Chitosan and chitosan/wheat gluten blends; properties of extrudates, solid films and bio-foams

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ABSTRACT

This thesis presents four different studies describing the characteristics and processing opportunities of two widely available biopolymers: chitosan and wheat gluten. The interest in these materials is mainly because they are bio-based and obtained as co- or by-products in the fuel and food sector.

In the first study, high solids content chitosan samples (60 wt.%) were successfully extruded. Chitosan extrusion has previously been reported but not chitosan extrusion with a high solids content, which decreases the drying time and increases the production volume. An orthogonal experimental design was used to assess the influence of formulation and processing conditions, and the optimal formulation and conditions were determined from the orthogonal experimental analysis and the qualities of the extrudates. The mechanical properties and processing-liquid mass loss of the optimized extrudates showed that the extrudates became stable within three days. The changes in the mechanical properties depended on the liquid mass loss.

In a separate study, monocarboxylic (formic, acetic, propionic, and butyric) acid uptake and diffusion in chitosan films were investigated. It is of importance in order to be able to optimize the production of this material with the casting technique. The time of the equilibration uptake in the chitosan films exposed to propionic and butyric acid was nine months. This long equilibration time encouraged us study the exposed films further. The uptake and diffusivity of acid in the films decreased with increasing acid molecular size. A two-stage absorption curve was observed for the films exposed to propionic acid vapour. The films at the different stages showed different diffusivities. The acid transport was also affected by the structure of the chitosan films. X-ray diffraction suggested that the crystal structure of the original films disappeared after the films had been dried from their acid-swollen state, and that the microstructure of the dried films depended on the molecular size of the acid. Compared with the original films, the dried films retained their ductility, although a decrease in the molecular weight of the chitosan was detected. The water resistance of the acid-exposed films was increased, even though the crystallinity of these films was lower.

The third study was devoted to chitosan/wheat gluten blend films cast from aqueous solutions. Different solvent types, additives and drying methods were used to examine their effects on the microstructures of the blended films. Chitosan and wheat gluten were immiscible in the aqueous blend, and the wheat gluten formed a discrete phase, and the homogeneity of the films was improved by using a reducing agent, compared with films prepared using only water/ethanol as cast media. Adding urea and surfactants resulted in a medium homogeneity of the films compared to those prepared with the reducing agents or with only water/ethanol. An elongated wheat gluten phase was observed in a film using glyoxal, in contrast to pure chitosan/wheat gluten blends. The opacity of the different films was studied. The mechanical properties and humidity uptake of the
films increased with increasing chitosan content. The films containing 30 wt.% of wheat gluten showed the most promising mechanical properties, close to those of the pristine chitosan films.

The final part describes the preparation and properties of a bio-foam composed of a blend of chitosan and wheat gluten. This foam was prepared without any porogen or frozen liquid phase to create porosity. A unique phase distribution of the chitosan and wheat gluten solutions formed without any agitation, and the foam was obtained when the liquid phase were withdrawn under vacuum. These foams showed high mass uptake of n-hexane and water in a short time due to their open pores and high porosity. The maximum uptake of n-hexane measured was 20 times the initial mass of the foam. The foams showed a high rebound resilience (94 % at 20 % compression strain) and they were not broken when subjected to bending.

**Keywords:** Chitosan, Extrusion, Film, Monocarboxylic acid, Uptake, Diffusivity, Wheat gluten, Blend, Foam, Microstructures, Opacity, Crystallinity, Mechanical properties
List of appended papers

This thesis is a summary of the following papers:

   Fei Chen, Fritjof Nilsson, Mikael Gällstedt, Mikael S. Hedenqvist
   *Polymers from Renewable Resources*, 2014, 5, 1–12.

B. Unusual Effects of Monocarboxylic Acids on The Structure and on The Transport and Mechanical Properties of Chitosan Films.
   Fei Chen, Mikael Gällstedt, Richard T. Olsson, Ulf W. Gedde, Mikael S. Hedenqvist

   Fei Chen, Xavier Monnier., Mikael Gällstedt, Ulf W. Gedde., Mikael S. Hedenqvist

D. A Novel Chitosan/Wheat Gluten Biofoam Fabricated by Mixing and Vacuum-drying.
   Fei Chen, Mikael Gällstedt, Richard T. Olsson, Ulf W. Gedde, Mikael S. Hedenqvist
   Manuscript

The author’s contributions to each of these papers are the following:

A. Performed the experimental work and manuscript writing.
B. Performed the experimental work (except for size-exclusion chromatography) and manuscript writing.
C. Performed most of the experimental work and manuscript writing.
D. Performed the experimental work and manuscript writing.
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1. PURPOSE OF THE STUDY

The use of renewable materials is a potential solution to problem of the limitation of petroleum resources. The purpose of this work was to study and develop properties and applications of polymer materials based on chitosan, a polymer from renewable resources. Although chitosan has received attention in the past decades, chitosan extrusion with a high solids content and the transport of acids in chitosan films under dry condition have not been tested. Studying processing and the properties can further develop the applications of chitosan-based materials.

Blend materials, from metals to polymers, are used in many applications. From a practical point of view, properties of chitosan materials could be improved by blending with wheat gluten, another renewable polymer. However, only few studies have been reported on blends of chitosan and wheat gluten blend and on a very limited content of chitosan and wheat gluten.

The goals of this study were:

(1) To develop chitosan extrusion with a high solids content and to study effects of formulation and processing conditions on the extrusion;

(2) To study the transport of acids in chitosan films, and to study the changes in these films as a result of exposure to the acids;

(3) To explore a new bio-blend film based on chitosan and wheat gluten, and to find methods to obtain fairly homogeneous films;

(4) To prepare a chitosan/wheat gluten blend foam using a facile method, to explain the principle mechanism of foam formation, and to characterize the properties of the foam.

All these goals serve to facilitate the use of bio-based polymers (wheat gluten and chitosan) as replacement of fossil-based polymers.
2. INTRODUCTION

2.1. Chitosan

Chitosan is a linear copolymer of β-(1, 4)-linked 2-acetamido-2-deoxy-β-d-glucopyranose and 2-amino-2-deoxy-β-d-glucopyranose (Figure 1). Chitosan is obtained from the deacetylation of chitin by alkaline treatment or by enzymatic hydrolysis. Chitin is the second most abundant polysaccharide in nature and it is readily obtained from natural resources such as the shells of crabs and shrimps, and from fungal mycelium [1–4]. The repeating unit contains one primary amino (-NH₂) group at the C-2 position and two hydroxyl (-OH) groups at the C-3 and C-6 positions. These groups provide active sites capable of reacting with other polymers or reagents. The -NH₂ groups can react with aldehydes or ketones to achieve alkylation [2,4–6]. For example, a copolymer of chitosan and poly(ethylene glycol)-aldehyde was obtained based on this reaction [7]. A crosslinking reaction can also occur between the -NH₂ of chitosan and a dialdehyde (glyoxal) [8–10]. Furthermore, by protonation of these amino groups under acidic conditions, it is possible to convert a neutral chitosan to a cationic biopolymer. These protonated amino groups are able to form chelate compounds with some heavy metals [5]. This cationic chitosan is also claimed to exhibit good antimicrobial activity against bacteria and fungi [11–16]. The amino and hydroxyl groups can also readily form strong inter- and intra-molecular hydrogen bonds, which gives chitosan a rigid crystalline structure [3,4,17].

![Structure of chitosan](image)

**Figure 1.** Structure of chitosan

It has been reported that chitin degrades between 150 and 600 °C [18]. A pristine chitosan sample starts to melt at a temperature of 120 ± 10 °C [19]. A chitosan film prepared in acid solution [20–22] usually shows a mass loss assigned to the evaporation of water at ca. 100 °C. The second mass loss, related to the evaporation of chemisorbed water and the elimination of -NH₂ groups, occurs at 130 – 140 °C. Because this temperature is close to the melting temperature of pristine chitosan, chitosan may, in fact, degrade before melting [1]. The decompositions of amine units, and the dehydration,
depolymerization and pyrolytic decomposition of the polysaccharide backbone occurs at 290 °C. Chitosan is completely degraded at 600 °C. Furthermore, the thermal properties of chitosan are dependent on the kind of chitin and on the methods used to deacetylate chitin [2].

All the characteristics and general properties of chitosan are influenced by the degree of deacetylation (DD, the percentage of glucopyranose units with amino groups) and the molecular weight [5]. For example, the solubility and antimicrobial property of chitosan increase with increasing DD [4], whereas the crystallinity decreases with increasing DD [4,23]. The biodegradability was improved by increasing the molecular weight. The DD is usually between 50 and 98 % [3,24,25]. Several approaches have been developed to determine the DD of chitosan, such as infrared spectroscopy (IR) [21,26,27], elemental analysis [28], nuclear magnetic resonance (NMR) [27,29], and UV-visible spectroscopy [30]. Molecular weights of chitosan have been reported over a wide range from 50 to 2000 kDa. The molecular weight can be assessed by size-exclusion chromatography (SEC, aqueous acid used as a solvent) or from intrinsic viscosity. The Mark-Houwink equation has been used to calculate the molecular weight from intrinsic viscosity measurements [5,26,30].

2.2. Chitosan extrusion

Extrusion has been widely applied to produce petroleum-based materials for many decades. The advantages of extrusion are its ability to efficiently process many types of raw materials at low cost, and to continuously produce products with complex cross-sections. However, only a few studies have considered the extrusion of chitosan. Blends of chitosan and poly(acrylic acid) (PAA) and chitosan and ethylene-vinyl-alcohol copolymer (EVOH) have been processed by melt extrusion [31,32]. Blends containing high chitosan contents (> 50 wt.% [31] showed poor properties, and since the extrusion had to be performed at a high temperature (at least 160 °C), thermo-oxidation of chitosan occurred [20–22]. Chitosan materials also can be processed by wet extrusion in which a processing liquid is added. Pellets were produced from chitosan and microcrystalline cellulose (MCC) using an extrusion/spheronization process [33–35]. However, when these pellets were applied as a drug release system, the presence of MCC led to a deteriorating in the drug-releasing properties, i.e., non-disintegration of the pellets and drug decomposition. In another study [36], it was difficult to extrude the blends of chitosan and hydrophilic polymers when the water content in the processing liquid was high. Therefore, it is important to further develop pristine chitosan extrusion. Pristine chitosan was extruded by Steckel and Mindermann-Nogly [37], and a large amount of water and acetic acid was used as processing liquid. Although the high content of processing liquid made a high chitosan content extrusion possible, it severely complicated the drying process.

The extrudability of chitosan materials with a high solids content (60 wt.%) was studied, and the molecular mass distribution of chitosan, the ratio of acetic acid in processing liquid, the barrel
temperature, and the screw speed were varied in the study. The experimental plan was based on an orthogonal design. Optimized formulations and processing conditions were first determined based on the initial torque in the extrusion. The final optimisation was based on the quality of the dry extrudate (homogeneity and surface finish).

2.3. Transport of acids in chitosan films and effects of acids on the properties of chitosan films

It has been reported that chitosan can be processed by various methods such as extrusion and injection moulding [2,23,38–40]. Because the amino groups in the chitosan chains are readily protonated in acids or their aqueous solutions, chitosan can be dissolved in these media. It has been reported that acids can change the structure and properties of chitosan materials [41–53]. However, little attention has been paid to the desorption kinetics of acids in chitosan materials. Ouattara and his colleagues [54] reported non-linear diffusion of acetic and propionic acid in chitosan films when the films were swollen by water. It was found [55] that a chitosan film cast from butyric acid solution took a longer time to reach a state free from any residual acid than a film cast from acetic acid, and that the drying kinetics were affected by the humidity. The thermodynamics and kinetics of the uptake of tannic acid and humic acid in chitosan-based composite beads were also studied under aqueous conditions [66]. All the experiments were performed with presence of water and solute-saturated samples were not used at the beginning of the diffusion, and it would be interesting to study acid uptake and acid diffusion of a saturated chitosan material under a low humidity condition.

In this study, the diffusion kinetics of concentrated formic, acetic, propionic, and butyric acid in chitosan films was assessed in a dry atmosphere. The reasons for choosing these acids were 1) that they are all used to prepare chitosan materials; and 2) that the effect on the diffusivity of increasing the size of the acid molecules can be examined. A short time for the acid uptake to saturation was expected, but the films exposed to propionic and butyric acids took a very long time to equilibrium, nine months, and a two-stage absorption was observed in the films exposed to propionic acid vapour. This sorption behaviour suggested structural changes in the chitosan films due to the acid uptake.

2.4. Chitosan blends

Several chitosan blends have been studied in recent decades. Blending chitosan with a hydrophilic polymer, such as poly(vinyl alcohol), poly(ethylene oxide), and poly(N-vinyl-2-pyrrolidone), is a feasible way to modify the physical and chemical properties of chitosan materials [56–61]. The interactions among the functional groups of the components favour the compatibility. Blends of chitosan and other polysaccharides (cellulose and starch) have a potential for use in food packaging,
because they are biodegradable and non-toxic, and have antimicrobial properties at a low cost [22,34,60–62].

2.4.1. Wheat gluten
Wheat gluten (WG) is the proteinaceous component of wheat flour, which is one of the abundant plant sources on the earth. Wheat gluten can be obtained as a by-product from wheat starch industries. Due to its high production volume and low cost, wheat gluten has been widely used. Wheat gluten is composed of two components: an alcohol-soluble monomeric protein fraction (gliadin) and a polymeric protein fraction, glutenin. There are thiol groups of cysteine residues in glutenin. Oxidation of the thiol groups can lead to disulphide crosslinks [63]. These disulphide bonds together with other interactions in the molecular chains are responsible for the insolubility of wheat gluten in neutral aqueous solutions. In order to process wheat gluten, reducing agents such as sodium sulfitte can be used to reduce the number of disulphide bonds, and the pH of the solutions can be altered above or below the isoelectric points of wheat gluten in order to more easily denature the protein [64–66].

2.4.2. Chitosan and wheat gluten blends in solid films
Only a few papers deal with blends based on chitosan and wheat gluten. Park and Bae [67] found that the mechanical, water vapour barrier and antimicrobial properties of wheat gluten samples were improved by adding only 3 wt.% of chitosan. The chitosan/wheat gliadin blends had a better water resistance than pristine chitosan, and the antimicrobial properties of the blend films increased with increasing chitosan content [68]. It was reported that chitosan/gliadin materials formed discrete domains in the blends with chitosan contents of 20 – 60 wt.%, and that phase inversion was observed at 40 wt.% chitosan [69]. The stiffness and strength, the water vapour permeability, and the water content of the blend increased with increasing chitosan content. Hence, blending chitosan and wheat gluten is an effective and efficient way to eliminate the drawbacks of the pristine materials. The flexibility and toughness of the blends are much greater than those of pristine wheat gluten. The blends also showed a greater long-term stability than plasticized wheat gluten, where there is a risk that the plasticizers may migrate from the bulk to the surfaces [70,71]. The sensitivity to moisture decreased in blends with wheat gluten. In addition, adding wheat gluten to chitosan can reduce the cost, because wheat gluten is cheaper than chitosan [72]. However, the reported studies concentrated on very low chitosan contents or the use of wheat gliadin rather than wheat gluten. From a commercial point of view, extracting gliadin from wheat gluten complicates the manufacturing process. Furthermore, the rheology of gliadin is different from that of gluten. It is, therefore, important to study blends of chitosan and wheat gluten with higher chitosan contents.

In this study, films of chitosan/wheat gluten blend were prepared with a number of additives (surfactants/reducing agents/plasticisers/denaturing agents/crosslinking agent) using different processing conditions. The effects of the additives and the processing conditions on the microstructures and properties of the blends were studied.
2.4.3. Bio-foams of chitosan and wheat gluten blend

Foams and porous materials based on both chitosan and wheat gluten have been widely studied. Chitosan-based porous materials can be used as separation filters for wastewater treatment or protein separation [73,74], as scaffolds for a wound dressing [75,76], as templates for porous ceramics [77], or as materials for tissue engineering [78–80]. The excellent foaming properties of wheat gluten have been extensively studied and have been a promising reason for the use of wheat gluten in the food industry for many years [81–83]. Recently, researchers explored wheat gluten foams as an alternative type of sound-insulation material [84–87]. A few studies have reported chitosan/wheat gluten blends [88], but no one has proposed a foam based on a chitosan/wheat gluten blend.

Porous materials can be prepared by different methods. The use of a porogen is a common way to create a porous structure in chitosan. For instance, inorganic particles (sodium chloride or silica [73,74]) or a hydrophilic polymer (polyethylene glycol [89]) were initially dispersed in the chitosan, and a pore structure was formed after a phase inversion process. In other studies [76,79,90], porous chitosan materials were obtained by gas/bubble foaming. Bubbles were created in a chitosan solution by mechanical agitation or blowing. Pore structures formed after the chitosan solution was dried. A cross-linking agent or alkaline treatment was required to increase the viscosity of the solution in order to prevent the bubbles from collapsing during drying. Wheat gluten foams were prepared by heating the wheat gluten solution with an emulsifier or fluid carbon dioxide to a temperature of at least 45 °C [84,85]. Besides these methods, freeze-drying is frequently used to fabricate both chitosan and wheat gluten foams/porous materials [75,77,78,80,86,87,91,92]. A basic procedure for freeze-drying as follows: a homogeneous chitosan or wheat gluten aqueous solution is frozen at low temperature (in a cold chamber or in liquid nitrogen), and the frozen liquid phase acts as a porogen in the matrix. Pore structures are subsequently formed after sublimation of the frozen phase under vacuum. Recently, chitosan foams with more complex structures were fabricated by an advanced technique, ice segregation induced self-assembly (ISISA) [93,94]. The use of a porogen, a gas foaming processing or a freeze-drying was thus necessary to prepare chitosan or wheat gluten foams.

In the work described in this thesis, chitosan/wheat gluten blend foams were prepared by a novel method that did not require the use of a porogen, injected gas, or frozen liquid phase. The chitosan and wheat gluten solutions were poured together to form a two-phase liquid, but the distribution of these two phases in the mixture changed with time, and a mixture with a new phase distribution formed after 40 min. Foams were obtained after this mixture was dried under vacuum. The formation of these porous structures is suggested, and the pore structure, porosity, sorption, and mechanical properties of samples with 100% foam were assessed.
3. EXPERIMENTAL

3.1. Materials

3.1.1. Materials for chitosan extrusion
The low molar mass chitosan (hereafter denoted LC, $\bar{M}_w \approx 12$ kDa, $\bar{M}_n \approx 1.6$ kDa) and the high molar mass chitosan (HC, $\bar{M}_w \approx 133$ kDa, $\bar{M}_n \approx 93$ kDa) were food grades supplied by Shanghai Nicechem Co., Ltd. Their degrees of deacetylation (DD) were 90 and 95 %, respectively. Before extrusion they were dried in a vacuum oven (50 mbar) at 70 °C for 12 h. Acetic acid (ACS, reagent grade 100 %) was purchased from EMSURE. Deionized water was used throughout.

3.1.2. Materials for chitosan films and chitosan/wheat gluten blends
The chitosan was provided by Sigma Aldrich and had a molar mass of: $\bar{M}_w = 790$ kDa and $\bar{M}_n = 210$ kDa. The degree of deacetylation (DD), as revealed by infrared spectroscopy, was 76 % [27]. Commercial wheat gluten powder was kindly supplied by Reppe AB, Lidköping, Sweden. According to the supplier, the gluten protein content (according to Mod NMKL nr 6, Kjeltec, Nx5.7) was 77.7 % and the starch content was 5.8 % (Ewers, polarimetric method). Anhydrous acetic acid (purity ≥ 99 %), polyethylene glycol sorbitan monolaurate (Tween 20), 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol (Triton-X), hexadecyltrimethyl ammonium bromide (HTLB), glyoxal (40 wt.% in H$_2$O), sodium sulfite (purity = 98 %), DL-dithiothreitol (DTT, purity > 98 %), 2-mercaptoethanol (purity ≥ 99 %) and sodium dodecyl sulphate (SDS, 20 % in H$_2$O, analytical) were obtained from Sigma Aldrich. Formic acid (purity = 97 %), propionic acid (purity = 99 %), butyric acid (purity ≥ 99 %), ammonia methanol solution (2 M), sodium acetate trihydrate (ACS, purity 99 %), sodium hydroxide (ACS, purity = 97 %), glyceral (ultrapure, HPLC grade), and drying agent (silica gel with moisture indicator) were supplied by Alfa Aesar. Potassium dihydrogen phosphate (GR for analysis, purity = 99.5 %) and urea (purity ≥ 99.5 %) were purchased from Merck. Ethanol (purity = 96 %) was supplied by VWR. n-Hexane (laboratory reagent grade) was supplied by Ficher Scientific. Deionized water was used in all the experiments.

3.2. Chitosan extrusion

3.2.1. Orthogonal experimental design
An orthogonal table was used to plan the experiments and to assess the influence of the different factors. An optimized combination of the factors and the levels can be obtained by range and variance analysis. In a trial, the chitosan content in the extrudates can be raised from 40 to 58 wt.%. It was also found that the ratio of low molecular weight chitosan (LC) to high molecular weight chitosan (HC), the content of acetic acid in the processing liquid, the barrel temperature, the screw speed and glycerol affected the extrusion process. Based on these findings, samples with 60 wt.%
solids content were extruded and glycerol was deleted from the experiment in order to limit the number of factors. Four factors each at four levels for each were arranged in an L16 orthogonal table (Table 2). The initial torque in the extrusion was adjusted to optimise the extrusion in the first step. The barrel temperatures (T) were 40, 50, 60 and 70°C and the screw speeds (S) were 40, 50, 60 and 70 rpm. The contents (H) of acetic acid (HAc) in the processing liquid were 10, 30, 50 and 70 wt.%. The LC/HC mass ratios (R) were 1/4, 2/3, 1/1 and 7/3.

3.2.3. Extrusion
The LC and HC powders were mixed at ambient conditions in a beaker by stirring with a rod, and water/HAc was then added using a mechanical mixer (IKA Labortechnik-RW20) for ca. 30 min (room temperature, 2000 rpm). The wet powder was then extruded in a laboratory counter-rotating twin-screw extruder (HAAKE MiniLab II Rheomex CTW 5) using a die yielding 3.760 ± 0.005 mm wide and 1.005 ± 0.005 mm thick tapes. The torque of the drive motor of the extruder was measured at the instant when 2 cm of tape had been obtained. This is referred to as the initial torque. The extrudates were cut into ca. 9 cm long pieces that were kept in a climate room (23 °C and 50 % RH) before testing.

3.3. Chitosan film preparation

3.3.1. Preparation of chitosan acetate films
Acetic acid at two different concentrations, 0.2 M and 1 M, was used to prepare the chitosan films, referred to as 0.2M and 1M, respectively. The chitosan powder was dissolved in the aqueous acetic acid to obtain a solution with a concentration of 1 g per 100 mL of solvent. The solution was stirred overnight and the pH of the solution was 4.0 ± 0.2. The chitosan films were obtained by drying the solutions in an oven at 26 ± 1 °C at 40 % RH, in which the air (volume = 256 L) was exchanged 40 times per hour. The chitosan films were stored in a desiccator with a silica desiccant for at least one week before being further studied. These chitosan acetate films are referred to as the original chitosan films.

3.3.2. Preparation of acetic-acid-free chitosan films
An acetic-acid-free film was prepared according to ref. [95]. A 0.2 M film was immersed in a bottle with an ammonia methanol solution. The bottle was shaken with a Yellowline OS 10 basic (SKAFTE) shaker for 15 min at 50 cycles/min. The solution was then replaced with water with an additional shaking for 15 min and the films were finally dried in a vacuum oven at 60 °C overnight. The chitosan films were stored in a desiccator with a silica desiccant for at least one week before being further studied. These films are referred to as the original buffered film.
3.4. Acid sorption and diffusion in the chitosan films

The materials and the experimental scheme used in this study are illustrated in Figure 2. The chitosan films were placed above the acid liquids in desiccators at 21 ± 2 °C. The mass of each film sample was measured by intermittently removing the sample from the desiccator and weighing it on a Precisa XR 205SM-DR balance. This process took 20 s. The saturated chitosan films were removed from the desiccator and placed in a ventilated fume hood (21 ± 2 °C, < 10 % RH) for desorption. The films were removed after different periods of time for weighing until constant mass was attained. The dried film is referred to as the vapour-exposed dried film.

3.5. Preparation of films of a chitosan/wheat gluten blend

It should be mentioned that wherever we refer to solutions in the thesis, these are mixtures with partly or fully dissolved polymers. The blend films were prepared according to different methods, as shown in Figure 3, from separate solutions of chitosan and wheat gluten. Chitosan was dissolved in aqueous acetic acid (acetic acid content: 0.05 mol/L) to a concentration of 1 g per 100 mL solvent. The solution was stirred overnight and the pH of the solution was ca. 4. The concentration of wheat gluten in the wheat gluten solution (based on water or water/ethanol) was 5 or 12 wt.%.
solution was prepared in various ways, as shown in Figure 3. One of the blends was prepared using ultrasonication (750 Watt Ultrasonic Processors – VCX Series). The treatment lasted for 5 min at a frequency of 20 kHz and an amplitude of 21% of the total power that the machine can supply.

Figure 3. The methodology to prepare films of a chitosan/wheat gluten blend

3.5.1. Films prepared using a water/ethanol solvent
Wheat gluten/chitosan blend films were made from a wheat gluten solution that was prepared with water and ethanol as solvent (2/1 by mass), according to Olabarrieta et al. [70] (systems 1 and 2, Table 5). The wheat gluten powder was added to the water/ethanol solution and the mixture was stirred for 20 – 40 min. The pH of the mixture was lowered to 4 by the addition of acetic acid, after which it was again stirred for 20 – 40 min. In one case, the mixture was then heated for 20 min to 70 °C and left at this temperature for 10 min (system 1, Table 5). Subsequently, with or without the heating step, the wheat gluten solution was poured into the chitosan solution and the mixture was stirred for 20 – 40 min. The solution was then filtered using a TexWipe TX309 cloth (118 × 60 threads per inch, pore size 100 – 200 µm) and then poured into petri dishes. Films were obtained by drying the solution in an oven at 26 ± 1 ºC at ca. 40 % RH (method O) or in a climate room at 23 ± 1 ºC, 50 ± 2 % RH (method C). In the oven, the air (256 l) was exchanged 40 times per hour. The
films kept in the oven dried overnight whereas the films in the climate room required approximately one more day to dry.

3.5.2. Films prepared with a reducing agent
The wheat gluten solution was prepared using water and a reducing agent and the procedure was partly adopted from refs. [72,96]. The water and reducing agent were mixed together and stirred for 15-20 min and the wheat gluten powder was then carefully added to the solution. After 30 min stirring, the pH was lowered to 4 by the addition of acetic acid. This solution was subsequently stirred for 20 – 40 min and then poured into the chitosan solution that was then stirred for a further 20 – 40 min. The films were obtained from the wheat gluten/chitosan solution as described in Section 3.5.1. In the case of the sodium sulfite reducing agent, two different amounts (0.3 and 3 wt.%, systems 3 and 5 in Table 5) were used. The lower amount is the same as that used in ref. [97] and slightly higher than that used by Morel et al. [96]. Initially, a high concentration of sodium sulfite (3 wt.%) was used when sodium sulfite was used alone and also in systems that were analysed in parallel (some of the sodium sulfite/surfactant systems and the sodium sulfite/urea system). It was, however, found that the same good results were obtained with only 0.3 wt.% sodium sulfite. Hence 0.3 wt.% sodium sulfite was used in the remaining work. The other two reducing agents (2-mercaptoethanol and DTT) were added to a concentration of 0.3 wt.%.

3.5.3. Films prepared with a surfactant, with or without a reducing agent (sodium sulfite)
Surfactants were used alone or in combination with a reducing agent (systems 9 to 14 in Table 5) in order to see how these affected the film structure. There is always an issue as to how much surfactant to add. In the literature, the amount vary significantly. Wongsasulak et al. [98] added 10 – 23 wt.% Tween-40 surfactant to a blend of cellulose acetate and egg albumen. Ziani et al. [99] added 16 wt.% (based on the chitosan content) of Tween-20 to a chitosan/poly(ethylene oxide) blend. On the other hand, Stanescu et al. [100] added 0.04 and 0.4 wt.% of an alkyl polyglycoside surfactant when producing a starch/chitosan hydrogel. Here, it was decided to use 10 wt.% surfactant (based on the wheat gluten content). The film preparation was identical to that presented in section 3.5.2 except that the surfactant replaced the reducing agent. When both a surfactant and a reducing agent were used, these were added at the same time (before the addition of the wheat gluten powder).

3.5.4. Films prepared with urea, with or without sodium sulfite
Films were made as described in section 3.5.2 except that the reducing agent (sodium sulfite) was replaced with a urea or urea/reducing agent. Based on previous results, 10 wt.% urea (systems 15 and 16 in Table 5) was used [101,102].

3.5.5. Films prepared with glyoxal
A film based on wheat gluten (0.3 wt.% sodium sulfite) and chitosan was prepared as described in section 3.5.2, but glyoxal (system 17 in Table 5) was added and the mixture was stirred for 30 min before the solution was poured into petri dishes.
3.5.6. Films prepared with glycerol
Films were made as described in section 3.5.2 with the difference that glycerol was added together with the sodium sulfite (system 8, Table 5).

3.6. Preparation of chitosan/wheat gluten bio-foams
Figure 4 presents the experimental scheme. The chitosan acetic acid solution (CS) and WG solution (WGS) were prepared according to the method presented by Chen et al. [88]. Chitosan powder was dissolved in aqueous acetic acid (0.05 M) to obtain solutions with concentrations of 0.5, 1, 1.5 and 2 g chitosan per 100 mL of solvent. These solutions are referred to as 0.5, 1, 1.5 and 2 wt.%, respectively. The solutions were stirred (the speed was 800 – 1000 rpm for all stirring operations) overnight and the pH of the solution was 4.0 ± 0.2. 0.3 wt.% of sodium sulfite, based on dry WG, and water were mixed together and stirred for 15 – 20 min. Subsequently, the WG powder was added slowly to the solution. After 30 – 40 min stirring, the pH was lowered to 4 by the addition of acetic acid. This solution was then stirred for 30 – 40 min. The concentration of WG was 0.5, 1, 1.5, and 2 g per 100 ml of solution (0.5, 1, 1.5 and 2 wt.%).

After the two solutions had been filtered using a TexWipe TX309 cloth (118 × 60 threads per inch, pore size: 100 – 200 µm), the CS and WGS were poured together into a polystyrene Petri dish to form a mixture. The mixture of CS and WGS was termed e.g. 1C/1W, which corresponded to a mixture of the solutions of 1 wt.% CS and 1 wt.% WG. Ten combinations were tested: xC/1W, 1C/xW and xC/xW where x was 0.5, 1, 1.5 and 2. In order to keep the number of variables low, the mass fraction of pure chitosan and WG was always 50/50 in the mixtures. In addition, for each combination of CS and WGS, the total mass of the liquid mixture in the dish was kept at 10, 16 or 22 g. After adding CS and WGS together, the dishes were kept at 20 ± 1 °C for 20 – 40 min. The mixing behaviour during this time period was monitored using a NIKON-D40 camera. The dishes were subsequently placed in a desiccator connected to a SCANVAC coolsafe™ 100-9 PRO freeze dryer equipped with an EDWARDS-RV3 vacuum pump (pressure: 0.1 – 0.5 hPa; temperature of the cold trap: -96 ± 1 °C). Dried films were obtained after 20 – 24 h of vacuum treatment. The films were stored in a desiccator with a silica desiccant for at least 24 h before being further investigated.

Pouring the CS into the WGS was referred to as method A (Figure 4). It should be noted that pouring WGS into CS instead did not affect the final film structure. Method A was applied for all the mixture combinations, except for the 0.5C/1W mixture. For this formulation, the porous content varied from batch to batch and, in order to maintain a high content of porous material, CS was added dropwise to WGS. This is referred to as method B (Figure 4).
3.7. Material characterization

3.7.1. Mass loss and diffusion simulation of extrudate

The mass loss of the extrudates was obtained by weighing them after different storage times in the climate room and by subtracting the mass from the sample mass immediately after extrusion. In order to determine the mass-loss kinetics, finite element simulations (FEM) were performed using Comsol Multiphysics. Since the lengths of the polymer tapes were much greater than their other dimensions, a two-dimensional analysis was sufficient. For reasons of symmetry, only one quadrant of the tape needed to be included in the simulations, resulting in a rectangular computational domain with two reflecting boundaries ($\vec{N} \cdot (-D(c)\nabla c) = 0$) and two zero concentration boundaries ($c_r = 0$). The $\vec{N}$ symbol defines the normal direction and $\nabla$ is the nabla operator. A mesh with 15 boundary layers and a total of 9179 mesh elements was used. In this domain, the convection-free diffusion equation $\dot{c} = \nabla \cdot (D(c)\nabla c)$ was solved with FEM, using BDFs as time-dependent solvers and UMFPACK as linear solver.

3.7.2. Acid sorption in the chitosan films

The concentration of acid in the chitosan film, $C_A$ (g acid per g dry chitosan), was calculated according to:

$$C_A = \frac{m(t) - m_0}{m_0}$$

(1)
where \( m(t) \) is the sample mass after exposure to the acid vapour during a time period \( t \) and \( m_0 \) is the mass of dry sample. The final, “equilibrium” concentration of acid is denoted \( C_{A,eq} \). \( C_{A,eq} \) was expressed in wt.% or in mol acid/g dry chitosan (\( C_{A,eq}/M_A \), where \( M_A \) is the molecular weight of the acid).

3.7.3. Acid diffusion in the chitosan films

The acid diffusivity was obtained from acid desorption data analysed by different methods. The following expression provides a simple estimate of the acid diffusivity [103]

\[
D = \frac{i^2 \times 0.49}{t_{0.5}}
\]

(2)

where \( D \) is the diffusion coefficient, \( l \) is the thickness of the film and \( t_{0.5} \) is the time when the acid loss is half the saturation value. This method is only approximate, since it does not consider the acid-concentration-dependence of the diffusivity. The film thickness changes during sorption and desorption and both the dry and swollen thicknesses were therefore used in the calculations.

A more accurate model was based on the assumption that the diffusion depended on the acid concentration that, in accordance with other studies on polymer-solute systems [104,105], was assumed to follow an exponential relationship:

\[
D(C_A) = D_{co}e^{\alpha C_A}
\]

(3)

where \( C_A \) is the acid concentration, \( D_{co} \) is the zero-concentration acid diffusivity and \( \alpha \) is the acid plasticization power. Molecular dynamics simulations have shown that Eq. (3) is a good descriptor of the concentration dependence [106]. Fick’s second law of diffusion was solved for a film geometry. Only half the film thickness was considered, and the inner boundary coordinate was described as an isolated point i.e. the acid concentration gradient was set to zero at the middle point. At the outer boundary, the concentration was considered to be zero or described by the following evaporation condition [104]:

\[
-D(C) \frac{\partial C}{\partial x} = F_A C
\]

(4)

where \( x \) is the distance from the outer surface and \( F_A \) is the evaporation constant. This yielded the boundary condition:

\[
C_{A,0} = \frac{C_{A,1}}{1 + \frac{F_A}{p(C_A)}}
\]

(5)

where \( C_{A,0} \) and \( C_{A,1} \) are respectively the acid concentrations at the boundary and adjacent to the boundary at a distance \( \Delta x \) from the outer boundary. The first model (referred to as M1) included a concentration-dependent acid diffusivity (Eq. (3)), but the changes in film thickness due to migration of the acid were neglected. Model M2 was the same as M1 but included the change in thickness. The dependence of the film thickness (\( I \)) on the acid concentration is given by:
\[ l = l_d \left(1 + \frac{(\rho_2 - \rho_1)}{\rho_1} C_A^1\right)^{1/3} \]  

(6)

where \( l_d \) is the dry film thickness, \( C_A^1 \) is the average acid concentration in the film and \( \rho_1 \) and \( \rho_2 \) are respectively the densities of the acid and the polymer.

The third model (M3) was different from M2, in which the acid diffusivity was considered to change instantaneously at a certain acid concentration \( C_{A,2} \). At acid concentrations higher than \( C_{A,2} \), the acid diffusivity was assumed to follow Eq. (3), whereas at concentrations lower than \( C_{A,2} \), the acid diffusivity was assumed to be constant \( (D_2) \). In the M1 – M3 models, the evaporation of the acid was considered to be fast enough to maintain an acid concentration equal to zero at the outer boundary. A fourth model (M4) was in all respects similar to M3 except that the evaporation rate at the outer boundary was considered to be slower, in accordance with Eq. (4).

3.7.4. Moisture sorption of films of chitosan/wheat gluten blends

Films were dried in a desiccator containing silica gel at 23 ± 1 °C for at least one week. Moisture sorption data were obtained gravimetrically using a balance (Mettler AE 100, ± 0.1 mg) during which the films were exposed to 50 ± 2 % RH at 23 ± 1 °C.

3.7.5. Mechanical testing

Before the mechanical testing, all the samples were conditioned in a climate room (23 ± 1 °C and 50 ± 2 % RH) for 48 h, and all the tests also carried out in the climate room. A universal material testing machine with a 200 N load cell (ZWICK-Z010, 200 N maximum force selected) was used for assessing the tensile properties of the optimized chitosan extrudate after different storage time (0, 0.5, 1, 2, 3, 6, 9 days). Another Instron 5944 universal testing machine with 500 or 50 N load cells was used for testing the vapour-exposed dried film, the chitosan/wheat gluten film, and the blend foam. The tensile speed for the extrudate was 12.5 mm/min, and that for the exposed film and the blend film was 2 mm/min. Except for the extrudate, all the tensile testing specimens were punched out from the samples using a sample die (ISO 37, type 3), giving specimens where the narrow section had a width of 4 mm. The thickness was measured on each specimen at five locations on the specimen section between the grips using a Mitutoyo, IDC112B thickness gauge; the average thickness value being used for the stress calculations. All reported mechanical data are averages of at least five replicates.

The chitosan/wheat gluten biofoams were compression tested. Cylindrical specimens with a diameter of 14 mm were cut from the films using a cork borer. The thickness was measured using a digital caliper ruler (Absolute AOS DIGIMATIC, Mitutoyo, Japan). The specimens were tested at 23 ± 1 °C and 50 ± 2 % RH in an Instron 5944 universal testing machine with a 500 N load cell. The compression rate was 1 mm (min)\(^{-1}\) in accordance with ref. [80]. The maximum strain was set to 20 or 80 %. The rebound resilience was determined using equation, according to [107–109].

\[ R = \frac{t_2-t_1}{t_0-t_1} \]  

(7)
where \( t_0 \), \( t_1 \), and \( t_2 \) are the thicknesses before loading, under loading (20 or 80 % strain) and after unloading, respectively. The number of replicates was 5.

3.7.6. X-ray diffraction (XRD)
The XRD patterns of the chitosan films were recorded using a PANalytical Xpert PRO diffractometer employing CuKα radiation (\( \lambda = 0.1542 \) nm). The films were taped onto a silica plate, which was attached to the sample spinner (PW3064). The specimens were continuously scanned from 5 to 60° (2\( \theta \)) in 0.017° steps. The total scanning time for the swollen film samples was reduced to one third of the scanning time for the original and vapour-exposed dried films in order to minimize the evaporation of the absorbed acid.

3.7.7. Infrared (IR) spectroscopy
IR spectra were obtained with a FTIR spectrometer (Perkin-Elmer Spectrum 2000, Perkin-Elmer Inc., USA.) equipped with a single reflection ATR accessory (Golden Gate from Specac Ltd., Kent, England. The resolution was 4 cm\(^{-1}\) for all the spectra taken. 16 scans were used on the dried chitosan films, whereas 4 scans were used for the acid-swollen chitosan films in order to minimize the loss of absorbed acid during the experiment. At least 3 locations on the top and bottom surfaces of the foams of the chitosan/wheat gluten blend foams, 32 scans were used in this case.

3.7.8. Solubility of the chitosan films
This analysis was made in accordance with ref. [110]. A buffer solution of pH = 4.5 was prepared using sodium acetate trihydrate and acetic acid, and a second buffer solution of pH = 7 was prepared from sodium hydroxide and potassium dihydrogen phosphate. Small sample pieces were cut from the films and immersed in the pH = 4.5 buffer solution for 24 h. The remaining pieces of the samples were collected by vacuum filtration, rinsed with the solution buffered at pH = 7 and dried in a vacuum oven at 70 °C for 24 h. The film solubility (\( S_f \)) was calculated as the difference between the initial and final dry sample masses relative to the initial dry sample mass.

3.7.9. Size-exclusion chromatography (SEC)
A SEC instrument equipped with PSS Novema Max columns (8 × 300 mm), a PSS SECcurity 1100 HPLC pump and a PSS SECcurity 1100 refractive index detector was used. Standard dextran/pullulan samples were used for the calibration. Samples with a chitosan concentration of 1 g L\(^{-1}\) were injected into the column system with a flow rate of 1 mL (min\(^{-1}\)). NaCl (0.1 M) trifluoroacetic acid (0.1 %) was used as the eluent.

3.7.10. Optical microscopy (OM)
Rectangular strips cut from the chitosan/wheat gluten blend films were examined in a Leica IM50 optical microscope.

3.7.11. UV-visible spectroscopy
The optical properties of the films were obtained by UV-visible spectroscopy (SHIMADZU, UV-2550). The average opacity of each material (5 replicates), defined as the area under the absorbance
versus wavelength curve [111,112] was obtained by integration of the absorbance curve between 400 and 800 nm. The values were normalized with respect to the film thickness, which was obtained using a Mitutoyo, IDC112B thickness-meter.

3.7.12. Scanning electron microscopy (SEM)
Scanning electron microscopy (SEM, Hitachi TM-1000, Hitachi Science System, Ltd., Japan) was used to characterize the chitosan/wheat gluten film, and a field-emission SEM (FE-SEM, Hitachi S-4800, Hitachi Science System, Ltd., Japan) was used to characterize the blend foam. Before insertion in the SEM, samples were placed in a Denton Vacuum chamber and coated with platinum using an Agar High resolution Sputter Coater (208RH), equipped with a platinum target/agar thickness controller. The thicknesses of the platinum layer for the solid film and the foam were 5 and 20 nm, respectively. Specimens frozen in liquid nitrogen were broken and their cross-sections were examined. In the foam case, the surfaces of the sample were observed. The surface exposed to the vacuum drying is referred to as the top surface, whereas the surface touching the Petri dish is referred to as the bottom surface. The size of the particles in the blend film and the pores in the foam was obtained by measuring the diameter in random directions of more than 100 and less than 250 particles or pores. The average thickness of the foam obtained by measuring the cross section on the FE-SEM micrographs.

3.7.13. Viscosities of the chitosan, wheat gluten, and their mixed solutions
A Brookfield Cap 2000+ viscometer, calibrated with a viscosity standard (CAPOL) of 56.1 cP, was used to assess the dynamic viscosities ($\eta_d$) of the solutions. Because forced mixing occurs when the cone spindle of the viscometer is spinning, well-blended mixtures of CS and WGS, obtained by vigorous stirring with a magnetic stirrer, were prepared and used in the viscometer. The rotation speed and measuring time were set to 500 rpm and 45 s, respectively. A higher rotation speed made it difficult to measure on the most dilute chitosan solution and a lower rotation speed increased the risk of having inaccurate data. In addition, the rotation speed was in the range where the viscosity was essentially independent of shear rate [113]. An average dynamic viscosity for each mixture combination was calculated from a minimum of 3 replicates. Because the viscosity of pure acetic acid (1.14 cP) and water (1 cP) [114] were similar under ambient conditions, the acetic acid/water viscosities were assumed to be 1 cP in further calculations.

3.7.14. Density and porosity measurement on chitosan/wheat gluten foams
A Mettler Toledo balance (AL104, reading accuracy = 0.1 mg), equipped with a density determination kit, was used for the density measurements according to the Archimedes principle. The density of the solid films ($\rho$) was calculated according to:

$$\rho = \rho_1 \left( \frac{A}{A-\delta} \right)$$

(8)
where \( \rho_i \) is the density of the n-hexane; \( A \) is the weight of the sample in air and \( B \) is the weight of the sample in the n-hexane. Since the solid films were prepared from the same formulations as the corresponding foams, although with a different loading (16 g instead of 22 g), the densities \( (\rho) \) reported for the solid films in Table 7 were taken to be the solid-phase as bulk densities \( (\rho_b) \) of the foams. Apparent densities \( (\rho_a) \) of the foams were obtained by assessing the mass and volume of the foam samples, which were cut into cylindrical specimens with a diameter of 14 mm using a cork borer. A digital caliper ruler (Absolute AOS Digimatic, Mitutoyo, Japan) was used to measure the size of the samples, and the average thickness and diameter was used in the volume calculation.

The porosity \( (P) \) was determined from the ratio of the apparent density \( (\rho_a) \) to the bulk density \( (\rho_b) \) of the samples according to:

\[
P = (1 - \frac{\rho_a}{\rho_b})
\]

3.7.1.5. Liquid uptake measurement in foams of the chitosan/wheat gluten blend

The initial mass of the foam samples was measured on a Precisa, XR 205SM-DR balance. They were then immersed in n-hexane or water for 1 s and 1 min. The wet mass was recorded within 10 s. Because the foams disintegrated during 1 min immersion in water, no data on 1 min water uptake were collected.
4. RESULTS AND DISCUSSION

4.1. Extruding chitosan at a high solids content
In a trial extrusion, when the solids content was more than 50 wt.%, the high viscosity of the samples exerted a very high force on the screws of the extruder and finally the screws stopped rotating. Since the viscosity was significantly affected by the molecular mass distribution of the chitosan [115], mixing low molecular mass chitosan with the high molecular mass chitosan solved this problem. The initial torque was found to be the most useful parameter to describe the extrudibility.

4.1.1. Extrusion based on the orthogonal experimental design
Four controllable variables, the mass ratio of LC/HC, the acetic acid (HAc) content in processing liquid, the screw speed and the barrel temperature, each at four levels, are listed in Table 1. The initial torque values for each experiment are presented in Table 2. The range analysis was used to assess the influence of the factors. The parameter $K$ was calculated according to:

$$K_{IT(S,H,R)} = \frac{\sum_{i=1}^{4} M_{IT(S,H,R)}}{4}$$

(10)

where $i$ represents the levels ($i = 1, 2, 3, 4$); $M_{IT(S,H,R)}$ is the initial torque at level $i$ for factor T, S, H, or R.

The Range is the difference between the maximum and minimum $K$ values for a given variable, calculated according to:

$$Range = \max\{K_{IT(S,H,R)}\} - \min\{K_{IT(S,H,R)}\}$$

(11)

The range thus indicates the extent to which a change in the value of the variable concerned affects the initial torque. For each variable, the F value is the ratio of the sum of squares of deviation of that variable from the mean to the error sum of squares.

The results of the range analysis are shown in Table 3 that also shows the result of an Analysis of Variance. In this case, a F value greater than 9.28 is significant at the $p < 0.05$ level. The results thus clearly show that the LC/HC ratio is the variable that has the most significant impact on the extrudability. The initial torque decreased with increasing fraction of the low molecular mass chitosan. It was very difficult to extrude the sample containing the highest fraction of HC (LC/HC = 1/4). The influence of the other factors decreased in the order: HAc content (H) > screw speed (S) > barrel temperature (T). The optimal formulation and conditions giving the lowest initial torque were $T_1S_3H_1R_4$: barrel temperature = 40 °C, screw speed = 60 rpm, HAc concentration = 10 wt.% and LC/HC ratio = 7/3.
Table 1. Variables and their levels

<table>
<thead>
<tr>
<th>Variables investigated</th>
<th>Levels for each variable</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>T: Barrel temperature (°C)</td>
<td>40</td>
</tr>
<tr>
<td>S: Screw speed (rpm)</td>
<td>40</td>
</tr>
<tr>
<td>H: HAc content in processing liquid (wt.%)</td>
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<td>R: Mass ratio of LC/HC</td>
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Table 2. The orthogonal design experiment and the initial torque

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Variable</th>
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<td>Screw speed (S, rpm)</td>
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Table 3. Range analysis and variance analysis of the initial torque in the orthogonal experiments

<table>
<thead>
<tr>
<th>Variable</th>
<th>Barrel temperature</th>
<th>Screw speed</th>
<th>HAc content in processing liquid</th>
<th>Mass ratio of LC/HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range analysis (M, N×cm)</td>
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<tr>
<td>K1</td>
<td>172.5</td>
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<td>0.718</td>
<td>0.033</td>
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</tr>
</tbody>
</table>

However, the extrudates prepared under these conditions showed cracks and an uneven surface (Figure 5 (a)). A LC/HC ratio = 1/1 (R2) and a HAc content = 30 wt. % (H2) were therefore selected, because they yielded the second lowest initial torque in the range analysis. The torque values for T1 and S3 were close to those of T2 and S2, and the latter the levels yielded a better extrudate quality. Hence, samples were extruded according to T2S2H2R3. These extrudates showed a smooth surface without cracks (Figure 5 (b)). Thus, the final optimization was barrel temperature = 50 °C, screw speed = 50 rpm, HAc = 30 wt.% in the processing liquid and LC/HC ratio = 1/1. The mass loss and mechanical data reported below were obtained from this optimized extrudate.

4.1.2. Mass loss from the extrudates

Although the content of processing liquid (40 wt.%) was low in the formulation, some liquid remained in the samples after extrusion. The mechanical properties of the extrudates changed during storage and this meant the remaining processing liquid evaporated. It is thus important to...
evaluate the properties when the extrudate has stabilized. A desorption curve of the optimal extrudate is shown in Figure 6, where most of the mass loss occurred within three days. The zero-concentration diffusivity \( D_{c0} \) and the plasticisation power \( \alpha \) were calculated using Eq. (3) and by applying a finite element simulation (FEM) analysis to the experimental data. The average diffusivity over the whole concentration range \( \bar{D} \) was calculated according to:

\[
\bar{D} = \left(\frac{1}{C_s}\right) \int_0^{C_s} D_{c0} e^{\alpha c} dc
\]

where \( C_s \) is the mass loss (solute per polymer, g/g). The calculations yielded \( D_{c0} = 6.0 \times 10^{-9} \text{ cm}^2/\text{s} \), \( \alpha = 23.8 \text{ g/g} \), and \( \bar{D} = 5.8 \times 10^{-8} \text{ cm}^2/\text{s} \).

**Figure 6.** Normalized mass loss from chitosan extrudate \( (T_2S_2H_2R_3) \) as a function of storage time. Filled circle is the experimental data, and line is the simulation.

### 4.1.3. Mechanical properties of extrudates

The changes in the mechanical properties of the extrudates with storage time are shown in Figure 7. The modulus increased from 18 to 830 MPa, whereas the elongation at break decreased from 17 to 3 % during the first three days, within which most of the mass loss of the extrudates occurred. The modulus and elongation at break were essentially constant after three days. This indicates that the presence of the processing liquid not only affected the extrudability but also had an impact on the mechanical properties of the extrudates.
Figure 7. Modulus of elasticity and elongation at break as functions of storage time for sample $T_3S_3H_3R_3$

4.2. Effects of monocarboxylic acids on the structure and properties of chitosan films
Since many studies [43,45,116,117] have used 1 – 2 % (v/v) acetic acid solution (equal to 0.175 – 0.35 M) to dissolve chitosan, 0.2 M acetic acid solution was used in this study. 1 M acetic acid solution was used to examine whether the properties of the chitosan films are affected by the concentration of the solvent. It has been reported that a chitosan film cast from acetic acid solution was transformed into chitosonium acetate [116–120]. According to the degree of deacetylation (DD) and average molecular weight of the chitosan powder, the theoretical content of residual acetic acid in the chitosan films should be 21 wt.%. Indeed, mass losses of $24.4 \pm 1.6$ (0.2M) and $26.5 \pm 0.1$ wt.% (1M) were recorded after the films were buffered in an alkaline solution (cf. 3.3.2). The experimental acid content was slightly higher than the theoretical one ($24-21 = 3$ wt.% for 0.2M, 26-21 = 5 wt.% for 1M) due to the presence of water in the crystalline phase [121,122] and/or non-protonated acetic acid.

4.2.1. Acid sorption in the chitosan films
Data for acid uptake and solubility are listed in Table 4. Differences between the 0.2M and 1M films were small, and no obvious trend was observed. The acid uptake decreased with increasing molecular size of the acid, mainly due to the difference in saturation vapour pressure of the acids. The chitosan films exposed to formic acid vapour gained seven times the initial mass, resulting in a gel-like sample. The solubility coefficient is defined as the ratio of the equilibrium concentration of acid in the solid film ($C_{A,eq}$) to the equilibrium partial pressure of the acid vapour above the liquid acid ($p_A^*$. Butyric acid showed the highest solubility coefficient, whereas the solubility coefficient of acetic acid was the lowest. It was worth noting that the thin chitosan films (0.1 mm) took a longer time to reach a final saturation in the concentrated acid vapour than they were expected, indicating that structural changes occurred in the chitosan films during the acid uptake. For the films exposed
to formic and acetic acid, a levelling off occurred after 16 and 40 days, respectively. The films exposed to propionic and butyric acid took a considerably longer time to reach equilibrium.

Table 4. Sorption of acids in chitosan films

<table>
<thead>
<tr>
<th>Acids</th>
<th>0.2M</th>
<th></th>
<th>1M</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( p'_A ) (kPa)</td>
<td>wt. %</td>
<td>mol/g</td>
</tr>
<tr>
<td>Formic</td>
<td>5.0¹</td>
<td>710</td>
<td>0.154</td>
<td>142</td>
</tr>
<tr>
<td>Acetic</td>
<td>1.9¹</td>
<td>213</td>
<td>0.036</td>
<td>112</td>
</tr>
<tr>
<td>(Propionic-62)</td>
<td>(0.4²)</td>
<td>(88)</td>
<td>(0.012)</td>
<td>–</td>
</tr>
<tr>
<td>Propionic-283</td>
<td>0.4²</td>
<td>161</td>
<td>0.022</td>
<td>402.5</td>
</tr>
<tr>
<td>Butyric</td>
<td>0.1³</td>
<td>105</td>
<td>0.012</td>
<td>1050</td>
</tr>
</tbody>
</table>

¹Lange's Handbook of Chemistry, 10th ed.  
²Sigma Aldrich/PubChem.  
³A reference[123]  
\( g_{sol} \) is the mass of acid; \( g_{poly} \) is the mass of the dry films

Figure 8. Mass increase in the chitosan films exposed to propionic and butyric acid as a function of the square root of time. (■): 0.2M film exposed to propionic acid vapour; (☐): 1M film exposed to propionic acid vapour; (○): 0.2M film exposed to butyric acid vapour; (△): 1M film exposed to butyric acid vapour.

Figure 8 shows the two-stage sorption behaviour of chitosan films exposed to propionic acid. The chitosan films apparently reached the first saturation after 1500 h. Unexpectedly, the samples started gaining weight on further exposure. The second mass uptake continued until 6700 h later.
This anomalous sorption behaviour suggests that the structure of the chitosan films gradually changed during the exposure to the propionic acid. Propionic-62 and propionic-281 refer to the samples after 62 days (1500 h) and 281 days (6700 h) exposure, respectively. A very short plateau was also found for the samples exposed to butyric acid after a similar time. The propionic and butyric acid uptake rates were almost identical after 84 days of exposure.

The propionic and butyric acid mass uptake was greater in the 1M films than in the 0.2M films at the beginning of sorption. The difference in uptake rate disappeared, after the films had been exposed for 13 days to propionic acid and for 60 days to butyric acid. This suggests that the initial structure was different in the 0.2M and 1M film. Different initial uptake rates were not observed in the 0.2M and 1M films exposed to formic and acetic acid vapour, perhaps because the curves converged before a mass uptake value was recorded.

In addition, the acetic acid uptake in the buffered film was higher and the equilibrium time was shorter than that of the original film, due probably to more available amino groups and a more amorphous structure in the buffered film (cf. XRD data in section 4.2.3).

4.2.2. Acid diffusion in the chitosan films

In general, the desorption rate decreased with increasing molecular size of the acid. The desorption rate of the propionic–281 film was faster than that of the propionic-62 film. Average diffusion coefficients of the films were estimated using Eq. (2) using initial thicknesses and swollen thicknesses in the equation. Figure 9 shows that the logarithm of the diffusivities decreased linearly with increasing molar volume of the acids, and that the diffusivities of the 0.2M and 1M films were essentially the same.

![Figure 9. Diffusion coefficient of the chitosan films obtained by Eq. (2) as a function of molar volume of acids, (○): 0.2M film and dry thickness; (.DataGridView): 1M film and dry thickness; (■): 0.2M film and swollen thickness; (▲): 1M film and swollen thickness; (▲): 0.2M film (propionic-62) and dry thickness; (Δ): 1M film (propionic-1) and swollen thickness; (▼): 0.2M film (propionic-62) and swollen thickness; (▽): 1M film (propionic-62) and swollen thickness.](chart.png)
Because the best desorption of the acid desorption curves was obtained by using M4, the zero-concentration diffusivity and plasticization power were obtained from M4. Figure 10 (a) shows that the $D_\infty$ of formic acid was ca thousand times higher than that of the other acids, due to the high saturation vapour pressure of formic acid. The $D_\infty$ of the 1M films for propionic-62 was significantly lower than that for propionic-281, indicating that the structures of the films were more permeable to propionic acid at the second plateau than at the first plateau. The plasticization power ($\alpha$) describes the plasticization efficiency of the solute, which can be related to the excess free volume in the polymer added by the solute. Figure 10 (b) shows that the plasticization power ($\alpha$) increased with increasing molecular size of the acid and that the $\alpha$-values were similar for the 0.2M and 1M films. The higher $\alpha$ for the larger acid molecules indicates that the larger molecules create more free volume in the chitosan films than the smaller acid molecules. The highest $\alpha$-value of the propionic-62 film suggest that the structural changes are massive in the first plateau, which may be a structural transition similar to that of a polymer at a glass transition temperature (Tg). Furthermore, the $D_\infty$ of acetic acid in the buffered film was greater than that in the original film, while the plasticization power ($\alpha$) in the buffered film was lower than that in the original film. This may be attributed to a higher acetic acid mass uptake in the buffered film than that in the original film.

4.2.3. XRD analysis

It has been reported that two hydrated crystal forms exist in chitosan materials, type I and type II. Type II is usually observed in the chitosan materials prepared with monocarboxylic acids [50,121,124,125], it has been shown that different acids yield the same unit cell in the type II crystal form [50,125]. The XRD pattern of the original 0.2M films shows the type II form (Figure 11 (1)). Three peaks at $2\theta = 11.5$, ca. 18.5 and 22.5° were evident. The peak at 11.5° is assigned to a hydrated
or “tendon” crystal form in the chitosan film [121,122]. Compared with the 0.2M films, the 1M films exhibited only a slight difference in the XRD pattern, in which a small peak was observed at ca. 9°

Only a broad peak at 23°, originating from the amorphous part of chitosan [126], was observed in the XRD pattern of the films saturated with acetic acid (Figure 11 (2)). No characteristic peaks of the type II crystal form appeared again in the vapour-exposed dried films, suggesting that amorphous structures were dominant after desorption. Fewer crystal structures were also found in the IR spectrum of the vapour-exposed dried films, in which a band (653 cm⁻¹) related to crystalline of chitosan films [41,127–129] became much broader in the dried films than in the spectrum of the original film. A structural transition in chitosan materials has been reported in other studies [50,121,124]. Chitosan acetate materials were prepared by immersing chitosan powder in acetic acid/isopropanol solution for several hours, or by exposing the powder to the vapour of acetic acid for several days at room temperature. A spontaneous water-removing action of acid occurred when the materials were stored at 100 % RH. This action caused a transition from a hydrated to a dehydrated polymorph, which showed a sharp peak at ca. 15° in XRD. This transition was not observed in this study, perhaps because the acid-saturated films were dried at a low RH.

![Figure 11. XRD curves of the 0.2M films: (1) original chitosan film; (2) chitosan film saturated in acetic acid; (3) vapour-exposed dried chitosan film; (4) original chitosan powder.](image-url)
Interesting differences in the XRD patterns of all the vapour-exposed dried films were found at low scattering angles (5° to 10°). The \( d \)-spacings showed a linear increase with increasing molecular size of the acids (Figure 12). These \( d \)-spacings were larger than the values reported for anhydrous and hydrated chitosan crystals [124]. Since the increase in the value of the \( d \)-spacings (2.5 Å) was larger than the increase in molecular size (1.5 Å) from formic to acetic and from acetic to propionic acid etc., water molecules may be involved in the new structure. In addition, all the vapour-exposed dried films showed XRD patterns different from those of films cast from aqueous acetic, formic or propionic acid [119,130]. All these differences suggest that the long-term exposure of chitosan to the acid vapour yielded a “new” material.

The type II crystal form was absent in the buffered film (Figure 13 (1)). The characteristic peaks of the chitosan acetate film at 1540 and 1408 cm\(^{-1}\), assigned to protonated amino groups and acetate anion [43,48,117,119,131,132], were absent in the IR spectrum of the buffered film. The spectrum was similar to that of pristine chitosan powder. After the buffered film had been exposed to acetic acid vapour, the XRD pattern was essentially the same as that of the acetic-acid-exposed dried film (Figure 11(4) and Figure 13 (4)).
4.2.4. Mechanical properties

The mechanical properties of the original and vapour-exposed dried films are shown in Figure 14. The original 0.2M showed mechanical properties similar to that of the original 1M film, only small differences being observed between the vapour-exposed dried 0.2M and 1M films, except for the strain at and energy at break of the formic-acid-exposed dried films. The propionic-281 and butyric-acid-exposed dried films had the highest strain and energy at break, although the modulus and yield stress of these samples were lower than those of the other samples. This suggests that long-term exposure of the chitosan films to propionic and butyric acid yielded more extensible and tougher materials. It has been reported that chitosan films cast from aqueous formic, acetic, propionic and butyric acid do not show apparent differences in their tensile properties [47]. On the other hand, a much lower tensile strength and greater strain at break were observed in films cast from solution of acids with a larger molecular volume, such as lactic and citric acids [45].

It has been reported that the tensile stress at break of the chitosan films increases with increasing chitosan molecular weight (range of the $\tilde{M}_w$: 370 – 1800 kDa) [51]. In the present study, the molecular weight of the acetic-acid-exposed dried films was lower than that of the propionic-62 dried film, but the modulus and strain at break of the former were greater than those of the latter films. This suggested that the mechanical properties of the vapour-exposed dried films were more sensitive to the type of acid and the exposure time than the polymer molecular weight.
Figure 14. (a) Elastic modulus ($E$), (b) tensile strain at break, (c) tensile stress at yield, and (d) energy at break of (1) the original chitosan film and the dried films previously saturated in the vapour of (2) formic acid, (3) acetic acid, (4) propionic acid (propionic-62), (5) propionic acid (propionic-201) and (6) butyric acid. Grey and white columns refer to the 0.2M and 1M films, respectively.

4.2.5. Solubility in a buffer solution

The original films showed 100% solubility in the buffer at pH = 4.5, whereas only small proportions of the vapour-exposed dried films were soluble (Figure 15 (a)). Even after 72 h immersion in the buffer, the dried films still retained their original shape, as shown in Figure 15 (b). This improvement in water resistance could be attributed to that the long-term acid exposure introduced structural changes. In other studies, the solubility of chitosan materials in water was decreased by neutralizing/buffering the materials, by blending with other polymers, and by adding a cross-linking agent [133,134]. Although the solubility of the buffered film was lower than that of the original film (Figure 15 (a)), the buffered films broke to pieces when they were collected from the buffer solution.
A mass increase was observed in the formic-acid-exposed dried film. The extra mass originated from the species in the buffer solution, which were absorbed by the gel-like sample formed in the buffer solution. The formation of the gel sample in the buffer may be attributed to new or more intense electrostatic interactions between the chitosan and the formic acid molecules, which may form physical crosslinking sites in the sample. These interactions were suggested by the IR spectrum of the formic-acid-exposed dried film, where a few bands assigned to chitosan (1655, 1560, 1346 and 1319 cm⁻¹) shifted to some extent (not shown).

\[\text{Figure 15.} \quad \text{(a) The solubility in the pH 4.5 buffer solution of (1) the original chitosan film and the dried films previously saturated in the vapour of: (2) formic acid, (3) acetic acid, (4) propionic acid (propionic-62), (5) propionic acid (propionic-281), and (6) butyric acid. Also shown are the solubility of (7) the original buffered film, and (8) the dried buffered film previously saturated in the vapour of acetic acid. (b) Illustration of the original chitosan (CS) film dissolved completely in the buffer and of the dried chitosan film previously saturated in acetic acid vapour. The films were immerced in the buffer solution for 72h.}\]

4.3. Chitosan and wheat gluten blend films

Table 5 lists the systems for preparing chitosan/wheat gluten blend films. Their microstructure and homogeneity were studied by the morphology and the opacity of the blend films. In addition, the mechanical properties of the blend films prepared by system 3 were studied with respect to the component contents.

4.3.1. Blend films prepared using a water/ethanol solvent

For the film cast from water/ethanol, the photomicrograph showed that discrete brown phase/particles, with sizes from 40 to 200 µm, distributed randomly in a transparent matrix (Figure 16 (a)). These particles were referred to as “large” particles. The SEM micrograph of the film showed not only the “large” particles but also small particles with a size of ca. 1 µm dispersed uniformly in
the cross section. These particles (the size < 10 μm) were referred to as “small” particles. The pristine wheat gluten films were dark yellow or brown, whereas pristine chitosan films were transparent, and the number of the “large” and “small” particles increased with increasing content of wheat gluten. Hence, the discrete phase/particles were rich in wheat gluten (perhaps 100 wt.%).

When the concentration of the wheat gluten solution was reduced from 12 to 5 wt.% in early preliminary experiments, both the number and the size of the large particles decreased. Hence, 5 wt.% wheat gluten solutions were used for all the blend film preparations. The size of the small particles was slightly increased (Figure 18) when the heating procedure was omitted. The photomicrograph showed no differences in the blend films cast under the two different conditions (method O (fast drying) and C (slowly drying)).

![Photomicrographs of the blend films](image)

**Figure 16.** Photomicrographs of the blend films (wheat gluten/chitosan mass ratio: 50/50) prepared according to method C: (a) system 1 (water/ethanol), (b) system 3 (sodium sulfite) and (c) system 11 (HTLB).
### Table 5. The different chitosan/wheat gluten systems

<table>
<thead>
<tr>
<th>System</th>
<th>Type(^1)</th>
<th>Amount(^2) (% w/w)</th>
<th>Solvent</th>
<th>Special treatment</th>
<th>Particles(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>water/ethanol</td>
<td>Heating</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>water/ethanol</td>
<td>No heating</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>sodium sulfite</td>
<td>0.3</td>
<td>water</td>
<td>-</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>sodium sulfite</td>
<td>0.3</td>
<td>water</td>
<td>Ultrasonication</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>sodium sulfite</td>
<td>3</td>
<td>water</td>
<td>-</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>2-mercaptoethanol</td>
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<td>water</td>
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<td>7</td>
<td>DTT</td>
<td>0.3</td>
<td>water</td>
<td>-</td>
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</tr>
<tr>
<td>8</td>
<td>sodium sulfite/glycerol</td>
<td>0.3</td>
<td>water</td>
<td>-</td>
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<td>9</td>
<td>Triton X-100</td>
<td>10</td>
<td>water</td>
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<td>10</td>
<td>Tween-20</td>
<td>10</td>
<td>water</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>HTLB</td>
<td>10</td>
<td>water</td>
<td>-</td>
<td>Yes</td>
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<td>sodium sulfite/Tween-20</td>
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<td>water</td>
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<tr>
<td>13</td>
<td>sodium sulfite/Triton X-100</td>
<td>0.3</td>
<td>water</td>
<td>-</td>
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</tr>
<tr>
<td>14</td>
<td>sodium sulfite/HTLB</td>
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<td>water</td>
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<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>urea</td>
<td>10</td>
<td>water</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>sodium sulfite/urea</td>
<td>3</td>
<td>water</td>
<td>-</td>
<td>No</td>
</tr>
<tr>
<td>17</td>
<td>sodium sulfite/glyoxal</td>
<td>0.3</td>
<td>water</td>
<td>-</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^1\)Type of additive. Except for system 17, the additive has been added to the wheat gluten solution before blending with chitosan. The wheat gluten/chitosan weight ratio was 50/50, except for systems 1 and 3 where 30/70 and 70/30 ratios were also investigated.

\(^2\)Amount of additive based on weight of wheat gluten. The surfactant content (Tween-20, Triton X-100, HTLB) was based on the weight of wheat gluten and surfactant.

\(^3\)Films containing "large" particles (Yes) or no/very few large particles (No).
Figure 17. SEM micrographs of the blend film (wheat gluten/chitosan: 50/50, system 1, prepared according to method C; arrow in Figure 17 (a) points to a large wheat gluten particle.

Figure 18. Average size of the small particles in blend films (wheat gluten/chitosan: 50/50). The distribution of particle sizes is shown as error bars (standard deviations).
4.3.2. Films produced using a reducing agent
Reducing agents decrease the number of the disulphide bonds, and this lead to changes in rheology of wheat gluten solution. The large particles disappeared completely in the film prepared with sodium sulfite (Figure 16 (b)). However, the small particles (1.1 μm), slightly larger than those in the system with water/ethanol (0.7 μm), were still observed, indicating that the chitosan and wheat gluten were an essentially immiscible system. Figure 19 shows that the number of the small particles in the film prepared using chitosan/wheat gluten at a mass ratio of 30/70 was apparently greater than that in the 70/30 film, suggesting that the particles were rich in wheat gluten as in the water/ethanol system. A larger amount (3 wt.%) of sodium sulfite and two other reducing agents (0.3 wt. % 2-mercaptoethanol and DL-dithiothreitol (DTT)) yielded somewhat larger “small” particles (ca. 1.7 μm) than the system with 0.3 wt.% of sodium sulfite. In addition, glycerol (plasticizer) and ultrasonication barely changed the microstructures of the blend films prepared using sodium sulfite (0.3 wt.%).

Figure 19. SEM micrographs of the sodium sulfite system (system 3, method O)); wheat gluten/chitosan: (a) 30/70 and (b) 70/30.

4.3.3. Blend films produced using surfactants, urea, or glyoxal with or without sodium sulfite
Severe phase separation occurred when the wheat gluten solution containing sodium dodecyl sulphate (SDS) and the chitosan solution were mixed. A blend film could not be prepared, because the two separated phases were difficult to blend well by agitation. On the other hand, it was possible to prepare blend films using Triton-X 100 and Tween 20 (two non-ionic surfactants), HTLB (a cationic surfactant), urea (a protein denaturant and plasticizer), and glyoxal (a cross-linking agent). Based on the preliminary results with sodium sulfite and a non-toxic property of sodium in food packaging applications [135], sodium sulfite was used as a component combined with the surfactants in the study. As shown in Figure 18, the use of all the additives alone or in combination with sodium sulfite, except for glyoxal, yielded larger small particles than 0.3 wt.% sodium sulfite.
The large particles observed in the films prepared with all surfactants, urea, and glyoxal were smaller and the number was less than those in the water/ethanol-prepared film, and a photomicrograph of the film prepared with HTLB is shown in Figure 16 (c). In the system with glyoxal, all the small particles had an oval shape along the film plane. The average width and thickness of the small particles were 2.5 and 0.6 µm, respectively. The large aspect ratio (4.2) of the small particles may be attributed to cross-linked molecules in the blend. The cross-linked films were more brittle than the other films.

For most of the systems, the blend films prepared by the different drying methods (method C and method O) showed no differences in morphology or particle size, but it was difficult to distinguish the boundaries of the small particles in the films prepared using glyoxal when dried by method O.

4.3.4. Opacity of blend films

Figure 20 shows the opacity of the blend films as a function of the small particle size. Because the opacity measured in this study was the “apparent” light absorption originating from the absorption by the material and from light scattering at the surfaces and internal heterogeneities of the films, the opacity of the blend films was expected to be greater than that of the pristine wheat gluten (Figure 20, (4)). However, the films prepared with glyoxal had an opacity clearly lower than that of the wheat gluten film. In general, the opacity of the films cast by method C (slow drying) was lower than that of the films cast by method O (fast drying). The opacity of the films increased with increasing small particle size, especially when the small particle size was more than 1.5 µm. This opacity analysis excluded the large particles, because they were too few to affect the light scattering. For example, the water/ethanol-prepared films contained more large particles than the HTLB-prepared film, but the latter had a higher opacity (refer to samples 1 and 3 in Figure 20, respectively). A large standard deviation of the opacity indicated a poor homogeneity of the blend films. The most heterogeneous film was the one prepared using HTLB and dried by method O (Figure 20, (3)).

The opacity of the films prepared by system 3 (0.3 wt.% of sodium sulfite) was assessed at different wheat gluten contents. The results revealed that the opacity increased with increasing wheat gluten content. However, the highest opacity was observed in the films with 30 and 50 wt.% of wheat gluten that were dried using the method O.
4.3.5. Mechanical properties, moisture sorption and desorption of the blend films prepared with sodium sulfite

The tensile properties of the blend films prepared with 0.3 wt.% of sodium sulfite are summarized in Figure 21. The modulus of the tested samples was almost constant regardless of the chitosan content and drying method. The other mechanical parameters of the films essentially declined with increasing wheat gluten content. The pristine wheat gluten films were too brittle to be measured. It is worth noting that with 30 wt.% of wheat gluten in the blend, the film had mechanical properties similar to those of the pristine chitosan film, and that yielding was observed in the stress-strain curve. All the mechanical parameters of the slowly dried films (method C) were slightly greater than those of the films dried rapidly (method O). In order to examine the effects of the small particle size and the homogeneity of the blend films on the mechanical properties, the most heterogeneous films prepared with HTLB (chitosan/wheat gluten = 50/50) were also measured. It was observed that the strain and energy at break of HTLB-prepared films were similar to those of the films prepared with sodium sulfite, whereas the modulus and yield stress of the former were lower than those of the latter films. This suggests that HTLB acted as a plasticizer that overcame the effects of heterogeneity to the blend films.
Figure 21. Mechanical properties of the sodium sulfite (0.3 wt.%, system 3) films: (a) modulus and strain at break as a function of chitosan content. (b) Energy at break and maximum stress as a function of chitosan content. Error bars are standard deviations. Filled and open symbols indicate drying according to methods C and O, respectively.

The films containing 0.3 wt.% of sodium sulfite were used to assess the moisture uptake and diffusion kinetics. The moisture uptake decreased with increasing wheat gluten content, as shown in Figure 22. Diffusivities of the films were estimated according to Eq. (2). The diffusivities of all the blends were lower than that of the pristine chitosan film ($1.4 \times 10^{-9}$ cm$^2$/s), and the diffusivities of the blend films with 30 and 50 wt.% of wheat gluten ($4.2 \times 10^{-10}$ and $3.0 \times 10^{-10}$ cm$^2$/s) were lower than that of the pristine wheat gluten film ($6.8 \times 10^{-10}$ cm$^2$/s). It has been reported that water diffusivity in a polyethylene/starch blend is lower than that in either of the two polymers [136].
These results may be explained by that the interface between the wheat gluten particles and chitosan matrix which could trap water molecules.

\[ \text{Figure 22. Moisture uptake in the different films dried according to methods C (○) and O (●).} \]

### 4.4. Preparation and properties of foams of chitosan and wheat gluten blends

Chitosan/wheat gluten blend films were prepared by the fast and slow drying methods (method O and method C) described in section 4.3. In order to examine the effects of a faster drying method, vacuum drying was used. Surprisingly, a few films became porous after drying, despite the fact that no porogen was added and the solution was not previously frozen. However, it was very difficult to reproduce the porous samples according to the systems and methods used in section 4.3. A breakthrough was made when a sandwich-like structure was observed in the cross section of the porous samples. The structures of the denser surface regions and porous middle regions suggested that the blend foam was a heterogeneous biphasic system. Since mechanical agitation yielded a homogeneous solution on a gross scale, allowing no agitation/blending was a way to achieve the biphasic state, and the porous samples were indeed reproduced by a method without agitation, as shown in Figure 23 (a). Effects of concentrations of the two solutions and vacuum drying on the pore formation are discussed in the following sections.
Figure 23. (a) A foam and (b) solid film prepared from 1C/1W. The scale bar is 0.5 cm.

4.4.1. Effects of different combinations of chitosan solution (CS) and wheat gluten solution (WGS) on foam formation

The method to create the chitosan/wheat gluten foam is outlined in Figure 4. Three non-blended mixtures with different total masses were used: 22 g (maximum mass which the dish can contain), and two lower masses (16 and 10 g), which were studied to examine whether or not the foam formation was dependent on the total mass. The results showed that most of the 22 g non-blended mixtures yielded a porous structure, whereas only a few parts of the films dried from 16 g mixtures were foamed and no foams were obtained from the 10 g mixtures. Therefore, the 22 g mixtures were mainly used in the study.

<table>
<thead>
<tr>
<th></th>
<th>WGS (wt. %)</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (wt. %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>100</td>
<td>75</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td>1.5</td>
<td>–</td>
<td>25</td>
<td>75</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 6. Porous volume (%) for the different combinations

1 To simplify the calculations, the circular film was divided into four quarters and the extent of the foaming was, based on these four quarters, 0, 25, 50, 75 and 100.

Because it was easy to distinguish solid (transparent/translucent) and foamed (white) parts, foam contents were calculated as the proportion of porous volume with respect to the total film area. Table 6 shows that only 0.5C/0.5W, 0.5C/1W and 1C/1W produced the fully foamed samples. The porous volume decreased with increasing concentration of CS in the case of xC/1W.
foam formation involved different steps and factors, it was necessary to study them individually. The first important factor was the total polymer content in the mixture. Because the solid mass fractions of the two components were 50/50 and the total mass in the dish were constant (22 g), the total polymer content was actually 0.7, 1, 1.2, and 1.3 wt.% in the cases of 1C/xW and xC/1W (x = 0.5, 1, 1.5, 2). In the case of xC/xW, the values were 0.5, 1, 1.5, and 2 wt.%. The second finding was that the viscosities of the mixtures apparently changed with changes in the concentration of CS. The viscosities of the CS, WGS and their combinations are discussed in the next section.

4.4.2. Viscosity of solutions
The dynamic viscosity of CS increased from 20 to 120 cP with increasing concentration of CS from 0.5 to 2 wt.%. A gel-like sample was observed at 2 wt.% of CS. The dynamic viscosity of all the WGS was too low to be tested because of a limitation in the viscometer, but it has been reported that the viscosity of a 1 wt.% WGS at a pH of 3.5 – 5 is 1.95 – 2.1 cP [83]. The dynamic viscosity of the mixing solution increased with increasing concentration of CS/WGS. The viscosity data for the well-blended solution could not however represent the real situation in the non-blended mixtures. It was meaningful to use the viscosities to examine whether the well-blended mixtures were miscible or not. If CS and WGS were miscible, the viscosity of the well-blended solution could be estimated from their individual viscosities and their fractions in the mixture according to [137]:

\[
\ln \eta_{\text{bienda}} = \ln \eta_{\text{CS}} \cdot \omega_{\text{CS}} + \ln \eta_{\text{WGS}} \cdot \omega_{\text{WGS}}
\]  

(13)

where \( \eta_{\text{CS}} \) and \( \eta_{\text{WGS}} \) are the viscosities of CS and WGS; and \( \omega_{\text{CS}} \) and \( \omega_{\text{WGS}} \) are the mass fraction.

![Figure 24](image_url) Figure 24. Calculated (Δ) and measured (●) dynamic viscosity.
Any differences between the calculated and experimental results indicated immiscibility of the two solutions or two polymers. Taking $x_C/1W$ as an example, the calculated viscosity decreased slightly with increasing concentration of CS, but the experimental viscosities increased over the tested concentration range (Figure 24). The obvious differences suggested that the well-blended CS/WGS mixtures were immiscible on a finer scale, although vigorous agitation mixed CS and WGS uniformly from an optical viewpoint. This micro-scale phase separation may be one of key factors affecting the pore formation.

4.4.3. Spontaneous mixing and effects of vacuum drying on samples

The first important step in the foam formation was a change in the phase distribution, shown in Figure 25. Because the phase distribution changed with time, this step was referred to as spontaneous mixing. A view from above (Figure 25 (a)) showed that, at first, a non-blended mixture had two phases, in which the milky/opaque part was WGS and the transparent part was CS. The WGS phase diffused into the CS phase gradually and spontaneously. Mixed phase structures formed at the end. Viewed from a side, the CS phase was beneath the WGS phase at the beginning. Streams of the WGS phase flowed down with time. Finally, a very thin CS-rich layer formed at the top surface. The spontaneous mixing for $1C/1W$ was over within 40 min. The procedure for $0.5C/0.5W$ was much shorter than that for $1C/1W$ (15 min), and for $0.5C/1W$, the procedure took ca. 30 min. In contrast, spontaneous mixing never occurred in $2C/2W$ due to the high viscosity of 2 wt.% CS. The notable findings were that it was impossible to obtain a foam from a well-blended solution or from a mixture without the spontaneous mixing, such as $2C/2W$. Driving forces for the mixing may be include gravity, viscosity of the solutions, and interactions between the solutions and polymers.

The second indispensable step was vacuum drying. None of the spontaneously mixed mixtures yielded a pore structure at normal pressure. Effects of the vacuum drying are indicated in Figure 26. According to the conclusion in section 4.3, a solid film of chitosan/wheat gluten blend was an immiscible system where the wheat gluten particles (average size = 1.3 $\mu$m) uniformly dispersed in the chitosan. In the film from a mixture undergoing spontaneous mixing but dried at normal pressure, the cross section showed agglomerates of a wheat-gluten-rich phase with irregular shapes randomly dispersed close to the bottom surface (Figure 26 (a)). In contrast, the agglomerates spread and almost dominated the whole cross-section of the film (16 g total mass) dried under vacuum (Figure 26 (b)). This suggested that forced drying was capable of changing the phase distribution of the two components in the mixture. This ability was required for foam formation. Besides the agglomerates, in the two cases, it was found that small wheat gluten particles with a size of 1 – 4 $\mu$m were well dispersed in the chitosan phase. The temperature of the desiccator during the vacuum drying was recorded. Initially, the temperature was 14 °C and it was 20 °C (room temperature) at the end. Hence the liquid phase was not frozen during the vacuum drying.
Figure 25. Photographs of the mixing of WGS and CS (1C/1W) taken (a) from the top and (b) from the side. The extension of the WGS into the CS is marked by black arrows, and the final thin CS-rich layer is marked by a white arrow.
Figure 26. FE-SEM micrographs of cross-sections of solid films of 1C/1W dried (a) under ambient conditions and (b) under vacuum. Arrows point to WG-rich agglomerates. The inset figure is a magnification of part of an agglomeration.

4.4.4. Morphology of the foam and pore structure/foam formation

Figure 27 shows that the morphologies are evidently different in the top and bottom surfaces of the foam prepared from 1C/1W. Crater-like holes with a diameter about 0.4 mm appeared at the top, whereas a smooth surface with cracks and tiny holes was observed at the bottom. The crater-like structure could be remainder of gas bubbles created during the vacuum drying. Many oval holes at the bottom of the crater-like structure could be vents to evaporate the liquid phase. Besides the different geometry, the colour pattern of the top surface differed from that of the bottom. The top surface had a bright and homogeneous colour, whereas the bottom surface had many dark regions, which were wheat-gluten-rich domains, as indicated by the IR data of the top and bottom surfaces. In the IR spectra (not shown), the characteristic peak of chitosan was presented only at the top whereas the characteristic peak of wheat gluten was observed only at the bottom. It was estimated that the concentration of wheat gluten molecules in the bottom surface was more than twice that in the top surface (55 and 20 wt.%, respectively).
**Figure 27.** FE-SEM micrographs of (a) the top surface and (b) the bottom surface of a porous film prepared from the 1C/1W mixture. The white arrow points at the base of a crater with holes and the black arrows point at WG-rich domains.

Figure 28 (a) shows that the pores in the foam were open and irregular, and that the pores close to the bottom surface were less elongated than those close to top surface. The foam was covered by the skin-like top surface, which was a chitosan-rich phase. When the walls of the pores were examined, the dark regions similar to the bottom surface were found in the regions close to the bottom (Figure 28 (c-1)). The number of these regions decreased from the bottom to the top. They were absent in the pore walls close to the top. However, small wheat gluten particles with diameters of 1–3 µm were well dispersed in all the pore walls. The phase distribution suggested that the micro-scale phase separation formed after the spontaneous mixing was preserved after vacuum drying.

A scheme of the foam formation is suggested in Figure 29. The distribution of the CS and WGS phases changes in the horizontal and vertical dimensions during the spontaneous mixing, resulting in a mixed phase of CS and WGS covered by a thin CS-rich layer. This phase distribution is preserved until the mixture is dried in vacuum. The mixture becomes more viscous and starts to solidify as the liquid phase rapidly evaporated in the vacuum. Bubbles, formed in the mixed phase, are prevented from growing too large to collapse completely by the high viscosity of the chitosan-rich layer. The final pore structure forms after all the liquid phase evaporates from the oval holes at the top surface. The WGS phase contributes to the bubble formation due to its good foaming properties, and the CS phase provides stiffness to maintain the bubbles/closed pores until the later stages when the bubbles collapse and form crater-like structure.
Figure 28. (a) FE-SEM micrograph of the cross-section of the porous 1C/1W film, the shapes of the pores being indicated by crossed arrows. The white arrow points at the top surface skin. (b) Images taken from the region close to the bottom (1), further from the bottom (2 and 3) and close to the top (4). Black arrows mark wheat-gluten-rich regions and white arrows mark chitosan-rich regions. Scale bars are 50 μm in (b)
4.4.5. Dimensions of the foams

Dimensions of the foams with a 100% porous structure are shown in Table 7. The foam prepared from 1C/1W was approximately 15 times thicker than the film prepared from the same formulation and cast at normal pressure. As in the previous discussion in 4.4.1, the total polymer content was different in the different combinations. Table 7 shows that the thickness increased with increasing polymer content. The bulk/solid density of the foams also increased with increasing polymer content, probably because molecular packing was more compact in the films prepared from high polymer content. The apparent densities of the foams were quite low and decreased with decreasing the polymer content. Differences in the pore sizes of the three foams were only small, whereas the variation within the foam was large. The average pore sizes were similar to those of other wheat gluten foams [87,92] and were in the same range as that of chitosan-based foams (20–350 µm) [73,76,78,79]. The porosity (ca. 96%) of the foams was greater than that of other chitosan foams, and was comparable to that of other wheat gluten foams [87,92].

Table 7. Dimensions of the foams

<table>
<thead>
<tr>
<th>Foam</th>
<th>Thickness (µm)</th>
<th>Bulk density(^1) (kg m(^{-3}))</th>
<th>Apparent density(^2) (kg m(^{-3}))</th>
<th>Pore size (µm)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5C/0.5W</td>
<td>409 ± 83</td>
<td>1207 ± 60</td>
<td>46 ± 5</td>
<td>75.9 ± 35.6</td>
<td>96.1 ± 0.4</td>
</tr>
<tr>
<td>0.5C/1W</td>
<td>468 ± 110</td>
<td>1303 ± 59</td>
<td>52 ± 6</td>
<td>70.3 ± 39.8</td>
<td>96.0 ± 0.4</td>
</tr>
<tr>
<td>1C/1W</td>
<td>1054 ± 140</td>
<td>1328 ± 20</td>
<td>54 ± 6</td>
<td>81.6 ± 37.7</td>
<td>95.9 ± 0.5</td>
</tr>
</tbody>
</table>

\(^1\) Density of the solid material using the Archimedes principle

\(^2\) Density of the foams
4.4.6. Liquid uptake in the foams

Figure 30 shows that all the foams can sorb large amounts of n-hexane (non-polar solvent) and water (polar solvent). The 1C/1W foams absorbed more than 9 times the initial sample mass when immersed in n-hexane for one second. A longer immersion in n-hexane (one minute) did not greatly increase the uptake, indicating the uptake of the n-hexane reaches an equilibrium in a very short time due to the open pores and high porosity. Water was sorbed not only into the pores but also into the pore walls; as a result of which the foams weakened and collapsed after being immersed in water for 1 min. The reasons for the higher n-hexane uptake than water could be attributed to the different densities of the solvents and different surface tension. The liquid uptake in the foams was much greater than that of a wheat gluten foam (90 wt.%) immersed in limonene for three seconds [87].

On the other hand, water or buffer solution uptake by chitosan foams reach 1800 wt.%, but the immersion time in these cases was at least 30 min [76,80,90]. Furthermore, films with a thickness of ca. 2 mm were occasionally obtained from 0.5C/1W and 0.5C/0.5W. Such thick foams exhibited an uptake of n-hexane and water of 20 and 8 times the initial weight respectively in one second.

This uptake was considerably larger than that in the foam obtained from 1C/1W. Although a method to prepare these foams has not been developed fully, it shows that it is thus possible to increase the uptake of the blend foams further.

**Figure 30.** Uptake in foams immersed in n-hexane and water for different times
4.4.7. Compressive properties of foams

The stress-strain curve of the 1C/1W foam under compression showed three separate regions, which included elastic deformation, pore collapse and densification. It was difficult to divide the stress-strain curves of the 0.5C/1W and the 0.5C/0.5W foams into such regions, but they still showed similar compression features. The mechanical properties of the foams are summarized in Table 8. The modulus and compressive strength increased with increasing polymer content in the mixture. It has been reported that the tensile modulus increased with increasing concentration of chitosan due to concentration-dependent chitosan molecular conformations and packing during film formation [138]. Although the foams were soft, their modulus was far greater than that of a chitosan/gelatin bio-foam [80]. The rebound resilience at strains of 20 and 80 % showed a monotonous decrease with increasing polymer content. High rebound resilience (~90 %) at a strain of 20 % suggested that the elastic deformation occurred at the beginning of the pore collapse region. It is worth noting that the blend foams, without any plasticizer, are more flexible than pristine wheat gluten films.

Table 8. Compressive data of the foams

<table>
<thead>
<tr>
<th>Foam</th>
<th>Modulus (MPa)</th>
<th>Compressive stress (MPa)</th>
<th>Rebound resilience (%)</th>
<th>Rebound resilience (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5C/0.5W</td>
<td>0.3 ± 0.03</td>
<td>0.1 ± 0.01</td>
<td>64 ± 13</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>0.5C/1W</td>
<td>0.6 ± 0.1</td>
<td>0.2 ± 0.04</td>
<td>41 ± 5</td>
<td>92 ±12</td>
</tr>
<tr>
<td>1C/1W</td>
<td>1.2 ± 0.2</td>
<td>0.3 ± 0.05</td>
<td>39 ± 5</td>
<td>89 ± 10</td>
</tr>
</tbody>
</table>

1 At a strain of 80 %
2 The resilience was assessed after a strain of 80 %
3 Resilience assessed after a strain of 20 %
5. CONCLUSIONS

The main objective of the work described in this thesis was to investigate the possibilities of processing chitosan by adjusting the formulation and processing conditions. Extrusion and film casting were primarily used as processing methods, but also vacuum drying of chitosan in the liquid state. The properties of the film were further improved by blending chitosan with wheat gluten.

Chitosan materials were successfully extruded at a high solids content (60 wt.%) when water/acetic acid was used as processing liquid. A range analysis revealed that the molecular weight of the chitosan strongly influenced the quality of the extruded product. The best results were obtained with a mixture of 12 and 133 kDa chitosan in 1/1 molar ratio extruded at 50 °C from a 30 wt.% aqueous acetic acid solution. The influence of additional processing factors showed that acetic acid concentration, barrel temperature and screw speed (in a decreasing order of importance) also affected the extrudate quality, as determined by the surface finish and evenness of the samples. Analysis of the residual processing liquid within the extrudate showed that most of the mass loss occurred via diffusion within the first three days. After three days, the mechanical properties of the extrudate also became stable, with a ca. 10 times higher modulus than that of the newly extruded sample and a strain at break of ca. 3 %.

The effect of the acid was further investigated in chitosan films cast from aqueous acetic acid solutions, and the films formed were evaluated after exposure to different monocarboxylic acids (formic, acetic, propionic, and butyric acids). Compared with the films exposed to higher molecular weight acid vapours, the films exposed to formic acid vapour rapidly showed the largest acid uptake. In contrast, very long sorption times and different initial absorption rates were observed in the films exposed to propionic and butyric acid vapours. It was therefore concluded that the acid uptake and desorption kinetics were primarily related to molecular size of the acid, but also to the structure of the film. None of the acid-exposed dried films became brittle, although the molecular weight of the films decreased somewhat after exposure to the acid vapour. The toughness of the films exposed to propionic acid increased with longer exposure time, based on measurements after 62 and 281 days. Interestingly, the solubility of the dried films after exposure to the different monocarboxylic acids decreased in an aqueous acetate solution of pH = 4.5, in which the original films were completely dissolved. In addition, the uptake and loss kinetics of acetic acid vapour increased in the chitosan film free of acetic acid ions, which was determined from measurements on an acetic-acid-casted film treated by an ammonia solution.

A biphasic system, where the wheat gluten was the discrete phase and the chitosan was the continuous phase, was explored as cast materials. The wheat gluten formed large particles 40 – 200 µm in size in the blend films prepared from water/ethanol, urea, and surfactants, which significantly decreased or disappeared completely when the reducing agents (sodium sulfite, dithiothreitol-DTT, and 2-mercaptoethanol) were used, leading to a much greater homogeneity of the films. The resulting materials contained only small particles (0.7 – 3.7 µm in size), and
subsequently a much finer discrete phase. The use of surfactants did not, in general, lead to further improvements in homogeneity, even though the size of the wheat gluten particles was decreased when sodium sulfite was combined with the surfactants. The use of glyoxal cross-linking agent resulted in a more brittle film material with small and elongated wheat gluten particles. Overall, the opacity of the blend films increased with increasing size of the small particles and with increasing content of wheat gluten in the blend. A system containing only 0.3 wt.% sodium sulfite was then used to prepare films evaluated for their moisture uptake and mechanical properties. The moisture uptake decreased with increasing wheat gluten content, and the mechanical properties of the blend films with 30 wt.% of wheat gluten were similar to those of the pristine chitosan films.

Finally, chitosan/wheat gluten bio-foams were prepared without the use of any porogen or the traditional freeze-drying that are often used for the preparation of porous materials. Open pores (ca. 75 µm) and very low density of the blend foam was achieved solely as a result of vacuum drying from a mixture of the chitosan and wheat gluten solutions. The porosity of the foams was shown to depend on the polymer concentration in the chitosan and wheat gluten solutions. These foams could rapidly sorb large amounts of n-hexane and water, but immersion in water resulted in their disintegration after only 1 minute. However, in the dry state the foams without adding any plasticizer showed good toughness and high rebound resilience at a strain of 20%, compared with pristine wheat gluten solid films or foams.
6. FUTURE WORK

Several challenges have been identified on the basis of the findings presented in this thesis:

It is interesting to extrude a chitosan sample without use of processing liquid, and a plasticizer is added to vary the rheology of the extruded sample.

Besides exposing chitosan films to concentrated acid vapour, acid transport in chitosan films exposed to dilute acid vapours should be studied to give a full picture of the acid sorption/desorption kinetics in the chitosan materials.

A blend material of chitosan and wheat gluten should be processed by extrusion or injection rather than by casting.

In order to expand potential applications of chitosan/wheat gluten foams, their mechanical properties could be improved by adding plasticizers. The effects of plasticizers on the microstructures in the foam could also be studied. Methods to produce foams with even greater porosity could be developed. Antimicrobial properties of the blend foam should be characterized.
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Thank you!

Tack så mycket!
8. REFERENCES


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