequency of 1 Hz and a heating rate of 5 °C/min.

2.4.5. Color determination

The color of a film sample was determined by a colorimeter (HunterLab, model ColorQuest, Reston, VA) in terms of L^*, a^* and b^* values. Each film sample was measured at its center and four other positions along the strip; average color values were reported.

2.4.6. Phase separation detection

Dried chitosan films were stored in a desiccator in the presence of P_2O_5 at ambient temperature (≈30 °C) for 1 week. After that the samples were equilibrated for another week under the environment of saturated NaCl salt solution, which produced a relative humidity of 75%. The equilibrium moisture contents of the samples were used to establish the sorption isotherms. From the sorption isotherms, phase separation was defined as a point (or glycerol concentration) beyond which the moisture content of each film sample increased; the moisture content increased was due to the fact that free (excess) glycerol separated from the film matrix could bind with moisture adsorbed by the film from the environment (Godbillot et al., 2006).

2.5. Statistical analysis

A full-factorial experimental design was used to schedule the experiments. All experimental data were analyzed using the analysis of variance (ANOVA) using SPSS® software (version 17). The results were presented as mean values with standard deviations. Duncan's multiple range tests were employed to establish differences between mean values at a confidence level of 95%. All experiments were performed in triplicate.

3. Results and discussion

3.1. Drying characteristics of edible chitosan films

The drying curves of edible chitosan films plasticized with various concentrations of glycerol undergoing hot air drying are shown in Fig. 1. The detailed moisture content evolution of the films during the final period of drying are shown in Fig. 2. It is seen from these figures that the film samples dried at this control condition required very long drying time. Different concentrations of glycerol provided films of more or less the same initial moisture contents but different equilibrium moisture contents. The equilibrium moisture content of the films increased with the glycerol concentration. Since glycerol is a hydrophilic plasticizer, higher concentration of glycerol led to more binding of the film matrix with water, hence higher moisture content at the equilibrium (Talja et al., 2007).

The drying curves of chitosan films plasticized with various concentrations of glycerol undergoing vacuum drying and LPSSD at 90 °C and 10 kPa are shown in Figs. 3 and 4, respectively. It can be seen from Figs. 1, 3 and 4 that, at the same glycerol concentration, the films dried at control condition required longer drying time than the films dried by vacuum drying and LPSSD. This is because vacuum drying and LPSSD involved the use of higher drying temperature than in the case of hot air drying. The temperature gradients between the film samples and the drying media were thus higher, hence a greater driving force for heat transfer, which was also related to the rate of mass transfer. However, at the same drying temperature (90 °C) LPSSD required longer drying time than vacuum drying. This is possibly due to a higher humidity condition in the drying chamber of LPSSD, resulting in a lower removal rate of moisture than in the case of vacuum drying. Moreover, during vacuum drying, the electric heater was used more often since it was the only source of energy for drying, whereas there were two sources of energy in the case of LPSSD (electric heater and superheated steam). Hence, the amount of radiation from the electric heater that the films undergoing vacuum drying might absorb was higher, leading to higher drying rates (Devahastin et al., 2004).

In all cases, when comparing the films dried using the same drying method, the drying rate decreased with increased glycerol concentration. This is because the films with higher glycerol concentrations could bind more water (Talja et al., 2007). In particular, the hot air dried films with high glycerol concentrations (at 75% and 125% w/w chitosan) had very low drying rates. This is due to the combined effect of the use of lower drying temperature and higher glycerol concentrations.

The average drying time to reach the desired final moisture content of 14% (d.b.) as well as the equilibrium moisture contents of chitosan films prepared by different drying methods are listed in Table 1. Chitosan films plasticized with all glycerol concentrations had initial moisture contents in the range of 54.52–57.83 kg/kg (d.b.). At the same glycerol concentration, it was noted that differ-

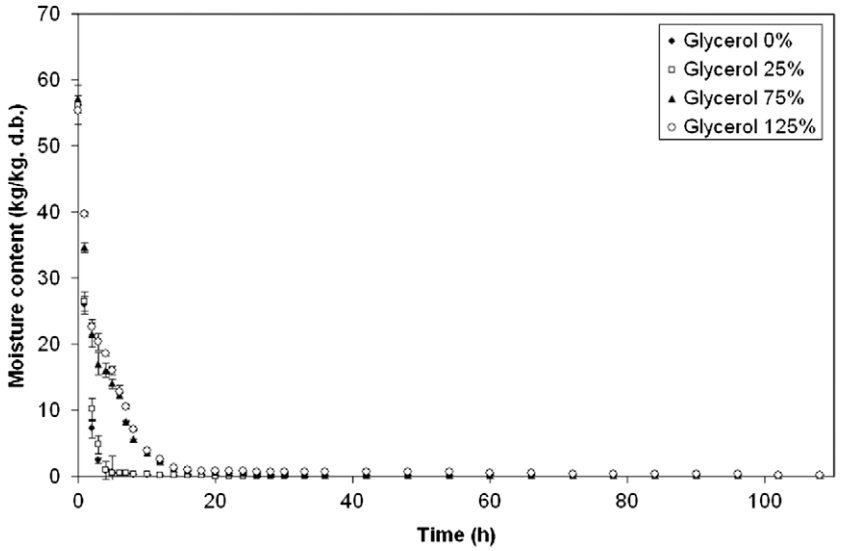

Fig. 1. Drying curves of edible chitosan films with various concentrations of glycerol undergoing hot air drying at 40 °C.

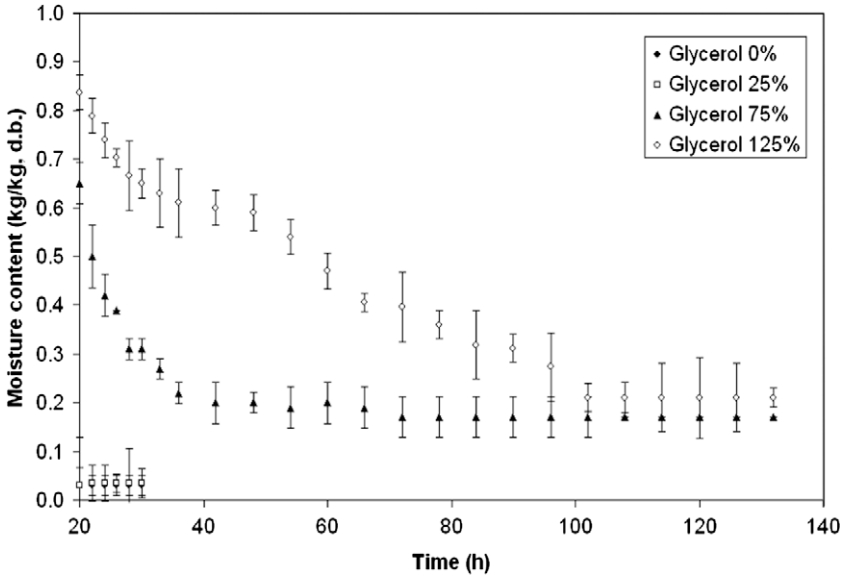

Fig. 2. Enlarged drying curves (during the final period of hot air drying) of edible chitosan films with various concentrations of glycerol.

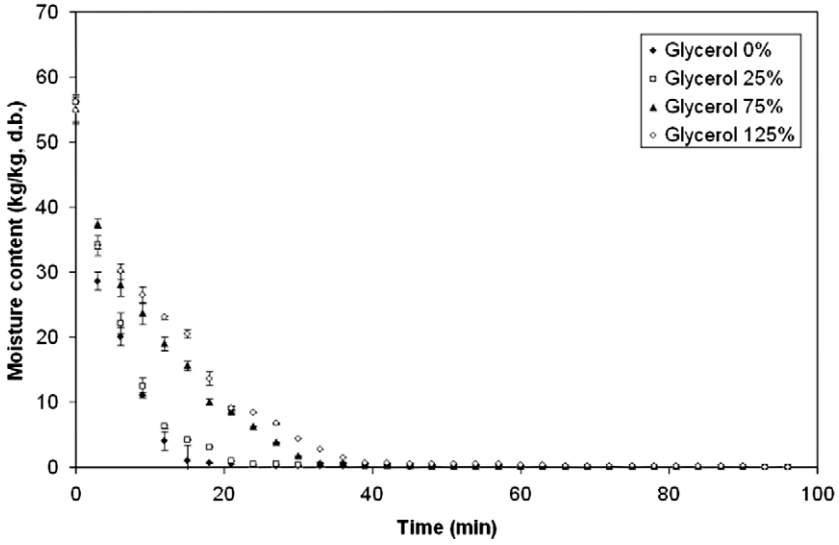

Fig. 3. Drying curves of edible chitosan films with various concentrations of glycerol undergoing vacuum drying at 90 °C, 10 kPa.

ent drying methods had significant effects on the required drying time to reach the desired final moisture content. It was also noted that at higher glycerol concentrations (at 75% and 125% w/w chitosan) it was not possible to air dry the films to the desired final moisture content; the equilibrium moisture contents of the films were higher than 14% (d.b.). However, in the cases of vacuum drying and LPSSD, due to the use of a higher temperature, the films could be dried to the desired final moisture content.

When comparing within the same drying method, it was noted that the time needed to dry films to 14% (d.b.) increased with the glycerol concentration. This is because glycerol is a hydrophilic plasticizer; films with a higher glycerol concentration could adsorb more water in their matrix and this led to increased drying time to reduce the film moisture.

The drying time of the films with 25% w/w glycerol in this study was shorter than that of the films prepared by Mayachiew and Devahastin (2008). This is because the mass of the film solution used in this study (16 g) was lower than that of the film solution used by Mayachiew and Devahastin (2008) (21 g).

3.2. Film thickness

The thickness of edible chitosan films plasticized with various concentrations of glycerol after conditioning at 75% relative humidity (RH) for 48 h is shown in Table 2. It was found that the glycerol concentration had a significant effect on the film thickness. In the case of hot air drying the thickness of edible chitosan films increased with the glycerol concentration. Since glycerol is a hydrophilic plasticizer, edible chitosan films with higher concentrations of glycerol adsorbed more moisture. Hence, these films swelled to a larger extent; this led to an increase in the film thickness. On the other hand, in the cases of vacuum drying and LPSSD, only the thickness of the films with a glycerol concentration of 125% w/w increased significantly. This is because the higher drying temperature in the cases of vacuum drying and LPSSD allowed for polymer chain arrangement and cohesion within the film matrix, resulting in a tighter and more compact structure (Perez-Gago and Krochta, 2000), hence less swelling of the films. The reader is referred to Section 3.4 for further discussion on this behavior. In

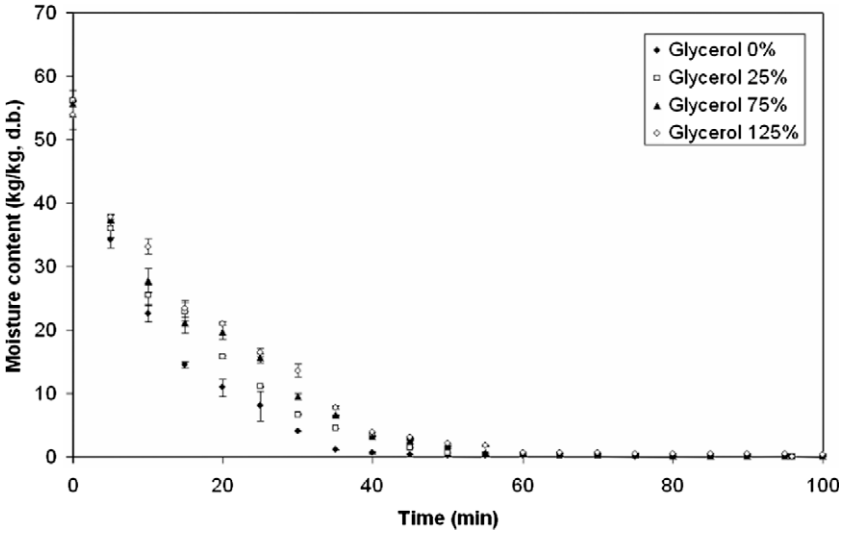

Fig. 4. Drying curves of edible chitosan films with various concentrations of glycerol undergoing LPSSD at 90 °C, 10 kPa.

Table 1
Average drying time to reach the final moisture content of 14% (d.b.) as well as equilibrium moisture content of edible chitosan films.

Drying condition/glycerol concentration (% w/w chitosan)	Average drying time to reach (14% d.b.)	Equilibrium moisture content (% d.b.)
Hot air drying (control ≈ 40 °C)		
0	10 h	4.3 ± 0.8[a]
25	12 h	4.3 ± 2.2[a]
75	72 h*	18.3 ± 3.3[b]
125	102 h*	22.4 ± 3.3[b]
Vacuum drying (90 °C, 10 kPa)		
0	45 min	4.4 ± 2.3[a]
25	55 min	4.8 ± 2.3[a]
75	80 min	4.3 ± 2.2[a]
125	95 min	5.2 ± 3.4[a]
LPSSD (90 °C, 10 kPa)		
0	60 min	3.3 ± 1.8[a]
25	80 min	4.2 ± 1.3[a]
75	95 min	3.8 ± 2.7[a]
125	110 min	4.8 ± 2.4[a]

Same letter in the same column means that the values are not significantly different at 95 % confidence level ($p > 0.05$).
* These films could not be dried to the desired final moisture content of 14% (d.b.); the time reported here is the time required to reach the equilibrium moisture content, which was higher than 14% (d.b.).

addition, heat treatment could also produce interchain cross-link and lead to amide formation, which reduced the amount of hydrophilic groups. The smaller number of hydrophilic groups led to less swelling (Mayachiew and Devahastin, 2010). Data on the film temperature evolution during drying are not shown here for the sake of brevity; the reader is referred to Suvarnakuta et al. (2005) for the detailed temperature evolution of any biomaterial undergoing the studied drying processes.

At the glycerol concentrations of 0%, 25% and 75% w/w the thickness of the films dried by all methods was not significantly different, except for the films with the glycerol concentration of 75% w/w and dried by hot air drying. In all cases, edible chitosan films with 125% w/w glycerol were thicker than the desired value of 15 μm; in the case of hot air drying the films with glycerol concentrations of 75% and 125% w/w suffered from this excessive thickness. Since all film forming solutions had similar solid contents, in the range of 54.52–57.83 kg/kg (d.b.), larger thickness of chitosan films with 125% w/w glycerol was most probably due to the higher concentration of glycerol. More glycerol implies that the films could adsorb more moisture; hence these films swelled to a larger extent.

3.3. Mechanical properties

In this study only two most relevant properties, namely, tensile strength and percent elongation, were tested. The mechanical properties of commercially available stretch films (Clean Wrap™ and M Wrap™) were also evaluated and compared with those of edible chitosan films after conditioning at 75% RH for 48 h. The results are shown in Table 2. It was noted that the glycerol concentration significantly affected the tensile strength and percent elongation of the films. In all cases, the films without glycerol had the highest tensile strength and lowest percent elongation. On the other hand, when glycerol was added, the films were more flexible. This is because glycerol penetrated through the polymer matrix and interfered with chitosan chains, decreasing intermolecular attraction and increasing polymer mobility, which led films to be more flexible (Ziani et al., 2008).

In the case of hot air drying the expected effect of glycerol on the tested mechanical properties, i.e., a decrease in the tensile strength and an increase in the percent elongation with increasing plasticizer concentration, was observed. On the other hand, in the cases of vacuum drying and LPSSD glycerol did not exhibit the conventional effect at the concentrations of 25–125% w/w; the tensile strength and percent elongation of the films plasticized at these glycerol concentrations were not significantly different. This might be due to the higher degrees of crystallinity and thermal cross-linkage that occurred more in the films during these two drying processes (Mayachiew and Devahastin, 2008). The intermolecular and intramolecular forces in the polymer chain thus increased and resisted polymer mobility. This led the vacuum and LPSSD dried films to be tighter and more compact than the hot air dried films (Perez-Gago and Krochta, 2000; Mayachiew and Devahastin, 2010). Again, the reader is referred to Section 3.4 for further discussion on this behavior. In addition, it was possible that the film matrix might be saturated at a plasticizer concentration of around 25% w/w, so further increase in the plasticizer concentration posed no additional effect on the matrix structure and hence the mechanical properties (Oses et al., 2009). Evidence on phase separation will be given and discussed in Section 3.6.

The changes of the tensile strength and percent elongation of the films prepared by vacuum drying and LPSSD did not agree with

the results of some earlier studies (Arvanitoyannis et al., 1998; Oses et al., 2009). In those earlier studies ambient drying and hot air drying were used to prepare films at low temperature and the tensile strength and percent elongation of those films generally decreased and increased with an increase in the plasticizer concentration, respectively.

The edible chitosan films prepared in this study had lower tensile strength and percent elongation than the films prepared by Mayachiew and Devahastin (2008). This is probably due to the use of a sonicator to degas the film solution. It is noted that the degassing process was the only difference between our films and the films of Mayachiew and Devahastin (2008). Sonication might induce chain scission of polymer (Kim and Lee, 2002). Hence, the films were ripped more easily than the films of Mayachiew and Devahastin (2008).

Based on the classifications of Krochta and Mulder-Johnson (1997) the films prepared in the present study had moderate mechanical properties (tensile strength and percent elongation of 10–100 MPa and 10–50%, respectively). On the other hand, the Thai Industrial Standard Institute (TISI, 1993) states that stretch films must have tensile strength and percent elongation of at least 4 MPa and 60%, respectively, for PVC; and of at least 3.3 MPa and 90%, respectively, for LDPE. From these standards, all edible chitosan films had lower percent elongation than that required by the TISI standard. However, all the films in this study had good quality in terms of tensile strength. Except for the hot air dried films plasticized with glycerol concentrations of 75% and 125% w/w, the tensile strength values of all other edible chitosan films were higher than those of the commercial stretch films. In all cases, percent elongation of edible chitosan films was lower than that of the commercial

Table 2
Moisture content, thickness, tensile strength and percent elongation of chitosan films and commercial stretch films.

Film type/glycerol concentration (% w/w chitosan)	Moisture content (% d.b.)		Thickness (μm)		Tensile strength (MPa)	Percent elongation (%)
	Before conditioning at 75% RH	After conditioning at 75% RH	Before conditioning at 75% RH	After conditioning at 75% RH		
Chitosan film dried by hot air drying at 40 °C						
0	14.4 ± 2.4^{b}	18.4 ± 4.3^{a}	15 ± 0.6^{b}	15 ± 0.6^{a}	64.3 ± 4.9^{e}	13.7 ± 2.0^{ab}
25	14.7 ± 1.8^{b}	19.3 ± 2.7^{a}	15 ± 0.6^{b}	15 ± 0.6^{a}	44.9 ± 3.1^{c}	16.9 ± 2.8^{ab}
75	18.3 ± 2.7^{bc}	26.2 ± 3.8^{bc}	29 ± 0.6^{c}	32 ± 3.2^{c}	15.8 ± 2.9^{b}	39.9 ± 3.6^{c}
125	22.4 ± 2.8^{c}	35.4 ± 4.2^{d}	45 ± 0.6^{d}	47 ± 4.2^{d}	8.7 ± 1.9^{a}	48.7 ± 5.6^{d}
Chitosan film dried by vacuum drying at 90 °C, 10 kPa						
0	14.2 ± 2.0^{b}	19.1 ± 1.7^{a}	15 ± 0.6^{b}	16 ± 0.6^{a}	60.5 ± 5.5^{de}	11.9 ± 3.3^{a}
25	15.3 ± 3.7^{b}	22.4 ± 3.2^{ab}	15 ± 0.6^{b}	15 ± 0.6^{a}	44.3 ± 5.9^{c}	14.2 ± 2.6^{ab}
75	14.3 ± 3.2^{b}	26.2 ± 2.8^{bc}	15 ± 0.6^{b}	15 ± 0.6^{a}	37.2 ± 1.8^{c}	14.8 ± 3.5^{ab}
125	14.2 ± 4.4^{b}	30.3 ± 3.3^{cd}	15 ± 0.6^{b}	19 ± 2.1^{ab}	39.6 ± 1.4^{c}	20.2 ± 5.2^{b}
Chitosan film dried by LPSSD at 90 °C, 10 kPa						
0	13.1 ± 3.3^{b}	18.1 ± 2.4^{a}	15 ± 0.6^{b}	15 ± 0.6^{a}	55.2 ± 4.0^{d}	12.1 ± 4.3^{a}
25	14.2 ± 4.2^{b}	20.6 ± 2.2^{a}	15 ± 0.6^{b}	15 ± 0.6^{a}	40.9 ± 2.9^{c}	19.3 ± 2.2^{b}
75	13.9 ± 3.4^{b}	20.3 ± 3.2^{a}	15 ± 0.6^{b}	15 ± 0.6^{a}	43.7 ± 1.9^{c}	19.0 ± 3.6^{b}
125	15.4 ± 2.7^{b}	28.7 ± 3.4^{c}	15 ± 0.6^{b}	20 ± 3.2^{b}	38.6 ± 2.8^{c}	20.2 ± 2.2^{b}
Clean Wrap™ (LDPE)	0.07 ± 0.02^{a}	–	13 ± 0.6^{a}	–	20.4 ± 2.0^{b}	67.8 ± 11.6^{e}
M Wrap™ (PVC)	0.06 ± 0.02^{a}	–	12 ± 0.6^{a}	–	22.0 ± 2.2^{b}	62.2 ± 10.0^{e}

Same letter in the same column means that the values are not significantly different at 95% confidence level ($p > 0.05$).

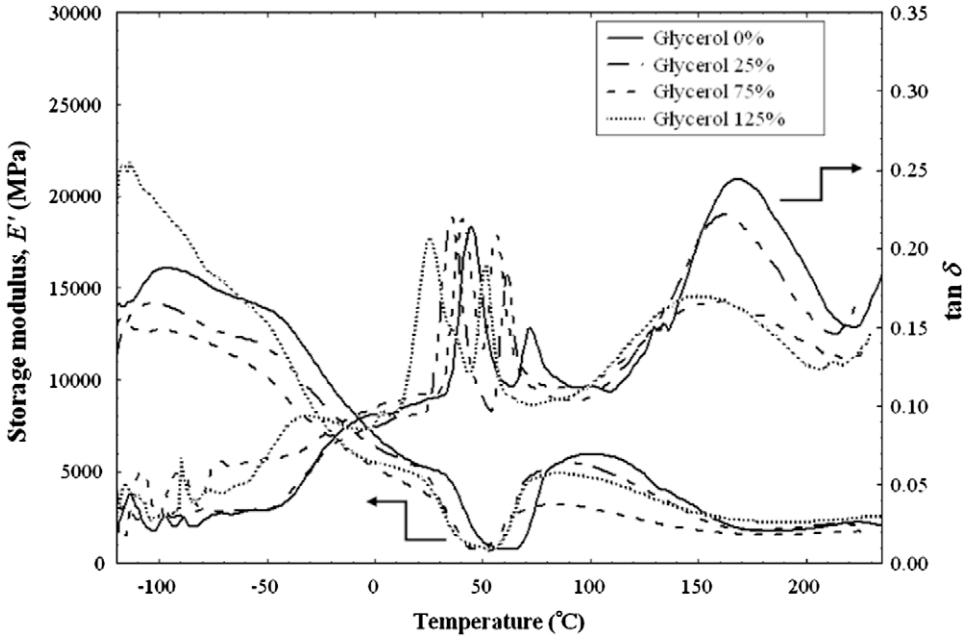

Fig. 5. Storage modulus (E') and tan δ of edible chitosan films with various concentrations of glycerol undergoing hot air drying at 40 °C.

stretch films. This means that the edible chitosan films in this study were stronger but less stretchable than the commercial stretch films.

Although hot air dried film with a glycerol concentration of 125% w/w had the highest percent elongation, it had the thickness and moisture content higher than the desired values. Thus, it could not still be used in practice.

3.4. DMTA

The storage modulus (E') and tan δ of chitosan films with various glycerol concentrations undergoing hot air drying at 40 °C, vacuum drying and LPSSD at 90 °C and 10 kPa are shown in Figs. 5–7, respectively. The glass transition temperature of each film sample was determined as the peak of tan δ (α-relaxation) (Kristo and Biliaderis, 2008); the glass transition temperature of the chitosan films are listed in Table 3. It was found that the glycerol concentration significantly affected the glass transition temperature of the films. In all cases, the films without glycerol had the highest glass transition temperature and the glass transition temperature decreased with increased glycerol concentration due to the plasticization process. These results agree with those of earlier studies (e.g., Quijada-Garrido et al., 2007).

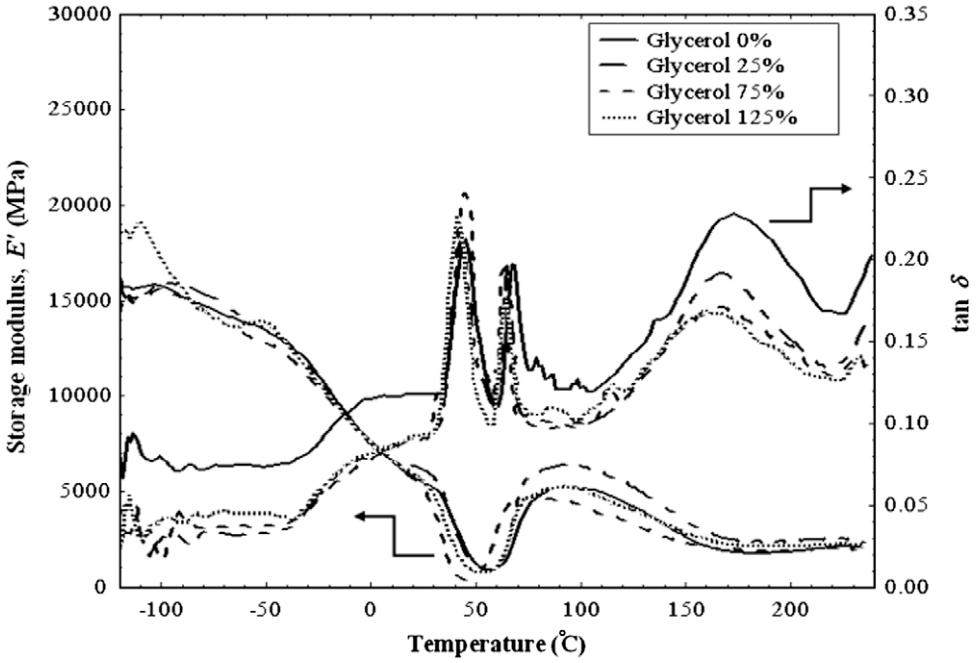

Fig. 6. Storage modulus (E') and tan δ of edible chitosan films with various concentrations of glycerol undergoing vacuum drying at 90 °C, 10 kPa.

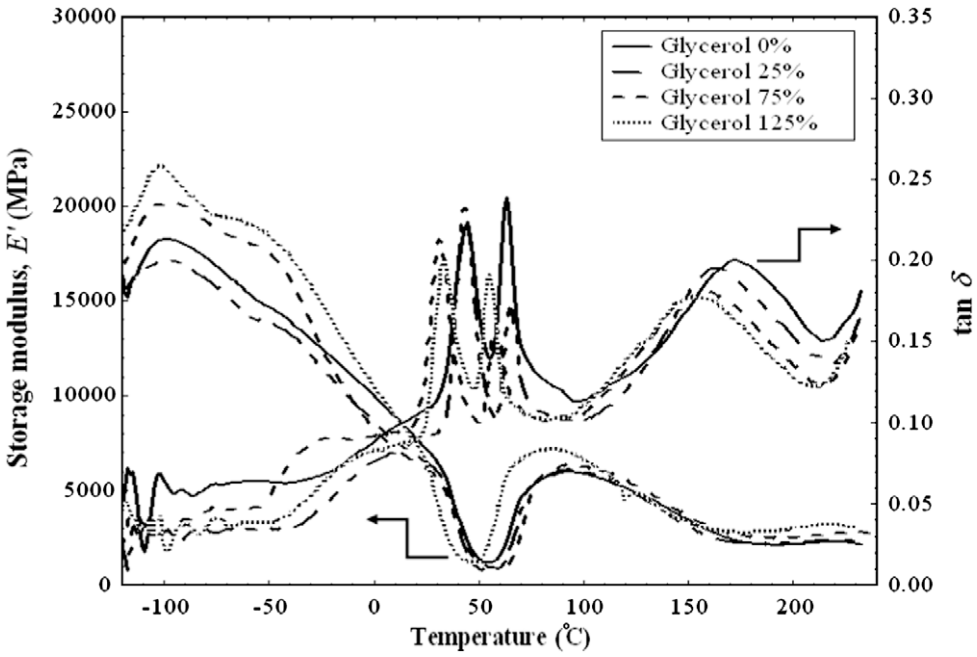

Fig. 7. Storage modulus (E') and tan δ of edible chitosan films with various concentrations of glycerol undergoing LPSSD at 90 °C, 10 kPa.

Table 3
Glass transition temperature of chitosan films.

Drying condition/glycerol concentration (% w/w chitosan)	Glass transition temperature (°C)
Hot air drying (control ≈ 40 °C)	
0	171
25	163
75	160
125	152
Vacuum drying (90 °C, 10 kPa)	
0	175
25	167
75	165
125	161
LPSSD (90 °C, 10 kPa)	
0	174
25	165
75	163
125	159

Table 4
Color of chitosan films and commercial stretch films.

Film type/glycerol concentration (% w/w chitosan)	Color		
	L^*	a^*	b^*
Chitosan film dried by hot air drying at 40 °C			
0	96.6 ± 0.1^{a}	-0.2 ± 0.1^{b}	1.4 ± 0.3^{bc}
25	96.6 ± 0.0^{a}	-0.3 ± 0.1^{bc}	1.5 ± 0.1^{bc}
75	97.0 ± 0.1^{a}	-0.4 ± 0.1^{c}	1.7 ± 0.4^{cd}
125	97.2 ± 0.0^{a}	-0.4 ± 0.1^{c}	1.7 ± 0.2^{cd}
Chitosan film dried by vacuum drying at 90 °C, 10 kPa			
0	96.8 ± 0.1^{a}	-0.3 ± 0.0^{bc}	1.4 ± 0.2^{b}
25	96.6 ± 0.0^{a}	-0.9 ± 0.0^{f}	3.3 ± 0.2^{de}
75	96.9 ± 0.1^{a}	-0.8 ± 0.1^{ef}	3.6 ± 0.6^{e}
125	96.8 ± 0.1^{a}	-0.7 ± 0.1^{ef}	3.2 ± 0.1^{de}
Chitosan film dried by LPSSD at 90 °C, 10 kPa			
0	96.5 ± 0.1^{a}	-0.5 ± 0.0^{d}	2.9 ± 0.1^{de}
25	96.4 ± 0.1^{a}	-0.7 ± 0.1^{e}	3.2 ± 0.3^{de}
75	96.6 ± 0.1^{a}	-0.4 ± 0.1^{c}	3.0 ± 0.3^{de}
125	96.7 ± 0.1^{a}	-0.3 ± 0.1^{bc}	2.7 ± 0.1^{d}
Clean Wrap™ (LDPE)	96.9 ± 0.2^{a}	0.02 ± 0.0^{a}	0.04 ± 0.0^{a}
M Wrap™ (PVC)	97.2 ± 0.2^{a}	0.02 ± 0.0^{a}	0.04 ± 0.0^{a}

Same letter in the same column means that the values are not significantly different at 95% confidence level ($p > 0.05$).

When comparing at the same glycerol concentration, the hot air dried films had lower glass transition temperature than the vacuum and LPSSD dried films. As mentioned in Section 3.2, this is probably due to the higher thermal cross-linkage that occurred more in the films during these two drying processes. The higher extents of thermal cross-linkage in vacuum and LPSSD dried films resulted in lesser extents of swelling due to more rigid chains, leading to higher glass transition temperature. In addition, thermal treatment of chitosan might lead to amide band formation, which reduced the amount of hydrophilic groups, thus decreased swelling of the films (Mayachiew et al., 2010). In addition, these results agree with the film thickness results of the present study; the hot air dried films with high glycerol concentrations had larger thickness than the vacuum and LPSSD dried films due to lower thermal cross-linkage and less compactness.

3.5. Color

The color of commercial stretch films (Clean Wrap™ and M Wrap™) and that of edible chitosan films with various concentrations of glycerol after conditionings at 75% RH for 48 h are shown in Table 4. In terms of the lightness (L^* value) the films of all cases were not significantly different. In terms of the redness (a^* value) chitosan films of all cases exhibited green color more than the commercial stretch films. In the case of yellowness (b^* value) the chitosan films prepared by all drying methods exhibited yellow color more than the commercial stretch films. This is because of the different natural colors of the starting materials; the starting color of chitosan film solution was slightly more yellow. However, among all drying methods and glycerol concentrations, the color of the chitosan films was not significantly different.

3.6. Phase separation

The sorption isotherms of edible chitosan films with various glycerol concentrations are shown in Fig. 8. Above the maximum concentration that glycerol could act as plasticizer, phase separation occurred and the equilibrium moisture content increased because extra moisture could bind with the free (or excess) glycerol in the films (Godbillot et al., 2006). It was noted in this study that the concentration of glycerol of 25% w/w seemed to be the maxi-

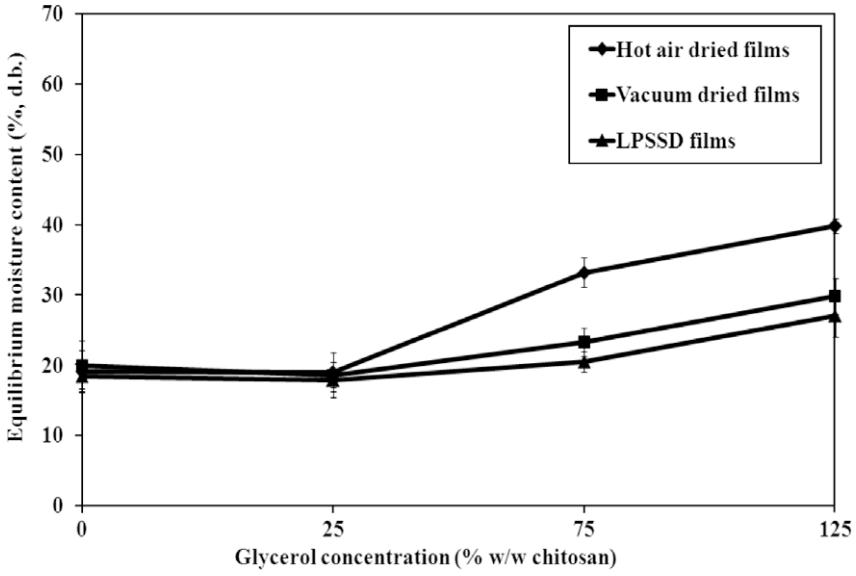

Fig. 8. Equilibrium moisture content of edible chitosan films with various concentrations of glycerol.

mum concentration beyond which phase separation started to occur.

4. Conclusion

The effect of glycerol concentration (0%, 25%, 75% and 125% w/w chitosan) on physical properties, mechanical properties and glass transition temperature of edible chitosan films prepared by hot air drying at 40 °C, vacuum drying and low-pressure superheated steam drying (LPSSD) at 90 °C, 10 kPa was investigated. The results showed that at the same glycerol concentration vacuum and LPSSD dried films required much shorter drying time than hot air dried films. Within the same drying method, the time used to dry the films increased with the glycerol concentration. The glycerol concentration had different effects on the thickness of edible chitosan films prepared by different drying methods.

The tensile strength and percent elongation of the hot air dried films generally decreased and increased, respectively, with an increase in the concentration of plasticizer. However, in the cases of vacuum and LPSSD dried films the tensile strength and percent elongation of the films plasticized at glycerol concentrations of 25–125% w/w were not significantly different, due to glycerol phase separation. Glycerol concentration had also found to significantly affect the glass transition temperature of the films. Within the same drying method, the glass transition temperature decreased with an increase of the glycerol concentration. At the same glycerol concentration, the glass transition temperature of hot air dried films was lower than that of vacuum and LPSSD dried films, indicating that these films were more compact than the hot air dried films. The color of chitosan films was not significantly different in all cases. Based on the results of this study, the optimum concentration of plasticizer and the best drying method that should be used are the concentration of 25% w/w and drying by low-pressure superheated steam drying.

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Comparative Evaluation of Mathematical Models for Release of Antioxidant from Chitosan Films Prepared by Different Drying Methods

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Antioxidants are often added to many types of food packagings, especially packaging films, to enhance their effectiveness in protecting food from the environment. This positive action is possible due to release of the agents from the film matrix into food to reduce oxidation, thus extending the product's shelf life. For effective design of antioxidant-added films, the release characteristics of antioxidants from the films under various conditions need to be known and predicted. The aim of this study was to compare various simple mathematical models for prediction of the release of antioxidants from edible chitosan films into distilled water at room temperature. Chitosan films were prepared via hot air drying, vacuum drying, and low-pressure superheated steam drying. Models with different expressions for the effective diffusion coefficient were tested. The model equations were solved numerically using COMSOL Multiphysics software (Comsol AB, Stockholm, Sweden). The prediction efficiency of the models was verified by comparing the predicted release kinetics of the antioxidant, in terms of the total phenolics content (TPC), with the experimental data available in the literature. It was found that the model assuming the effective diffusion coefficient as a function of the phenolics concentration gave the best agreement with the experimental results.

Keywords Distilled water; Effective diffusion coefficient; Hot air drying; Low-pressure superheated steam drying; Phenolics; Swelling; Vacuum drying

INTRODUCTION

Packaging films enriched with natural antioxidant and/or antimicrobial compounds have received significant research interest in recent years.[1–5] This is due to the ability of these active packagings to help prolong the shelf life of food products, either by retarding oxidation reactions or inhibiting growth of spoilage microorganisms.[6] Natural antioxidant and/or antimicrobial compounds are preferred to synthetic compounds due to the ability to satisfy the need of health-conscious consumers of the former group of compounds.[7,8]

In order to improve the effectiveness of antioxidant or antimicrobial films, it is necessary to be able to control the release of additives from the films into food at a predetermined rate to maintain the minimum required concentration of antioxidant or antimicrobial agents on the food surface over a desired period of time.[9] A number of investigators have studied the effects of various parameters on the release of additive agents from many types of films.[3,9–11] Among several factors, it is well recognized that drying is a step that significantly affects the film structure and hence the film release characteristics. Mayachiew and Devahastin,[3] for example, studied the effects of drying methods and conditions (i.e., ambient-temperature drying, hot air drying at 40°C, vacuum drying, and low-pressure superheated steam drying within the temperature range of 70–90°C at an absolute pressure of 10 kPa) on the release of phenolic compounds from chitosan films into distilled water. It was found that the drying methods and conditions had a significant effect on the release characteristics of the films due to the differences in the degree of film swelling as a result of the different levels of interactions between the film matrix and the added compounds.

In order to reduce the number of experiments that need to be performed to fully understand the effects of various parameters on the release behavior of films, the use of a mathematical model that allows prediction of the release behavior is an attractive alternative. A number of models are available and have been applied to various types of films under various release situations. For example, Yoshida et al.[10] applied the diffusion model to study the mechanism of potassium sorbate release from chitosan

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films as a function of immersion time in an aqueous solution. The diffusion coefficient was assumed to be constant. This model gave adequate agreement with their experimental data. However, these authors suggested that the effect of film swelling should be included in the model if a more realistic prediction of release from such hydrophilic films as chitosan films is to be made.

Mathematical models that take into account the effect of film swelling have actually been proposed and tested. Klier and Peppas,[13] for example, studied simultaneous theophylline release and water diffusion in swellable polymer (hydroxyethyl methacrylate-co-N-vinyl-pyrollidone) and found that swelling of a polymer slab could have a significant effect on the additive release behavior. A swelling term was added to the Fickian diffusion equation; the half thickness of the film was considered as a moving boundary. It was shown that swelling of a glassy polymer indeed had a significant effect on the release behavior of the additive.

Sadikoglu et al.[14] proposed a diffusion model with a time-dependent diffusion coefficient to describe potassium sorbate diffusion through whey protein films. The model was divided into two parts, consisting of solute (potassium sorbate) diffusion and solvent (water–glycerol) diffusion. The solute diffusion through a swelling film was assumed to obey Fick's law of diffusion with a time-dependent diffusion coefficient. On the other hand, the solvent diffusion coefficient was assumed to be constant. The change of the film thickness was not explicitly taken into account. The simulated and experimental release behavior agreed very well.

Although there have already been a number of studies using mathematical models to predict the release behavior of additives from different types of films, it is still of interest to investigate whether a simple liquid diffusion model could be used to predict the release behavior of an additive from swelling biopolymer films. In addition, it is of interest to determine which form of the diffusion coefficient could be used to best describe the release characteristics of those films. The effects of various drying methods that could be used to prepare films on the release characteristics of the films have also not yet been modeled. Therefore, the aim of this work was to test a simple liquid diffusion model with different expressions for the effective diffusion coefficient in modeling the release of an additive from edible chitosan films prepared by different drying methods and under different drying conditions. The validity of each model variation was tested by comparing the simulated release behavior with the experimental data available in the literature.

MODEL DEVELOPMENT

Model Description

In this study the release of antioxidants was monitored via following the evolution of the total phenolics content (TPC); phenolics were released from chitosan films prepared by various drying methods into distilled water. The concentration of the added antioxidant (Indian gooseberry extract) was fixed at 1% (w/w) and the initial film thickness was 20 μm in all cases. The simulated release profiles were compared with the experimental results of Mayachiew and Devahastin.[3] The geometry of a chitosan film sample is shown in Fig. 1.

To simplify the model, the following assumptions were made: chitosan film was assumed to be a homogeneous material, the initial distribution of phenolic compounds in chitosan film was uniform, diffusion of phenolic compounds from chitosan film into water was considered unidirectional in the x-direction at a constant temperature (ambient temperature), and no external mass transfer resistance was assumed to be significant because the release medium (water) was well stirred.

Three variations of the diffusion model were tested in this study. The major difference between each model variation is the different form (expression) of the effective diffusion coefficient:

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D_{\text{eff}}\frac{\partial c}{\partial x}\right) \tag{1}$$

where D_{eff} is the effective diffusion coefficient of an additive through a film sample (m^2/s), c is the additive concentration (mg/g film), t is time (s), and x is the distance along the film thickness (see Fig. 1).

Model 1: Constant Diffusion Coefficient

In the first model, variation of the effective diffusion coefficient of phenolic compounds through the film structure is assumed to be constant:

$$\frac{\partial c_{\text{PC}}}{\partial t} = \frac{\partial}{\partial x}\left(D_{\text{eff\_PC}}\frac{\partial c_{\text{PC}}}{\partial x}\right) \tag{2}$$

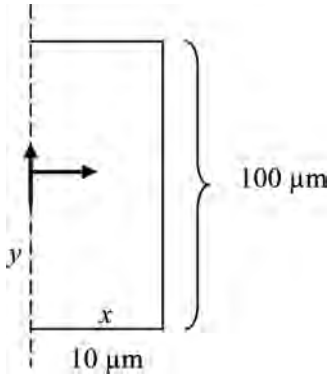

FIG. 1. Schematic diagram of chitosan film (half film thickness).

where c_{PC} is the concentration of phenolics in chitosan film (mg/g dried film). Initially, the phenolic compounds were assumed to be homogeneously distributed in chitosan film at a constant concentration c_{PC0}; the value of c_{PC0} depended on the drying methods that were used to prepare films (data not shown).

$$c_{PC}(x,0) = c_{PC0} \tag{3}$$

Due to symmetry, the phenolics concentration gradient at the center of the film sample was zero:

$$\frac{\partial c_{PC}(0,t)}{\partial x} = 0 \tag{4}$$

Because of the assumption of well-stirred surroundings, the concentration of phenolics at any location in distilled water was the same. Only the transport of phenolic compounds in chitosan film was therefore considered. At the interface between the film and water, the concentration of phenolics was assumed to be either zero or the equilibrium value. If the accumulation of phenolic compounds in water insignificantly affected the release of the compounds, the concentration at the film surface could be set equal to zero; this is generally called the *perfect sink assumption* (in other words, the water could infinitely take the phenolic compounds into its body). On the other hand, the equilibrium concentration should be used if the water could not take all the phenolic compounds into its body; the equilibrium concentration must be determined experimentally.[15]

$$\text{Perfect sink}: c_{PC}(\delta,t) = 0 \tag{5}$$

$$\text{Equilibrium}: c_{PC}(\delta,t) = c_{PC\_eq} \tag{6}$$

where δ denotes the film surface. Comparison between the perfect sink assumption and the equilibrium assumption will be later discussed.

Model 2: Phenolics Concentration-Dependent Diffusion Coefficient

The effective diffusion coefficient is assumed in this case to be a function of the phenolics concentration.[13]

$$D_{eff\_PC} = a \exp\left(-\left(1 - \frac{c_{PC}}{c_{PC\_eq}}\right)\right) \tag{7}$$

where a is an empirically determined constant.

Model 3: Moisture-Dependent Diffusion Coefficient

Water absorption by a film sample during the release of phenolic compounds is taken into account in this model variation. The model is separated into two transport processes; that is, water absorption by the film and release of phenolic compounds from the film. The thickness of chitosan film is nevertheless assumed to be constant while water is diffusing into the film; the effect of swelling is implicitly taken into account via the use of the effective diffusion coefficient.[16] The model for water absorption is given in Eq. (8): Q1

$$\frac{\partial c_w}{\partial t} = \frac{\partial}{\partial x}\left(D_{eff\_w}\frac{\partial c_w}{\partial x}\right) \tag{8}$$

$$D_{eff\_w} = a_1 \exp\left(-\left(1 - \frac{c_w}{c_{w\_eq}}\right)\right) \tag{9}$$

Initial and boundary conditions for water absorption:

$$c_w(x,0) = c_{w0} \tag{10}$$

$$\frac{\partial c_w(0,t)}{\partial x} = 0 \tag{11}$$

$$c_w(\delta,t) = c_{w\_eq} \tag{12}$$

where D_{eff_w} is the effective diffusion coefficient of water, c_w is the concentration of water in chitosan film (g/g dried film), c_{w0} is the initial concentration of water in chitosan film (g/g dried film), c_{w_eq} is the concentration of water in chitosan film at equilibrium (g/g dried film), and a_1 is an empirical constant. The initial concentration of water is approximately 0.14 g/g dried film based on the data in Mayachiew and Devahastin.[3]

The governing equation for the release of phenolic compounds is similar to that used to simulate the transport of water and is given in Eqs. (13–14):

$$\frac{\partial c_{PC}}{\partial t} = \frac{\partial}{\partial x}\left(D_{eff\_PC}\frac{\partial c_{PC}}{\partial x}\right) \tag{13}$$

$$D_{eff\_PC} = a_2 \exp\left(-\left(1 - \frac{c_w}{c_{w\_eq}}\right)\right) \tag{14}$$

Initial and boundary conditions for the release of phenolic compounds are given in Eqs. (15–17):

$$c_{PC}(x,0) = c_{PC0} \tag{15}$$

$$\frac{\partial c_{PC}(0,t)}{\partial x} = 0 \tag{16}$$

$$c_{PC}(\delta,t) = c_{PC\_eq} \tag{17}$$

where D_{eff_PC} is the effective diffusion coefficient of phenolic compounds, c_{PC} is the concentration of phenolic compounds in chitosan film (mg/g dried film), c_{PC0} is the initial concentration of phenolic compounds in chitosan film (g/g

dried film), c_{PC_eq} is the concentration of phenolic compounds in chitosan film at equilibrium (mg/g dried film), and a_2 is an empirical constant. The effect of water absorption and hence swelling on the release behavior is included in the effective diffusion coefficient D_{eff_PC}.

All model equations were solved by the finite element method using COMSOL Multiphysics version 3.5 (Comsol AB, Stockholm, Sweden). As mentioned earlier, only the half thickness of chitosan film was simulated. The direct (UMFPACK) linear system solver was used in the simulation. Mesh independence test was performed, and the results indicated that the use of 4,000 elements was sufficient to yield mesh-independent results.

MATERIALS AND METHODS

In addition to the experimental release profiles of phenolic compounds of Mayachiew and Devahastin,[3] experiments were performed in the present study to determine the water absorption kinetics of chitosan films. This was done to assess the predictability of Model 3.

Chitosan (molecular weight of 900,000 Da, degree of deacetylation of 90.2%) was obtained from S.K. Profishery Co., Ltd. (Bangkok, Thailand).

Preparation of Antioxidant Chitosan Films

Preparation of antioxidant chitosan films was conducted using a method described by Mayachiew and Devahastin.[3] Briefly, Indian gooseberry extract was added to the chitosan film solution at a concentration of 1% (w/w). The mixture was homogenized by a bench-top homogenizer (Ika Works [Asia], Model T 25 basic, Selangor, Malaysia) at 9,500 rpm for 2 min. The film solution (21 g) was poured onto an acrylic plate with the dimensions of 13×10 cm to cast an antioxidant film. Three methods were used to prepare films: hot air drying (HD) at 40°C, vacuum drying (VD) at 70°C, and low-pressure superheated steam drying (LPSSD) at 70°C; the two latter drying processes were conducted at an absolute pressure of 10 kPa.[17] After drying, each film sample was conditioned for at least 48 h at room temperature in a desiccator containing saturated salt solution of sodium chloride (Ajax Finechem, Seven Hills, Australia), which produced a relative humidity (RH) of 75% prior to further analysis.

Determination of Moisture Content of Antioxidant Films

An antioxidant film sample was cut into of 2×2 cm squares. The film thickness was measured at various locations using a micrometer (Mitutoyo, model 102-309, Tokyo, Japan) with an accuracy of ± 2 μm; only the film samples having a uniform thickness of 20 μm were taken to determine the moisture content. A film sample was enclosed by a wire screen to prevent curling when in contact with water. The film was weighed and subsequently placed in a beaker containing 30 mL of distilled water. At a predetermined sampling time, the film was taken out for moisture content evaluation. The film was blotted with tissue paper and put in a moisture can. After weighing, the whole can was placed in a vacuum oven (Sanyo, Model Gallenkamp/OM-09980, Loughborough, UK) at 70°C and absolute pressure of 100 mbar for 48 h. The mass of the dried film was then measured and the moisture content was calculated.

RESULTS AND DISCUSSION

Comparison of Interface Boundary Conditions

As mentioned earlier, two interface boundary conditions, that is, perfect sink (Eq. (5)) and equilibrium (Eq. (6)) conditions, could be used. A comparison between the use of the two boundary conditions is given in Figs. 2a and 2b.

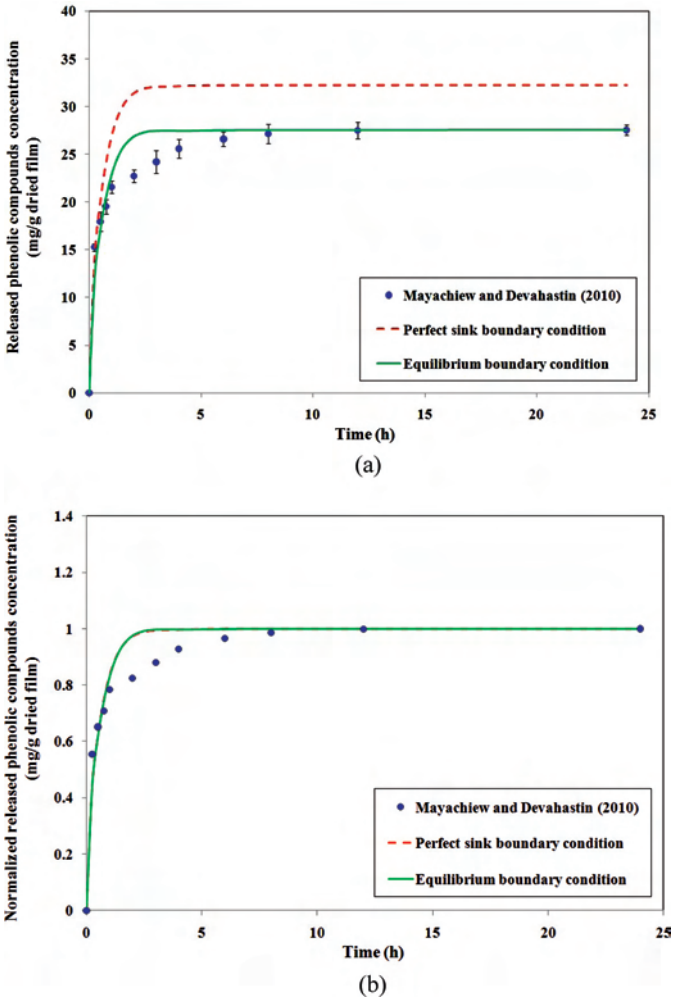

FIG. 2. (a) Predicted and experimental release profile of phenolic compounds from chitosan film prepared by HD at 40°C. Prediction was made by Model 1. (b) Predicted and experimental normalized release profile of phenolic compounds from chitosan film prepared by HD at 40°C. Prediction was made by Model 1 (color figure available online).

It is seen in Fig. 2a that the equilibrium boundary condition led to the simulated release profiles of phenolic compounds that are closer to the experimental data than the perfect sink boundary condition. However, if the normalized release profiles (ratio of the released phenolic compounds at any time t to the value at the equilibrium) were considered, it can be seen in Fig. 2b that the results obtained using either boundary condition are the same. The equilibrium boundary condition was nevertheless used in all subsequent simulations in this study.

Parameter Estimation

Three diffusion model variations were validated against the experimental results of Mayachiew and Devahastin.[3] Empirical constants in the expression for the effective diffusion coefficient of each model variation were adjusted to fit the experimental results; minimum mean square error (MSE) and maximum R^2 were used to indicate the best fit. After adjustment, the values of the constants were obtained and are listed in Table 1.

Information on the effective diffusivities of water and phenolic compounds in Model 3 for chitosan films prepared by VD and LPSSD at 80 and 90°C is not available; this is suggested for a future study. In the present study, simulated results were compared only to the experimental release data from the films prepared by vacuum and low-pressure superheated steam drying at 70°C to exemplify the use and validity of Model 3.

Comparison of Model Predictability

The difference between Model 1 and Model 2 is the use of constant effective diffusion coefficient and the diffusion coefficient that is a function of the phenolics concentration, respectively. In Model 3 the effect of polymer swelling on the release of phenolic compounds is considered; water absorption is implicitly included in the expression for the effective diffusion coefficient. The model could be used to predict the evolution of the film moisture content, as can be seen in Fig. 3, where the simulated film moisture content evolution is compared with the experimental data obtained in the present study. The predicted trends are in general agreeable with the experimental data; the water absorption reached the equilibrium within only a few minutes.

The simulated release profiles of phenolic compounds from different model variations are compared as shown in Figs. 4a–4c. It can be seen in these figures that the prediction of Model 2 more closely resembles the experimental results than Model 1 and Model 3 do; the predicted concentration of phenolic compounds rapidly increased and reached equilibrium faster than the experimental results when Model 1 and Model 3 were used to predict the results.

The results of Model 3 were supposedly better than those of Model 1 because the former also takes into account the effect of water absorption and hence film swelling. Thus, the release behavior as predicted by Model 3 was further investigated to determine why Model 3 gave the same results as Model 1. It was found that the diffusion coefficient of water increased rapidly and reached equilibrium only in a few seconds. This caused the diffusion coefficient of phenolic compounds to remain almost constant during the whole process (see Eq. (9)). Therefore, the release profiles as predicted by Model 3 were similar to those predicted by Model 1, which assumes a constant diffusion coefficient.

TABLE 1
Empirical constants in the expressions for effective diffusion coefficient

	Model 1	Model 2	Model 3	
	$D_{\text{eff\_}PC} = a$	$D_{\text{eff\_PC}} = a\exp\left(-\left(1-\frac{c_{\text{PC}}}{c_{\text{PC\_eq}}}\right)\right)$	$D_{\text{eff\_w}} = a_1\exp\left(-\left(1-\frac{c_{\text{w}}}{c_{\text{w\_eq}}}\right)\right)$	$D_{\text{eff\_PC}} = a_2\exp\left(-\left(1-\frac{c_{\text{w}}}{c_{\text{w\_eq}}}\right)\right)$
Drying method	a ($\times 10^{14}$)	a ($\times 10^{15}$)	a_1 ($\times 10^{12}$)	a_2 ($\times 10^{14}$)
HD				
40°C	1.80	2.59	2.20	1.78
VD				
70°C	1.35	3.82	5.25	1.40
80°C	1.32	4.70	N/A	N/A
90°C	1.11	4.94	N/A	N/A
LPSSD				
70°C	1.52	3.85	6.50	1.55
80°C	1.47	4.27	N/A	N/A
90°C	1.32	4.83	N/A	N/A

N/A = Not available.

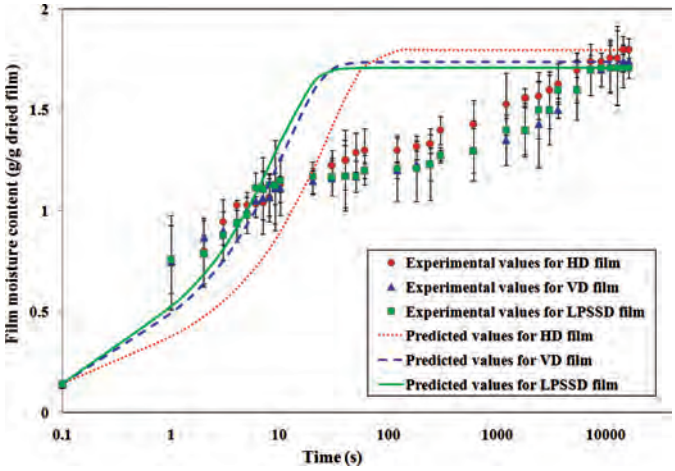

FIG. 3. Comparison between predicted and experimental moisture content evolutions of chitosan films prepared by HD at 40°C, VD and LPSSD at 70°C and 10 kPa (color figure available online).

Although Model 3 was not the best model for quantitative prediction of the release profiles, it probably provided a better explanation of the physical behavior of the release. In the first period, water absorption into chitosan films led to polymer chain relaxation, which led to easy release of the phenolic compounds. This mechanism is represented through the diffusion coefficient of the phenolic compounds, which is a function of the water (moisture) content. The increased diffusion coefficient of water led to an increase in the rate of release during the first period. From the experimental results, water was absorbed rapidly into chitosan films ($D_{\text{eff\_water}}$ is in the order of 10^{-12}). The effect of water absorption on the release of phenolic compounds was only short term when it was compared to the whole release process. Hence, it may be suggested that the release of phenolic compounds is not dominated by the absorption of water; the transport of phenolic compounds is the rate determining step. To support this hypothesis, the results of Model 2 are next considered.

In Model 2 it is assumed that the release rate of phenolic compounds is a function of phenolics concentration. From Figs. 4a–4c, the release profiles of phenolic compounds obtained from Model 2 matched well with the experimental results. Therefore, the assumption used in Model 2 may be considered reasonable. Again, like the release behavior of water, the diffusion coefficient of phenolic compounds in hot air–dried film used in Model 2 reached the maximum value in the first period. Afterwards, the concentration gradient of phenolic compounds decreased, leading to a decrease in the diffusion coefficient of phenolic compounds. The release process reached equilibrium when the concentration gradient of phenolic compounds was set equal to zero. The release of phenolic compounds as predicted by Model 2 is controlled only by the concentration

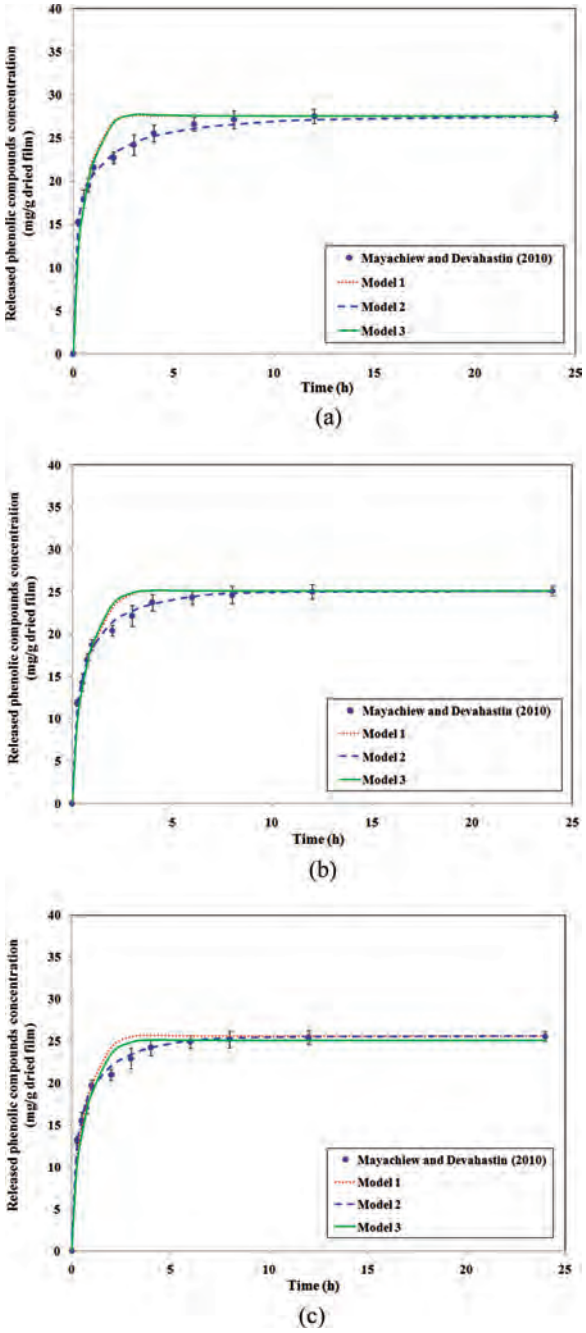

FIG. 4. Comparison between predicted and experimental release profiles of phenolic compounds from chitosan film prepared by (a) HD at 40°C, (b) VD at 70°C and 10 kPa, and (c) LPSSD at 70°C and 10 kPa (color figure available online).

gradient of phenolic compounds, which is in contrast with the assumption of Models 1 and 3. Because Model 2 gives the best prediction, it is possible to conclude that the release of phenolic compounds is dominated only by the diffusion of the phenolic compounds.

The effects of drying methods and conditions that were used to prepare chitosan films on the release behavior were

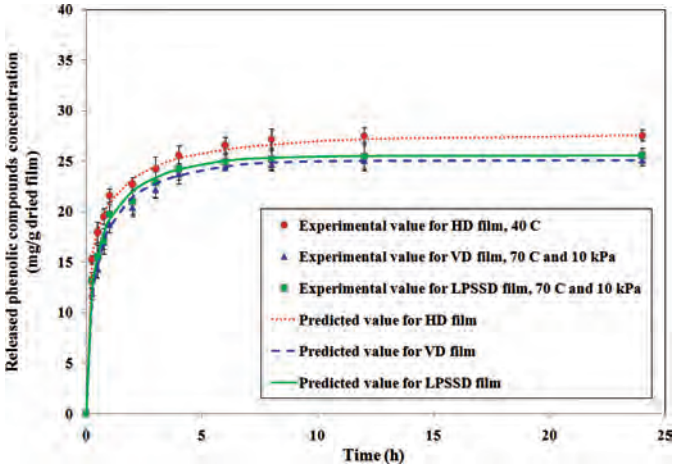

FIG. 5. Prediction of the release of phenolic compounds from chitosan films prepared by different drying methods. Prediction was made by Model 2 (color figure available online).

also investigated. The release profiles of the phenolic compounds from chitosan films prepared by three different drying methods are shown in Fig. 5. The release from HD film was faster than that from VD and LPSSD films; the amount of the released phenolic compounds from HD film was also the highest. As can be seen in Table 1, the diffusion coefficient of phenolic compounds in the case of HD film was the highest. The physical properties of chitosan films prepared by different drying methods and conditions indeed affected the release of phenolic compounds. Lower drying temperature induced lower interchain cross-linkage that resulted in easier transport of phenolic compounds through chitosan films.[3,7] Therefore, chitosan films prepared at lower drying temperatures released the phenolic compounds in greater amounts compared to chitosan films prepared at higher drying temperatures.

CONCLUSION

A simple liquid diffusion–based mathematical model that can be used to predict the release kinetics of antioxidant from edible chitosan films was tested. Three model variations, which have different expressions for the effective diffusion coefficient, were investigated. The predicted release profiles of phenolic compounds of all models were compared with the available experimental results to verify the accuracy of the prediction. The comparison unexpectedly revealed that Model 3, which includes the effect of film swelling and the use of variable effective diffusion coefficient of both water and phenolic compounds, gave exactly the same results as Model 1, which simply assumes a constant effective diffusion coefficient. This is because the diffusion coefficient of phenolic compounds in Model 3 rapidly became constant because the chitosan films absorbed water rapidly; this caused the film moisture content to reach equilibrium in only a few minutes. Water absorption was therefore noted to only slightly affect the phenolic compounds' release. Overall, the model that uses the phenolics' concentration-dependent effective diffusion coefficient gives the best prediction of the phenolic compounds release. The release of phenolic compounds from chitosan films is suggested to be dominated only by the concentration of the compounds.

NOMENCLATURE

a, a_1, a_2	Constants
c	Concentration (mg/g film)
c_{PC}	Concentration of phenolic compounds in chitosan film (mg/g dried film)
c_{PC0}	Initial concentration of phenolic compounds (mg/g dried film)
c_{PC_eq}	Concentration of phenolic compounds in chitosan film at equilibrium (mg/g dried film)
c_w	Water concentration (g/g dried film)
c_{w0}	Initial concentration of water (g/g dried film)
c_{w_eq}	Concentration of water in chitosan film at equilibrium (g/g dried film)
D_{eff}	Effective diffusion coefficient (m^2/s)
t	Time (s or h)
x	Distance along the film thickness (μm)

Greek letter

δ	Film thickness (μm)

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A COMPARATIVE EVALUATION OF DRYING METHODS AND CONDITIONS FOR ENHANCING THE PERFORMANCE OF ANTIMICROBIAL EDIBLE FILMS

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Keywords: Active packaging; Hot air drying; Low-pressure superheated steam drying; Natural antimicrobial agent; Vacuum drying

ABSTRACT

Antimicrobial edible films are an innovative food packaging to deliver high quality and extended shelf-life products to consumers in an environmentally responsible way. Antimicrobial edible films may be formed from edible biomaterial which incorporated with antimicrobial agent. To enhance the antimicrobial activity of chitosan films, galangal extract (GE) – a natural antimicrobial agent from traditional spice used in Thai foods – can be added to chitosan and form into antimicrobial edible film. Drying is the most challenging step in the production of such edible films. Although many works have reported studies on preparation of edible films and also of edible films enriched with antimicrobials, there are hardly any studies that report investigation of the effects of the methods and conditions of drying, which is one of the most important steps to prepare films, on the antimicrobial property. Drying under control conditions (ambient air drying at 30 ºC and hot air drying at 40 ºC), as well as vacuum drying and low-pressure superheated steam drying (LPSSD) at an absolute pressure of 10 kPa were carried out at different drying temperatures (70, 80, and 90 ºC). The antimicrobial activity of the films was tested against *S. aureus* using disc diffusion and viable cell count methods. The results showed that chitosan films containing GE possessed very good antimicrobial activity. The films dried under control conditions and LPSSD at 70 ºC exhibited greater antimicrobial activities in comparison with other drying conditions. Moreover, the antimicrobial films dried by LPSSD showed greater antimicrobial activities than films derived by vacuum drying.

INTRODUCTION

Food safety is becoming an increasingly important health issue for the food product since the increase in foodborne illnesses have been reported [1]. In addition, improving the shelf-life of food products has had an important economic impact by reducing the losses form spoilage. The safety and quality of food products can be enhanced by the use of new preservation technologies, most notably in packaging. Nowadays, there is a considerable interest in the possibility of using edible films to delay or to prevent growth of microorganisms.

Edible films can serve as carriers for a wide range of food additives, including antimicrobial agent, that can extend the shelf-life of foods. Among many materials that can be used to form edible films, chitosan (β-(1,4)-2-amino-2-deoxy-D-glucose) is one of the most promising as it has a good ability to form film and due to its biodegradability, biocompatibility and non-toxicity [2]. To further enhance the natural antimicrobial activity of chitosan films, spices, which are amongst the most promising natural antimicrobial agents, could also be incorporated into the films. Galangal (*Alpinia galanga* Linn.) or "khaa" in Thai is a traditional spice used extensively for flavoring and medicinal purposes. Galangal extract has also proved to be an affective natural antimicrobial agent against food poisoning bacteria, *S. aureus* [3-4].

Recently, various antimicrobial edible films have been developed to control the growth of spoilage and pathogenic microorganisms, resulting in an ability to prolong the shelf-life of foods. For example, lysozyme–chitosan composite film was examined the antimicrobial properties against *Escherichia coli* and *Streptococcus faecalis* [5]. Whey protein isolate films containing oregano, rosemary and garlic essential oils exhibited antimicrobial activity against *Escherichia coli* O157:H7, *S. aureus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Lactobacillus plantarum* [6]. The partially hydrolyzed sago starch and alginate that incorporated with lemongrass oil were effective in inhibiting the growth of *E. coli* [7]. Several factors should be considered when designing an antimicrobial packaging for food products. The concentration of antimicrobials and the ability to inhibit microorganism need to be considered.

Drying is one of the most challenging steps in the production of edible films. It is well known that different drying methods and conditions affect properties and functionalities of edible films [8-9]. However, no information is so far available on the effects of these (or any other) drying methods and conditions on the antimicrobial activity of chitosan films incorporated with GE.

The aim of this study was therefore to assess the effects of drying methods (i.e., ambient drying, hot air drying, vacuum drying, LPSSD) and conditions on the antimicrobial activity of chitosan films incorporated with

GE by both the disc diffusion and viable cell count methods.

MATERIALS AND METHODS

Materials

Chitosan (molecular weight of 900,000 Da and degree of deacetylation of 90.2%) was obtained from S.K. Profishery Co., Ltd. (Bangkok, Thailand). Galangal (*A. galanga*) rhizomes were purchased from local market.

Preparation of chitosan solution

1.5% (w/v) chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid under constant stirring at 300 rpm using a magnetic stirrer (Framo®-Gerätechnik, model M21/1, Eisenbach, Germany) at room temperature for 24 h. 25% glycerol (w/w chitosan) was then added into the chitosan solution; stirring was continued at room temperature for 1 h. After mixing the solution was centrifuged for 15 min at 12,400 rpm by a refrigerated centrifuge (Hitachi, model Himac CR21, Ibaragi, Japan) to remove undissolved impurities and bubbles in the solution.

Preparation of galangal extract

Galangal powder (10 g dry basis), dried by a tray dryer at 40 ºC with particle size between 125-425 µm, was extracted with 100 mL of 95% (v/v) ethanol. The extract was filtered through a filter paper (Ø110 mm, Cat. no. 1001 110, Schleicher and Schuell GmbH, Dassel, Germany); the filtrate was collected and concentrated by a rotary evaporator (Resona Technics, model Labo Rota 300, Gossau, Switzerland) at 40 °C for 10 min and kept at 4 °C in a dark bottle until its use.

Preparation of antimicrobial chitosan films

GE was added to the chitosan solution at concentrations of 0.3, 0.6 and 0.9 g/100 g. These concentrations were selected based on a minimum inhibitory concentration (MIC) against *S. aureus* (ATCC 25923) [3]. All mixtures were homogenized by a bench top homogenizer (Ika® Works (Asia), model T 25 basic, Selangor, Malaysia) at 9500 rpm for 2 min. The film solution (21 g) was poured on an acrylic plate with dimensions of 13×10 cm to cast an antimicrobial film.

Drying experiment

Drying of the film was performed by 4 methods, which are ambient air drying (~ 30 ºC), hot air drying at 40 ºC, vacuum drying and LPSSD at 70, 80 and 90 ºC at 10 kPa, following the methods of Mayachiew and Devahastin [8]. After drying the films were conditioned for at least 48 h in a desiccator containing saturated salt solution of magnesium nitrate (Ajax Finechem, Seven Hills, Australia), which produced a relative humidity (RH) of 53%.

Agar diffusion method

Quanlitative antimicrobial activity of the films was evaluated by the agar diffusion method [7]. The zone of inhibition assay on solid medium was used for determination of the antimicrobial effect of films against *S. aureus*. The inoculum (100 µL) of *S. aureus* containing approximately 10^6 CFU/mL was spread on the surface of Mueller Hinton agar plate. An edible film sample was cut into a 6-mm diameter disc and then placed on the agar plate. The plate was then incubated at 37 ºC for 24 h. The plate was then examined for zone of inhibition of the film disc. The total diameter of inhibition zone including the film disc was measured.

Viable cell count method

The biocide property of the antimicrobial films was evaluated by employing the macrodilution method recommended by the National Committee of Clinical Laboratory Standards [10]. About 0.3 g of each film specimen was placed in a sterilized flask in which 10 mL of *S. aureus* culture containing approximately 10^7 CFU/mL was added. The suspension was incubated at 37 ºC. 100 µL of the sample was taken at 0, 6, 12, 18 and 24 h and spread on TSA agar plate, which was incubated at 37 ºC for 24 h. The number of colonies was counted. The inhibition of bacteria growth was expressed as the reduction of cell number by log N/N_0. The test was performed in triplicate.

Statistical analysis

All data were subjected to the analysis of variance (ANOVA) using SPSS® software (Chicago, IL) and were presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95%. All experiments were performed in duplicate except when stated otherwise.

RESULTS AND DISCUSSION

Table 1 shows the antimicrobial activity of chitosan films incorporated with GE as tested by the agar diffusion method. The results showed that the chitosan films containing GE at 0.6% and 0.9% (w/w) were effective in inhibiting the growth of *S. aureus*. The diameters of the inhibition zones varied from 19.5 mm to 29.5 mm for chitosan films incorporated with 0.9% (w/w) GE and from 15.7 mm to 23.8 mm for the films with 0.6% (w/w) GE. No inhibition zone was observed when the extract concentration of 0.3% (w/w) was used. This could be ascribed to a limited GE release due probably to interaction between the extract and chitosan [11]. Another possible reason was the limit of detection of antimicrobial activity when using the disc diffusion method [12-13].

Regarding the effects of drying methods and conditions it was noted that drying methods and conditions had significant effects on the antimicrobial activity of chitosan films incorporated with GE. The results showed that ambient dried film had the highest antimicrobial activity; this was followed by LPSSD films and vacuum dried films. This may be due to the fact that the film temperature increased more rapidly and stayed at higher levels in the case of vacuum drying than in the case of LPSSD, thus inducing more thermal degradation of the antimicrobial compound [8, 14].

In addition, different intermolecular interactions also contributed to the observed results. The decrease in bacteria inhibition might be due to lower diffusion of the active agent into the agar medium as a result of higher interaction between chitosan and GE [11].

Table 1 Antimicrobial activity (in terms of inhibition zone diameter, mm) of antimicrobial films prepared by different drying methods and conditions against *S. aureus*

Drying method	Galangal extract concentration		
	0.3%	0.6%	0.9%
Ambient drying	-	23.8±0.6[a]	29.5±0.7[a]
Hot air drying	-	23.4±0.6[a]	29.2±0.4[a]
Vacuum drying			
70 ºC	-	20.5±1.0[b]	24.9±0.9[c]
80 ºC	-	15.7±0.5[c]	19.5±1.1[d]
90 ºC	-	-[d]	-[e]
LPSSD			
70 ºC	-	22.3±0.8[a]	27.1±1.0[b]
80 ºC	-	20.4±0.9[b]	23.6±0.7[c]
90 ºC	-	-[d]	-[e]

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

The antimicrobial films prepared at higher drying temperatures had lower antimicrobial activity, both in the cases of vacuum drying and LPSSD. The antimicrobial films prepared by LPSSD at 70 ºC had the highest antimicrobial activity compared with films prepared at other conditions of vacuum drying and LPSSD.

From viability test, the results indicated that the concentration of the GE significantly affected the cell viability of the *S. aureus* (Table 2). The results showed that the antimicrobial activity of the films increased with an increase in the extract concentration, as expected. Chitosan film incorporated with 0.9% (w/w) GE and prepared by ambient drying could reduce the number of *S. aureus* by about 3.66 log cycle within the contact time of 24 h. On the other hand, ambient dried film incorporated with 0.3% (w/w) GE exhibited lower cell reduction number of around 1.99 log cycle with lower decreasing rate of cell number (Figs. 1-3).

Considering the effects of the drying methods and conditions, the final bacterial counts when applying a film

Table 2 Antimicrobial activity (in terms of log N/N_0) of antimicrobial films prepared by different drying methods and conditions against *S. aureus*

Drying method	Galangal extract concentration		
	0.3%	0.6%	0.9%
Ambient drying	1.99±0.10[a]	3.14±0.16[a]	3.66±0.19[a]
Hot air drying	1.82±0.27[ab]	2.95±0.08[a]	3.42±0.31[a]
Vacuum drying			
70 ºC	1.50±0.09[bc]	2.26±0.23[bc]	2.66±0.16[bc]
80 ºC	0.97±0.23[de]	1.63±0.31[de]	2.19±0.25[cd]
90 ºC	0.64±0.23[e]	1.12±0.32[f]	1.57±0.34[e]
LPSSD			
70 ºC	1.72±0.10[ab]	2.69±0.10[ab]	3.10±0.13[ab]
80 ºC	1.14±0.27[cd]	1.91±0.13[cd]	2.41±0.23[cd]
90 ºC	0.78±0.20[de]	1.39±0.23[ef]	1.97±0.30[de]

Values in the same column with different superscripts mean that the values are significantly different ($p < 0.05$).

dried at control condition (ambient drying and hot air drying at 40 °C) and a film dried by LPSSD at 70 ºC were lower than those when using films prepared at other conditions of LPSSD and vacuum drying. In addition, the LPSSD films could decrease the cell number faster than the vacuum dried films prepared at the same drying temperature. This might be due to different intermolecular interaction between the extract and chitosan films, different microstructure of the films as well as different degrees of film swelling [11].

Antimicrobial films incorporated with 0.9% (w/w) galangal extract and prepared by ambient drying, hot air drying at 40 ºC and LPSSD at 70, 80 and 90 ºC resulted in an inhibition of *S. aureus* by up to 3.66, 3.42, 3.10, 2.41 and 1.97 log cycles, respectively. On the other hand, the decrease in the number of microorganisms in contact with vacuum dried films prepared at 70, 80 and 90 ºC was up to 2.66, 2.19 and 1.57 log cycles, respectively, during the same contact period of 24 h.

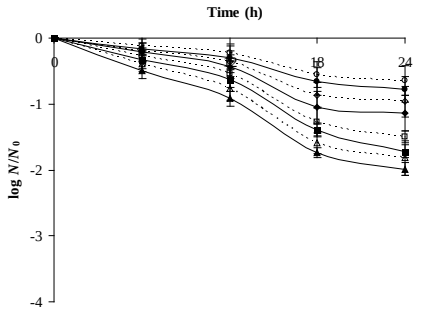

Fig. 1 Reduction of cell number in contact with chitosan films enriched with 0.3% (w/w) galangal extract and prepared by ambient drying (–▲–); hot air drying at 40 ºC (---△---); vacuum drying at 70 ºC (---□---), 80 ºC (---◇---), 90 ºC (---○---); LPSSD at 70 ºC (–■–), 80 ºC (–⬥–), 90 ºC (–●–).

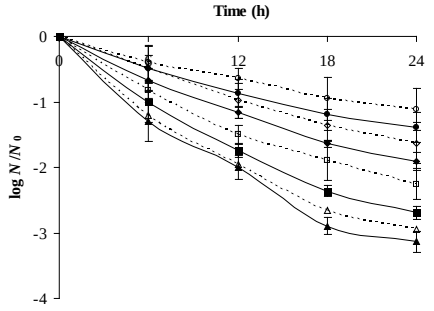

Fig. 2 Reduction of cell number in contact with chitosan films enriched with 0.6% (w/w) galangal extract and prepared by ambient drying (–▲–); hot air drying at 40 ºC (---△---); vacuum drying at 70 ºC (---□---), 80 ºC (---◇---), 90 ºC (---○---); LPSSD at 70 ºC (–■–), 80 ºC (–⬥–), 90 ºC (–●–).

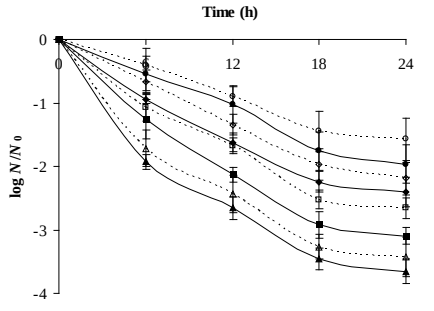

Fig. 3 Reduction of cell number in contact with chitosan films enriched with 0.9% (w/w) galangal extract and prepared by ambient drying (–▲–); hot air drying at 40 ºC (---△---); vacuum drying at 70 ºC (---□---), 80 ºC (---◇---), 90 ºC (---○---); LPSSD at 70 ºC (–■–), 80 ºC (–⬥–), 90 ºC (–●–).

The evaluation of the efficiency of chitosan films incorporated with galangal extract and prepared by different drying methods on *S. aureus* verified that the film incorporated with 0.9% (w/w) galangal extract and prepared by ambient drying, hot air drying at 40 °C and LPSSD at 70 ºC had the highest antimicrobial activity and could reduce the cell count by 3.66, 3.42 and 3.10 log cycles, respectively, after 24 h.

CONCLUSIONS

The experiments illustrated that an increase in the galangal extract concentration in chitosan films led to a higher antimicrobial activity against *S. aureus*. The antimicrobial activity was affected by the tested drying methods and conditions. Ambient drying, low-temperature hot air drying and LPSSD at 70 ºC led to films with higher antimicrobial activity may be due to lower intermolecular interaction. Optimization of antimicrobial characteristics of chitosan films incorporated with galangal extract will be useful for the design of active packaging films, which could help prevent surface growth of microorganisms.

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EFFECTS OF DRYING METHODS AND PLASTICIZER CONCENTRATION ON PHYSICAL AND MECHANICAL PROPERTIES OF EDIBLE CHITOSAN FILM

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ABSTRACT

In order to alleviate shortcomings of edible chitosan films (which have rigid and brittle nature), an idea of using advanced drying methods in combination with appropriate plasticizer to improve the mechanical properties of the films was proposed and tested. Physical (thickness and color) and mechanical (tensile strength and percent elongation) properties of edible chitosan films at four levels of glycerol concentrations (0, 25, 75 and 125% w/w chitosan) and prepared by three drying methods (hot air drying (≈40°C), vacuum drying and low-pressure superheated steam drying (LPSSD) (90°C, 10 kPa)) were investigated. It was found that the plasticizer concentration did not affect the thickness and final moisture content of the film samples at lower glycerol concentrations. The tensile strength and percent elongation of the hot air dried films generally decreased and increased with an increase in the concentration of glycerol, respectively. On the other hand, in the cases of vacuum drying and LPSSD, there was a limiting value for plasticizer concentration (25% w/w), above which the tensile strength and percent elongation of the films were not significantly different. In all cases, the color of edible chitosan films was not significantly different.

INTRODUCTION

Currently, the most popular packagings in food applications is plastics (e.g., low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polyvinyl chloride (PVC)) due to their non-breakable, light weight and easy handling characteristics. However, two serious problems of plastic packagings exist. First of all, the quantity of waste from these plastic packagings, which are not biodegradable and persist in the environment for a very long period of time, is now very high [1, 2, 3]. Another problem is that plastic materials are not completely inert. Chemical substances such as plasticizers and stabilizers can migrate from plastics to food and may lead to food quality changes and consumer health risk [1]. Therefore, in recent years, consumers have started to put more attention on edible biopolymer packagings as they are environmentally friendly and also naturally biodegradable [2]. Among many natural biomaterials that can be used to produce biodegradable and/or edible packagings, chitosan is one of most promising materials. Chitosan is a polysaccharide generally obtained by *N*-deacetylation of chitin. It is commercially available from a stable renewable source, that is, shellfish waste (shrimp and crab shell) of the seafood industry. Comparing with other polysaccharides, chitosan has several important advantages, including biocompatibility, biodegradability and non toxicity. Several studies on chitosan have reported this material as a potential candidate for edible films or coatings [4].

However, the nature of edible packaging films, which is rigid and brittle, causes limitations in food applications [5]. It is well recognized that mechanical properties, including tensile strength and percentage elongation, of synthetic packaging films are significantly better than those of edible films [3]. In order to improve the mechanical properties of edible biopolymer films, various types of plasticizers have been used. Among many possible plasticizers glycerol is most widely used.

Among many steps needed to prepare edible films, drying is considered one of the most important [6, 7, 8]. However, there are only a few reports on the combined effects of drying methods and plasticizer concentration on edible chitosan films. Hence, the aim of this study was to investigate the combined effects of drying methods and plasticizer concentration on selected physical (thickness and color) and mechanical (tensile strength and percent elongation) properties of edible chitosan films.

MATERIALS AND METHODS

Materials

Chitosan (molecular weight of 900,000 Da and degree of deacetylation of 90.20%) was purchased from S.K. Profishery Co., Ltd. (Bangkok, Thailand). Glycerol and acetic acid was obtained from Carlo Erba (Val de Reuil, Italy) and Merck (Darmstadt, Germany), respectively.

Film Preparation

The film preparation method is that of Mayachiew and Devahastin [9] with some modifications. Chitosan solution was prepared by dissolving 1.5% (w/v) chitosan and glycerol at 0, 25, 75 and 125% w/w chitosan in 1% (v/v) acetic acid under constant stirring using a magnetic stirrer (Framo®-Gerätechnik, model M21/1, Eisenbach, Germany) at 300 rpm at room temperature for 24 h. After mixing the chitosan solution was centrifuged for 15 min at 12,400 rpm by a refrigerated centrifuge (Hitachi, model Himac CR21, Ibaragi, Japan) to remove undissolved impurities in the solution. Later, the solution was degassed using a sonicator (Ultrawave, model U1350, Cardiff, UK) for 2 h. The chitosan solution (16 g) was then poured on an acrylic plate with dimensions of 13×10 cm to cast a chitosan film with a constant thickness of 15 μm for a drying experiment.

Film Drying

Hot Air Drying

A schematic diagram of a hot air dryer used in this study is illustrated in Fig. 1. The prepared film solution

was dried in the hot air dryer (≈40°C) at an inlet air velocity of 0.25 m/s. This drying process was used as a control drying process.

Vacuum Drying

For a vacuum drying experiment, the prepared film solution was dried at 90°C and 10 kPa in a dryer shown schematically in Fig. 2 [10]. The dryer consists of a stainless steel drying chamber and a liquid ring vacuum pump (Nash, model ET32030, Trumball, CT), which was used to maintain the vacuum in the drying chamber. An electric heater, rated at 1.5 kW was installed in the drying chamber to control the drying temperature. The change of the mass of the sample was detected continuously (at 60 s interval) using a load cell with an accuracy of ±0.2 g (Minebea, model Ucg-3 kg, Nagano, Japan).

Low-Pressure Superheated Steam Drying

For a low-pressure superheated steam drying experiment, the prepared film solution was also dried in a dryer shown schematically in Fig. 2; the same experimental setup as that used for the vacuum drying experiment was used but with an application of steam to the drying chamber [10].

Film Properties Determination

Chitosan films were prepared by various drying methods; their moisture content was set at approximately 14% (d.b.) [9]. The films were then conditioned for at least 48 h in a desiccator containing saturated salt solution of sodium chloride (Ajax Finechem, Seven Hills, NSW, Australia), which produced an relative humidity (RH) of 75% (an average relative humidity of the environment in Thailand).

Film Thickness Determination

The film thickness was measured using a micrometer (Mitutoyo, model 102-309, Tokyo, Japan) with an accuracy of ±2 μm. Each film sample was measured at its center and four other positions along the strip. The mechanical properties were calculated using the average thickness of each film sample.

Moisture Content Determination

The moisture content of a film sample was determined using the standard vacuum oven method [11]. The film was dried in a vacuum oven (Sanyo, model Gallenkamp/OM-09980, Loughborough, UK) at 70°C at a pressure of -900 mbar for 24 h.

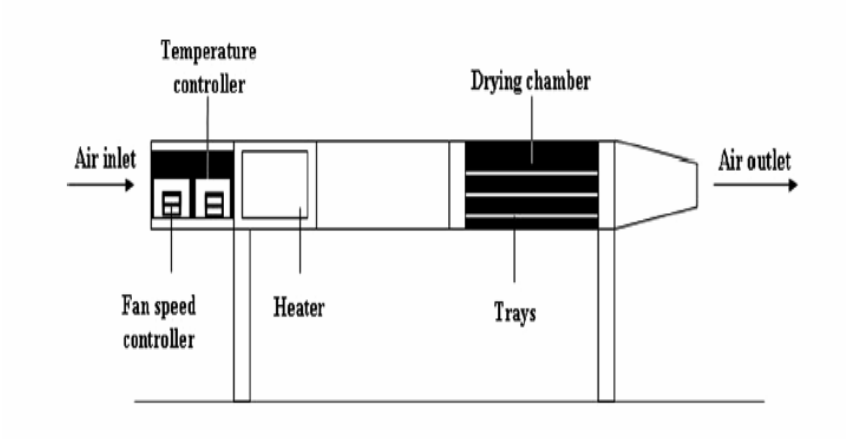

Fig. 1 A schematic diagram of hot air dryer and associated units

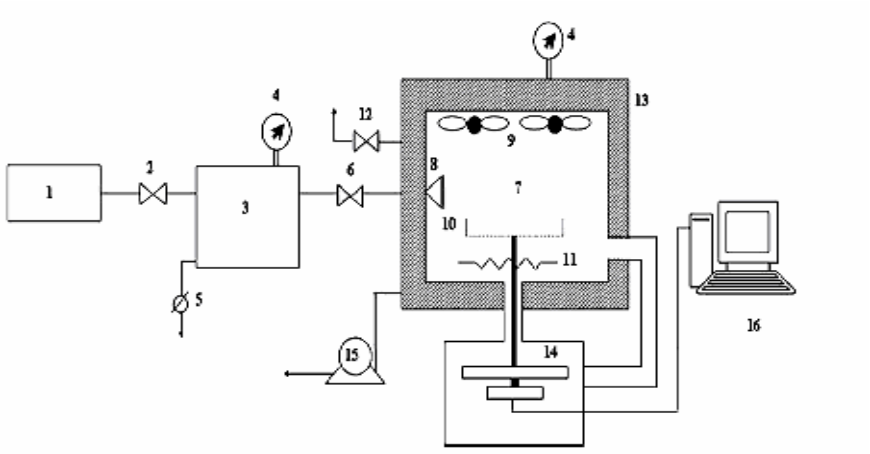

Fig. 2 A schematic diagram of vacuum or low-pressure superheated steam dryer and associated units (1, boiler; 2, steam valve; 3, steam reservoir; 4, pressure gauge; 5, steam trap; 6, steam regulator; 7, drying chamber; 8, steam inlet and distributor; 9, electric fans; 10, sample holder; 11, electric heater; 12, vacuum break-up valve; 13, insulator; 14, on-line weight indicator and logger; 15, vacuum pump; 16, PC with installed data acquisition card)

Mechanical Properties Determination

The measurement of the mechanical properties was carried out using a texture analyzer (Stable MicroSystem, model TA.XT.Plus, Surrey, UK). After conditioning chitosan films were cut into 10×2.54 cm strip and tested for tensile strength and percent elongation according to the ASTM Standard Method D882 (ASTM, 1995). Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum load for breaking the film by its cross-sectional area. Percent elongation was determined by dividing the film elongation at rupture by the initial grip separation.

Color Determination

The color of a film sample was determined with a colorimeter (HunterLab, model ColorQuest , Reston, VA) in terms of *L**, *a** and *b** values. Each film sample was measured at its center and four other positions along the film.

Statistical Analysis

All experimental data were analyzed using the analysis of variance (ANOVA) using SPSS® software. The results were presented as mean values with standard deviations. Duncan's multiple range tests were employed to establish differences between mean values at a confidence level of 95%. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Drying Characteristic of Edible Chitosan Films

The drying curves of edible chitosan films with various concentrations of glycerol undergoing hot air drying (control condition) are shown in Fig. 3. The detailed moisture content of the films during the final period of drying is enlarged and shown in Fig. 4.

It is seen from these figures that the film samples dried at control condition (hot air drying) required very long drying time. Different concentrations of glycerol provided films of more or less the same initial moisture contents but different equilibrium moisture contents. The equilibrium moisture content of the films increased with the glycerol concentration. Since glycerol is a hydrophilic plasticizer, higher concentrations of glycerol imply more water binding, leading to higher moisture contents of the films at equilibrium [12].

The drying curves of chitosan films with various concentrations of glycerol undergoing vacuum drying and low-pressure superheated steam drying (LPSSD) at 90°C and 10 kPa are shown in Fig. 5 and 6, respectively. It can be seen from Fig. 3, 5 and 6 that the films dried at control condition and LPSSD required longer drying time than the films dried by vacuum drying. The averages drying time to reach the desired final moisture content of % (d.b.) as well as the equilibrium moisture contents of chitosan films prepared by different drying methods are listed in Table 1.

Chitosan films plasticized with all glycerol concentrations had initial moisture contents in the range of 54.52-57.83 kg/kg (d.b.). At the same glycerol concentration, it was noted that different drying methods had significant effects on the required drying time to reach the desired final moisture content.

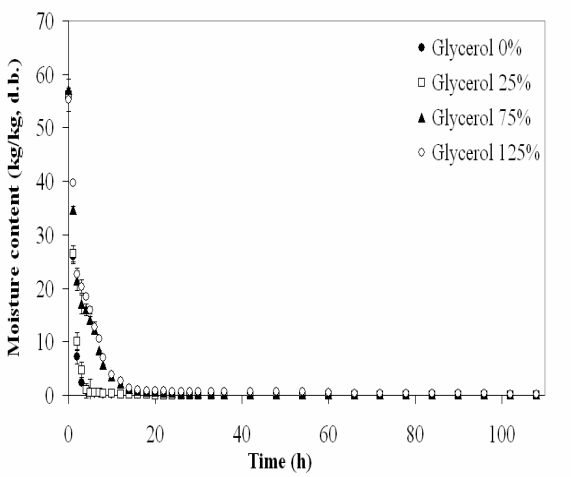

Fig. 3 Drying curves of edible chitosan films with various concentrations of glycerol undergoing hot air drying at 40°C

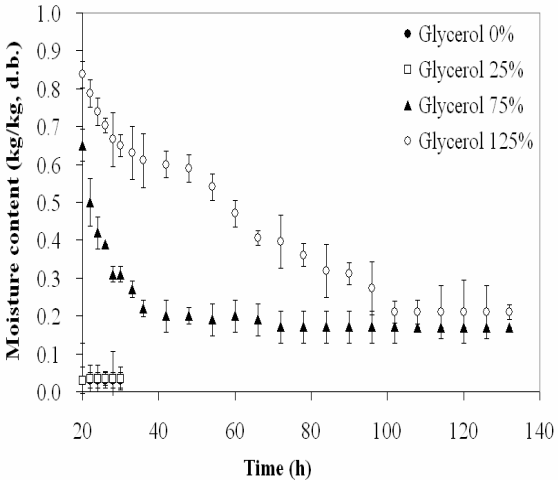

Fig. 4 Enlarged drying curves (during the final period of drying) of edible chitosan films with various concentrations of glycerol

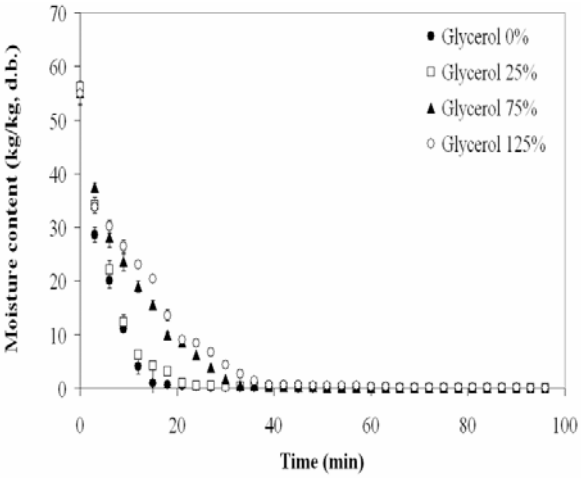

Fig. 5 Drying curves of edible chitosan films with various concentrations of glycerol undergoing vacuum drying at 90°C, 10 kPa

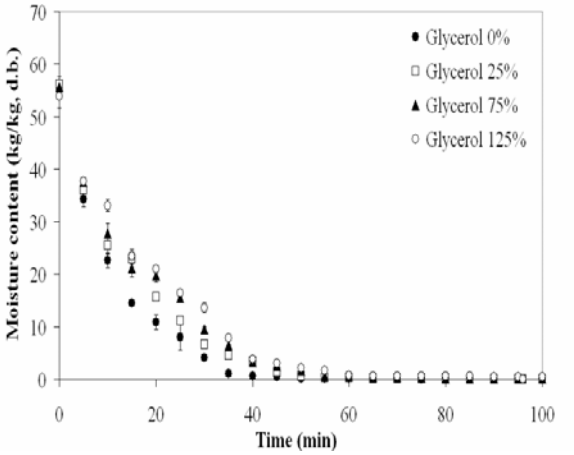

Fig. 6 Drying curves of edible chitosan films with various concentrations of glycerol undergoing low-pressure superheated steam drying at 90°C, 10 kPa

From Table 1 and Fig. 3 to 6, the hot air drying time was longer than that of LPSSD and vacuum drying. This is due to the use of lower temperature in the case of hot air drying. In addition, it was found that at the glycerol concentrations of 75 and 125% w/w chitosan it was not possible to dry the film to the desired final moisture content; the equilibrium moisture contents of the films were higher than 14 % (d.b.).

When comparing within the same drying method, it was noted that the time needed to dry films to 14 % (d.b.) increased with the glycerol concentration. This is because glycerol is a hydrophilic plasticizer; the film with a higher glycerol concentration could adsorb more water in their matrix and this led to increased drying time to reduce the film moisture.

Film Thickness

The thickness of edible chitosan films with various concentrations of glycerol after conditioning at 75% relative humidity (RH) for 48 h is shown in Table 2. It was found that drying methods had significant effects on the film thickness. In the case of hot air drying, the thickness of edible chitosan films increased with the glycerol concentration. Since glycerol is a hydrophilic plasticizer, edible chitosan films with higher concentrations of glycerol would adsorb more moisture. Hence, these films swelled, leading to an increase in the film thickness. On the other hand, in the cases of vacuum drying and low-pressure superheated steam (LPSSD), only the thickness of the films with a glycerol concentration of 125% w/w increased significantly. This is because the higher temperature in the cases of vacuum drying and LPSSD allowed for polymer chain arrangement and cohesion within the film matrix, resulting in a tighter and more compact structure [13].

Table 1 Average drying time to reach the desired final moisture content of 14% (d.b.) as well as equilibrium moisture content of edible chitosan films

Drying condition / Glycerol concentration (% w/w chitosan)	Average drying time to reach 14 % (d.b.)	Equilibrium moisture content (% d.b.)
Hot air drying (Control≈ 40 °C)		
0	10 h	4.3±0.8[a]
25	12 h	4.3±2.2[a]
75	72 h	18.3±3.3[b]
125	102 h	22.4±3.3[c]
Vacuum drying (90 °C, 10 kPa)		
0	45 min	4.4±2.3[a]
25	55 min	4.8±2.3[a]
75	80 min	4.3±2.2[a]
125	95 min	5.2±3.4[a]
LPSSD (90 °C, 10 kPa)		
0	60 min	3.3±1.8[a]
25	80 min	4.2±1.3[a]
75	95 min	3.8±2.7[a]
125	110 min	4.8±2.4[a]

Same letter in the same column means that the values are not significantly different at 95 % confidence level ($p > 0.05$)

The glycerol concentration had a significant effect on the film thickness. At glycerol concentrations of 0, 25 and 75%w/w, the thickness of films dried by all drying methods was not significantly different, except for the films with the glycerol concentration of 75% w/w and dried by hot air drying. In all cases, edible chitosan films with 125% w/w added glycerol had thickness higher than the desired value of 15 μm; in the case of hot air drying the films with glycerol concentrations of 75 and 125% w/w suffered from this excessive thickness problem.

Mechanical Properties

In this study only two most relevant properties, namely, tensile strength and percent elongation were tested. The mechanical properties of commercial stretch films (Clean wrap™ and M wrap™) were also evaluated and compared with those of edible chitosan films after conditioning at 75% RH for 48 h. The results are shown in Table 2. It was noted that different drying methods had significant effects on the tensile strength and percent elongation of the films. In all cases, the films without

Table 2 Moisture content, thickness, tensile strength and percent elongation of commercial stretch films and chitosan films

Film type / Glycerol concentration (% w/w chitosan)	Moisture content (% d.b.)		Thickness (μm)		Tensile strength (MPa)	Percent elongation (%)
	Before conditioning at 75% RH	After conditioning at 75% RH	Before conditioning at 75% RH	After conditioning at 75% RH		
Chitosan film undergoing hot air drying at 40°C						
0	14.4±2.4^{b}	18.4±4.3^{a}	15±0.6^{b}	15±0.6^{a}	64.3±4.9^{c}	13.7±2.0^{ab}
25	14.7±1.8^{b}	19.3±2.7^{a}	15±0.6^{b}	15±0.6^{a}	44.9±3.1^{c}	16.9±2.8^{ab}
75	18.3±2.7^{bc}	26.2±3.8^{bc}	29±0.6^{c}	32±3.2^{c}	15.8±2.9^{b}	39.9±3.6^{c}
125	22.4±2.8^{c}	35.4±4.2^{d}	45±0.6^{d}	47±4.2^{d}	8.7±1.9^{a}	48.7±5.6^{d}
Chitosan film undergoing vacuum drying at 90°C, 10 kPa						
0	14.2±2.0^{b}	19.1±1.7^{a}	15±0.6^{b}	16±0.6^{a}	60.5±5.5^{de}	11.9±3.3^{a}
25	15.3±3.7^{b}	22.4±3.2^{ab}	15±0.6^{b}	15±0.6^{a}	44.3±5.9^{c}	14.2±2.6^{ab}
75	14.3±3.2^{b}	26.2±2.8^{bc}	15±0.6^{b}	15±0.6^{a}	37.2±1.8^{c}	14.8±3.5^{ab}
125	14.2±4.4^{b}	30.3±3.3^{cd}	15±0.6^{b}	19±2.1^{ab}	39.6±1.4^{c}	20.2±5.2^{b}
Chitosan film undergoing LPSSD at 90°C, 10 kPa						
0	13.1±3.3^{b}	18.1±2.4^{a}	15±0.6^{b}	15±0.6^{a}	55.2±4.0^{d}	12.1±4.3^{a}
25	14.2±4.2^{b}	20.6±2.2^{a}	15±0.6^{b}	15±0.6^{a}	40.9±2.9^{c}	19.3±2.2^{b}
75	13.9±3.4b	20.3±3.2^{a}	15±0.6^{b}	15±0.6^{a}	43.7±1.9^{c}	19.0±3.6^{b}
125	15.4±2.7^{b}	28.7±3.4^{c}	15±0.6^{b}	20±3.2^{b}	38.6±2.8^{c}	20.2±2.2^{b}
Clean wrap™ (LDPE)	0.07±0.02^{a}	-	13±0.6^{a}	-	20.4±2.0^{b}	67.8±11.6^{e}
M wrap™ (PVC)	0.06±0.02^{a}	-	12±0.6^{a}	-	22.0±2.2^{b}	62.2±10.0^{e}
TISI (LDPE)	-	-	-	-	> 3.3	> 90
TISI (PVC)	-	-	-	-	> 4.1	> 60
Krochta and Mulder-Johnson (1997) (Moderate films)			-	-	10-100	10-50

Same letter in the same column means that the values are not significantly different at 95 % confidence level ($p > 0.05$).
TISI = Thai Industrial Standard Institute.

glycerol had highest tensile strength and lowest percent elongation. In the case of hot air drying, the expected effect of glycerol on the tested mechanical properties (decrease in the tensile strength and increase in the percent elongation with increasing plasticizer concentration) was observed. On the other hand, in the cases of vacuum drying and LPSSD the glycerol concentration did not exhibit the conventional effect of plasticizer at the glycerol concentrations in the range of 25-125% w/w; the tensile strength and percent elongation of the films plasticized at these glycerol concentrations were not significantly different. This could be due to the higher degrees of crystallinity and thermal cross-linkage that occurred more during these two drying processes [9].

Based on the classifications of Krochta and Mulder-Johnson [14] the films prepared in the present study had moderate mechanical properties (tensile strength and percent elongation about 10-100 MPa and 10-50%, respectively). On the other hand, the Thai Industrial Standard Institute (TISI) states that stretch films must have tensile strength and percent elongation of at least 4 MPa and 60%, respectively, for PVC, and of at least 3.3 MPa and 90%, respectively, for LDPE [15]. From these standards, all edible chitosan films had lower percent elongation than that required by the TISI standard. However, all the films in this study had good quality in terms of tensile strength.

Except for the hot air dried films with glycerol concentrations of 75 and 125% w/w, the tensile strength of all other edible chitosan films was higher than that of the commercial stretch films. In all cases, percent elongation of edible chitosan films was lower than that of the commercial stretch films. This means that the edible chitosan films in this study are stronger but less stretchable than the commercial stretch films.

Color

The color of commercial stretch films (Clean wrap™ and M wrap™) and that of edible chitosan films with various concentrations of glycerol undergoing hot air drying, vacuum drying and LPSSD after conditionings at 75% RH for 48 h are shown in Table 3. In terms of the lightness (L^* value), the films of all cases were not significantly different. In terms of the redness (a^* value), chitosan films of all cases exhibited green color more than the commercial stretch films. In the case of yellowness (b^* value), the chitosan films prepared by all drying methods exhibited yellow color more than the commercial stretch films. This is because higher temperatures led to higher level of Maillard browning reactions. However, among all drying methods and glycerol concentrations, the color of the films was not significantly different.

Table 3 Color of commercial stretch films and chitosan films

Film type / Glycerol concentration (% w/w chitosan)	Color		
	L^*	a^*	b^*
Chitosan film undergoing hot air drying at 40°C			
0	96.6±0.1^{a}	-0.2±0.1^{b}	1.4±0.3^{bc}
25	96.6±0.0^{a}	-0.3±0.1^{bc}	1.5±0.1^{bc}
75	97.0±0.1^{a}	-0.4±0.1^{c}	1.7±0.4^{cd}
125	97.2±0.0^{a}	-0.4±0.1^{c}	1.7±0.2^{cd}
Chitosan film undergoing vacuum drying at 90°C, 10 kPa			
0	96.8±0.1^{a}	-0.3±0.0^{bc}	1.4±0.2^{b}
25	96.6±0.0^{a}	-0.9±0.0^{f}	3.3±0.2^{de}
75	96.9±0.1^{a}	-0.8±0.1^{ef}	3.6±0.6^{e}
125	96.8±0.1^{a}	-0.7±0.1^{ef}	3.2±0.1^{de}
Chitosan film undergoing LPSSD at 90°C, 10 kPa			
0	96.5±0.1^{a}	-0.5±0.0^{d}	2.9±0.1^{de}
25	96.4±0.1^{a}	-0.7±0.1^{e}	3.2±0.3^{de}
75	96.6±0.1^{a}	-0.4±0.1^{c}	3.0±0.3^{de}
125	96.7±0.1^{a}	-0.3±0.1^{bc}	2.7±0.1^{d}
Clean wrap™ (LDPE)	96.9±0.2^{a}	0.02±0.0^{a}	0.04±0.0^{a}
M wrap™ (PVC)	97.2±0.2^{a}	0.02±0.0^{a}	0.04±0.0^{a}

Same letter in the same column means that the values are not significantly different at 95 % confidence level ($p > 0.05$)

CONCLUSIONS

In this study, the effect of glycerol concentration (0, 25, 75 and 125% w/w chitosan) on physical and mechanical properties of edible chitosan films prepared by hot air drying at 40°C, vacuum drying and low-pressure superheated steam drying (LPSSD) at 90°C, 10 kPa was investigated. The results showed that at the same glycerol concentration, the films dried by hot air drying required longer drying time than the films dried by LPSSD and vacuum drying. Within the same drying method, the time used to dry the films increased with the glycerol concentration.

The glycerol concentration had different effects on the thickness of edible chitosan films prepared by different drying methods. The thickness of hot air dried films increased with glycerol concentration whereas only the thickness of vacuum dried films and LPSSD films with glycerol concentration of 125% was significantly higher than that of the other films.

The tensile strength and percent elongation of the films generally decreased and increased with an increase in the concentration of plasticizer in the case of hot air drying. However, in the cases of vacuum drying and LPSSD, the tensile strength and percent elongation of the films with glycerol concentrations of 25-125% were not significantly different. Although hot air dried films with a glycerol concentration of 125% had the highest percent elongation, it had the thickness and moisture content higher than the preset values. Thus, it cannot still be used in practice. The color of chitosan films was not significantly different in all cases. Based on the result of this study, the optimum concentration of plasticizer and the best drying method that should be used is the concentration of 25% w/w and drying by low-pressure superheated steam.

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