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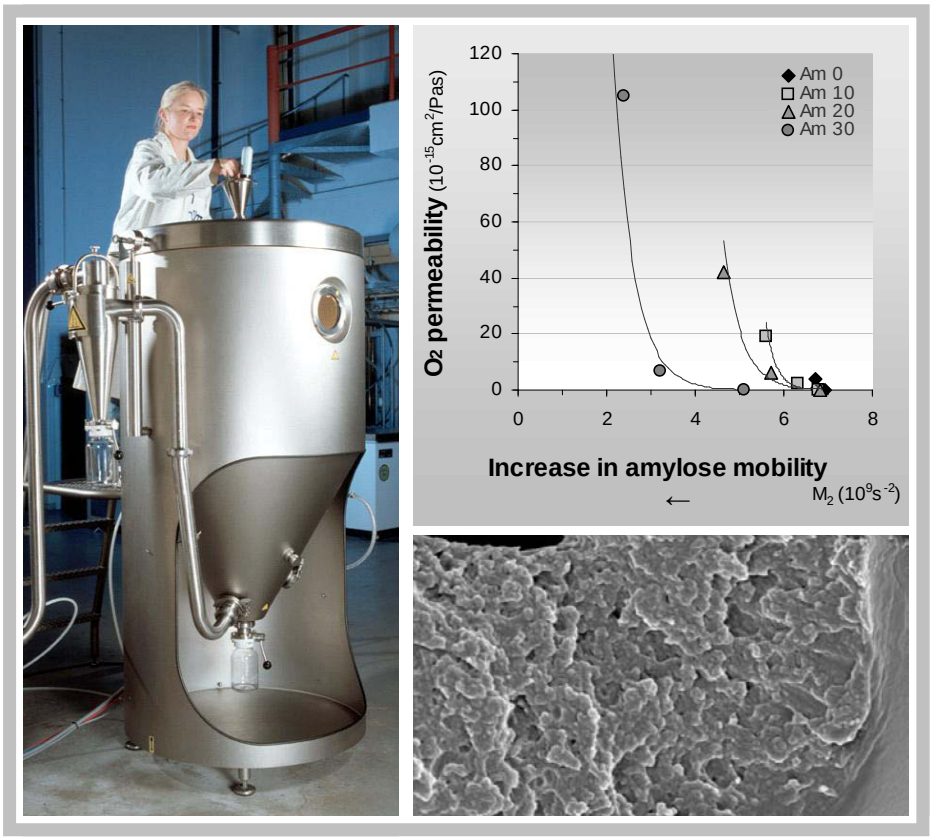

Riitta Partanen

Mobility and oxidative stability in plasticised food matrices

The role of water

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Riitta Partanen

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Keywords mobility, oil oxidation, plasticisation, starch, protein

Abstract

The importance of water in food structure and stability is well known, and the role of water as a plasticiser for biopolymers has been extensively studied during the last 25 years. Recently, understanding of the mechanisms of water plasticisation of glassy carbohydrate matrices at the molecular level has increased and its relevance to the rate of mass transfer has been emphasized. There appears to be a lack of such studies with food proteins, although the water factor is similarly recognised for example in protein-based edible films. Furthermore, food stability is currently evaluated on the basis of water activity and the physical state of the matrix. Therefore, it would be important to consider whether the major food polymers, starch-based carbohydrates and proteins, really act similarly with respect to water. In the present work, the role of water in system stability and biopolymer interactions was studied in two different systems: cast films with and without plasticiser and spray-dried particles with a dispersed lipid phase.

Plasticisation of amylose films by glycerol and water was studied by proton NMR relaxometry. In glassy amylose the proximity of T_g did not strongly affect the amylose mobility. The second moment M_2, which is a measure of strong dipolar interactions and decreases with increasing distance between the protons contributing to it, decreased slightly with increasing water content. It was thus suggested that glassy state swelling occurred in amylose film. Swelling behaviour is probably important when mass transfer in the matrix is considered. In rubbery state, high concentration (30%) of glycerol increased the mobility of amylose despite the phase separation that occurs in these systems already at much lower plasticiser content. The data on mobility of plasticised amylose was combined with results presented earlier on oxygen permeability of these films. Although increasing mobility generally resulted in increased permeability,

conditions were found in which the plasticiser induced segmental motions in amorphous amylose without appreciable loss in oxygen barrier properties.

In powder particles, the stability of embedded lipid phase was studied in traditional carbohydrate carriers, i.e. hydrolysed (maltodextrin with gum Arabic as an emulsifier) and modified (octenyl succinate derivative) starches, and in whey protein isolate. Powders with oils rich in volatile flavour or in polyunsaturated fatty acids were prepared by spray-drying and characterised by laser diffraction and scanning electron microscopy for oil distribution and by differential scanning calorimetry for their glass transition temperatures (T_g). The powders were stored under controlled conditions, and the effect of relative humidity on the rate of oxidation was studied by following the increase in peroxide value during storage. Formation of hydroperoxides is linked with oxygen transfer in the system, as it is the oxygen consuming step of the reaction. In the case of powders with embedded oil rich in volatiles, the release of limonene and carvone was studied as a function of time and temperature.

The starting hypothesis of this work was that a higher water vapour sorption at higher humidity would increase oxygen permeation in the matrices and lead to an increased rate of oxidation. This was in fact found to be the case in carbohydrate matrices during storage at 20^{o}C, at which temperature the rate of oxidation in matrices was higher at RH 54% than at RH 11%. An opposite behaviour was found for bulk oil, suggesting that the effect of water in matrix-dispersed oil was due to matrix properties. At elevated temperatures, a difference was found between hydrolysed and modified starches. The stability of oil in modified starch still correlated with the proximity of T_g, whereas the hydrolysed starch completely lost its barrier properties at 50^{o}C, which could not be explained by the T_g of the matrix. When volatiles release was studied at elevated temperature (70^{o}C), little release from dry matrices was found. Intense release was found in the proximity of the glass transition temperature in all the systems. In whey protein isolate matrix, oxidation of matrix-embedded oil was retarded compared to that of bulk oil at all humidities, but followed almost the same pattern as bulk oil with respect to humidity. The rate of oxidation was high at low humidities (RH 0% and RH 11%), was retarded at intermediate humidities (RH 50% and RH 75%) and again increased at high humidity (RH 90%), at which caking of the powder was observed as an indication of physical instability. Thus, it appeared that water did not have a similar role in the matrix

formed of globular proteins as it had in a glassy carbohydrate matrix. The importance of storage conditions and matrix properties for relating oxygen transfer to the rate of oxidation was demonstrated. Furthermore, it was proposed that the high solubility of the volatiles in non-volatile triglyceride phase was the reason for the retention of limonene and carvone in the matrices at elevated temperatures.

Partanen, Riitta. Mobility and oxidative stability in plasticised food matrices: the role of water [Hapettumisstabiilisuus ja molekyylien liikkuvuus elintarvikematriisien pehmittyessä. Veden merkitys]. Espoo 2008. VTT Publications 697. 92 s. + liitt. 43 s.

Keywords mobility, oil oxidation, plasticisation, starch, protein

Tiivistelmä

Veden merkitys elintarvikkeiden rakenteessa ja säilyvyydessä on suuri, ja veden pehmitinvaikutusta elintarvikkeiden rakennepolymeereihin on tutkittu runsaasti 25 viime vuoden aikana. Hiilihydraattimatriiseissa pehmittymisen mekanismia on alettu viime vuosina ymmärtää molekyylitasolla paremmin. Pehmittymisen vaikutusta erityisesti aineensiirtoon on painotettu. Lasimaisia proteiinimatriiseja on tutkittu vähemmän, vaikka proteiinien kalvosovelluksissa veden merkitys on yhtä lailla tunnistettu. Tällä hetkellä elintarvikkeiden säilyvyyttä arvioidaan vedenaktiivisuuden ja matriisin fysikaalisen tilan perusteella. Tästä syytä olisi tärkeää ymmärtää, vaikuttaako vesi samalla tavoin tärkeimpiin elintarvike-polymeereihin, tärkkelyspohjaisiin hiilihydraatteihin ja proteiineihin. Tässä väitöskirjatyössä veden merkitystä systeemin säilyvyydessä ja osuutta biopolymeerien vuorovaikutuksiin tutkittiin kahdessa eri matriisissa: valukalvoissa pehmittimen kanssa ja ilman pehmitintä sekä sellaisissa sumutuskuivatuissa partikkeleissa, jotka sisältävät dispergoidun lipidifaasin.

Amyloosikalvojen pehmittymistä veden ja glyserolin vaikutuksesta tutkittiin protoni-NMR-relaksaatiomittauksilla. Lasimaisen amyloosin liikkuvuus riippui lasisiirtymän etäisyydestä vain vähän. Toinen momentti M_2, joka mittaa dipoli-vuorovaikutusten voimakkuutta ja vähenee vuorovaikuttavien protonien etäisyyden kasvaessa, väheni hiukan vesipitoisuuden kasvaessa. Tulokset saattavat viitata amyloosikalvon amorfisten alueiden turpoamiseen lasitilassa. Turpoamisella on merkitystä, koska se oletettavasti vaikuttaa aineensiirtoon kalvon läpi. Kumitilassa suuri glyserolipitoisuus (30 %) lisäsi amyloosin liikkuvuutta huolimatta faasi-erottumisesta, joka tapahtuu jo huomattavasti alhaisemmassa pitoisuudessa. Mittaustulokset amorfisen amyloosin liikkuvuuden lisääntymisestä pehmittymisen tuloksena yhdistettiin aiempiin, kalvojen hapenläpäisymittauksista

saatuihin tuloksiin. Vaikka polymeeriketjujen lisääntyvä liike yleisesti johti läpäisyn lisääntymiseen, tarkastelu osoitti, että kaasunpidätyskyvyn selvä heikkeneminen ei ollut selitettävissä verkoston pehmittymisellä.

Dispergoidun lipidifaasin hapettumisstabiilisuutta tutkittiin perinteisissä hiilihydraattikantajissa – hydrolysoidussa (maltodekstriinissä arabikumi emulgaattorina) ja muunnellussa (oktenyylisukkinaattijohdannaisessa) tärkkelyksessä – sekä heraproteiini-isolaatissa. Jauheet, joiden sisältämä lipidifaasi koostui pääosin joko haihtuvista yhdisteistä tai monityydyttymättömistä rasvahapoista, valmistettiin sumutuskuivaimella. Lipidifaasin jakautumista partikkeleissa tutkittiin laserdiffraktiolla ja pyyhkäisyelektronimikroskoopilla (SEM), ja matriisin lasisiirtymälämpötilat määritettiin kalorimetrisesti (DSC). Jauheet säilytettiin säädetyissä olosuhteissa, ja ympäristön suhteellisen kosteuden (RH) vaikutusta hapettumisnopeuteen tutkittiin määrittämällä öljyn peroksidilukua säilytyksen aikana. Hydroperoksidien muodostuminen kytkeytyy hapen kulkuun matriisissa, koska se on pilaantumisreaktion happea kuluttava vaihe. Haihtuvien aineiden osalta tutkittiin limoneenin ja karvonin vapautumista säilytysajan ja lämpötilan funktiona.

Työn lähtöoletuksena oli, että suurempi vesihöyryn sorptio suuremmassa kosteudessa lisää hapen kulkua matriisissa ja siten hapettumisnopeutta. Tämä piti paikkansa hiilihydraattimatriisien osalta 20 ^{o}C:ssa, jossa hapettuminen oli kosteissa olosuhteissa (RH 54 %) nopeampaa kuin kuivissa (RH 11 %). Öljy hapettui sellaisenaan kuivissa olosuhteissa nopeammin kuin kosteissa, joten on todennäköistä, että veden vaikutus matriisiin dispergoidun öljyn hapettumiseen kohdistui nimenomaan matriisiin. Korotetussa lämpötilassa (50 ^{o}C) hydrolysoitu ja modifioitu tärkkelys käyttäytyivät eri tavoin, eikä eroa voitu selittää lasipistelämpötilojen välisillä eroilla. Haihtuvia yhdisteitä vapautui 70 ^{o}C:ssa vain vähän matriisista riippumatta. Voimakkaampi vapautuminen havaittiin lähellä lasipistelämpötilaa molemmissa matriiseissa. Haihtuvien yhdisteiden stabiilisuutta matriiseissa voidaan selittää sillä, että ne liukenevat lipidifaasin haihtumattomiin triglyserideihin.

Heraproteiinimatriisi hidasti öljyn hapettumista kaikissa olosuhteissa, mutta kosteuden vaikutus hapettumisnopeuteen oli sama kuin sellaisenaan tutkitussa öljyssä. Öljy hapettui nopeasti kuivissa olosuhteissa (RH 0 % ja RH 11 %), hitaammin keskikosteuksissa (RH 54 % ja RH 75 %) ja jälleen nopeammin

kosteissa olosuhteissa (RH 90 %), joissa jauhe paakkuuntui osoituksena fysikaalisista muutoksista. Siten vedellä ei voitu havaita hapettumiseen tutkitussa proteiinimatriisissa vastaavaa vaikutusta kuin hiilihydraattimatriisissa. Proteiinin muodostamalla tiiviillä rajapintakerroksella (öljy-matriisi-rajapinta) on mahdollisesti muuta matriisia tärkeämpi rooli aineensiirron säätelijänä.

Preface

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I thank my parents Leena and Jarmo for their love and encouragement throughout my life. I am grateful to my sister Marja for the special bond we share and for the joyful moments between our families. I warmly thank my parents-in-law Tuula and Seppo for so willingly helping as grandparents during these years. I have enjoyed the friendship of Inkku, Taru and Anuleena: this year will always be remembered as the year of finishing the theses (all four of us!). Finally, I thank my husband Veli Matti for his love throughout the years and for the numerous times he compromised his own comfort, especially during this last year. To our sons Esa and Olli, who are the salt and pepper of my life, I owe a million hugs.

List of publications

I Partanen, R., Marie, V., MacNaughtan, W., Forssell, P. and Farhat, I. (2004). ^{1}H NMR study of amylose films plasticised by glycerol and water. Carbohydrate Polymers 56, 147–155.

II Partanen, R., Yoshii, H., Kallio, H., Yang, B. and Forssell, P. (2002). Encapsulation of sea buckthorn kernel oil in modified starches. JAOCS 79(3), 219–223.

III Partanen, R., Hakala, P., Sjövall, O., Kallio, H. and Forssell, P. (2005). Effect of relative humidity on the oxidative stability of microencapsulated sea buckthorn seed oil. Journal of Food Science 70(1), E37–E43.

IV Partanen, R., Raula, J., Seppänen, R., Buchert, J., Kauppinen, E. and Forssell, P. Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. Journal of Agricultural and Food Chemistry 56, 5717–5722.

V Partanen, R., Ahro, M., Hakala, M., Kallio, H. and Forssell, P. (2002). Microencapsulation of caraway extract in β-cyclodextrin and modified starches. European Food Research and Technology 214, 242–247.

The author's contribution to the appended publications

I The author planned the work together with Dr. Imad Farhat, carried out the experiments and was responsible for the data-fitting. She participated in the interpretation of the data, and wrote the paper together with Dr. Pirkko Forssell and Dr. Imad Farhat.

II The work was planned together with the other authors. The author set up the methods together with Prof. Hidefumi Yoshii and supervised the experimental work. She also had main responsibility for interpreting the data and writing the paper, together with Dr. Pirkko Forssell.

III The author planned the work together with Dr. Pirkko Forssell and Prof. Heikki Kallio. She supervised the experimental work together with Piia Hakala. Dr. Olli Sjövall was responsible for the storage test at elevated temperature. The author had main responsibility for interpreting the data and writing the paper, together with Dr. Pirkko Forssell.

IV The author had main responsibility for planning the work, together with Dr. Pirkko Forssell. She conducted part of the analytical work. Dr. Janne Raula was responsible for the scanning electron microscopy and Dr. Rauni Seppänen for the chemical analysis by electron spectroscopy (ESCA). The author had main responsibility for interpreting the data and writing the paper, together with Dr. Pirkko Forssell.

V The author planned the work together with the other authors. She was responsible for the thermal analysis and the sorption isotherms, whereas the FT-IR analysis was performed by Dr. Mikko Ahro and the analysis of volatile contents using head-space-gas chromatography by Hannele Virtanen. The data interpretation was carried out together with the other authors. The author had main responsibility for writing the paper together with Dr. Mari Hakala and Dr. Pirkko Forssell.

Contents

Appendices of this publication are not included in the PDF version.
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List of symbols

Am	amylose
a_w	water activity
CAO	caraway extract
CLSM	confocal laser scanning microscope
DE	dextrose equivalent
DSC	differential scanning calorimeter
FID	T_2 relaxation as a free induction decay
FSO	flaxseed oil
FT-IR	Fourier transform infra-red spectroscopy
ESCA	electron spectroscopy for chemical analysis
ESEM	environmental scanning electron microscope
GA	gum Arabic
HiCap	commercial octenyl succinate starch derivative
HS-GC	head-space gas chromatography
IDF	International Dairy Federation
M_2	the rigid lattice second moment, a measure of dipolar interactions
MD	maltodextrin

MW	molecular weight
NMR	nuclear magnetic resonance
PUFA	polyunsaturated fatty acid
RH	relative humidity
SBO	sea buckthorn oil
SEM	scanning electron microscope
T_2	spin-spin relaxation time, de-phasing of the precessing spins
T_g	glass transition temperature
w_g	glass transition water content
WPI	whey protein isolate

1. Introduction

Real foods are systems with great complexity, which arises from their non-equilibrium state as well as their heterogeneity in structure and composition. The current expectation for long shelf-life of food products is a challenge for product stability. The interplay between active, i.e. available water and food stability was modeled in the early 1970s (Labuza, Tannenbaum & Karel, 1970). Labuza's concept of water activity (Figure 1) became widely used in the industry due to its practical value in quality assurance (Schmidt, 2004). The scientific limitations of the concept have been under debate (Franks, 1982; Slade and Levine, 1991), the main argument being that the theory is based on the assumption of an equilibrium state, which is not valid in most food systems. Directly after processing, water activity may differ between the phases of the product. In time, chemical and physical instability may change water activity. Since these observations, a considerable effort relating instability to water-induced physical changes in foods has been made.

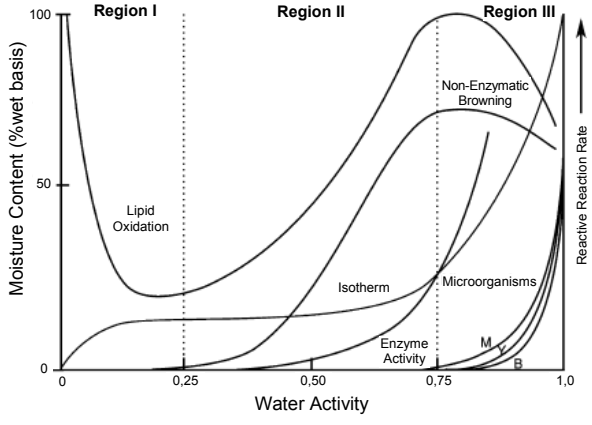

Figure 1. The original food stability map, in which reaction rates, microbial growth, enzyme activity and sorption isotherm are plotted as a function of water activity (Labuza et al., 1970). Reproduced with permission from Schmidt (2004).

1.1 Structure in solid biopolymer systems

A common feature in foods is that they consist of multiple phases, some of which are continuous, and some dispersed. Structure can be defined at different

levels, which may all influence the stability of the system. The structural features of the systems which are of importance to this thesis are presented in Table 1 at microscopic and molecular levels. Water activities are included, as water is a major determinant of structure. According to Lillford (1988), molecular structure and conformation are especially important in biopolymer-water interactions, when structures with sorption water ($a_w < 1$) are considered. However, the properties of large three-dimensional structures with high water contents ($a_w \sim 1$) are strongly influenced by microscopic dimensions and are not determined only by the molecular structure or composition. The macroscopic behaviour of the matrix can be studied empirically, but the mechanisms causing instability operate at the molecular level. Studying the interactions between water and food molecules is difficult for two main reasons: water is a molecule with unique self-association behaviour (Schmidt, 2004), and there is a lack of techniques for measuring the interactions or so-called "binding" of water (Lillford, 1988). The major macromolecules in foods are carbohydrates and proteins. There are some considerable differences in their structure formation, which are outlined below. The work of this thesis focused on starch-derived carbohydrates and on whey proteins and the review is therefore limited to these components.

Table 1. Structural levels for multi-component biopolymer systems of the present study.

STRUCTURE CHARACTERISTICS		
MACROSCOPIC	MICROSCOPIC	MOLECULAR
POWDER PARTICLE WITH DISPERSED LIPIDS $0 < a_w < 1$	Solid-gas interface: particle size distribution, porosity and morphology Solid-liquid interface: oil droplet size distribution and non-emulsified lipids	Physical interactions, level of aggregation and organisation
PLASTICISED FILM $0 < a_w < 1$	Homogeneity, compatibility of components, surface roughness	Molecular network, physical interactions and chain entanglements, crystallinity

1.1.1 Structure in starch-based matrices

Starch consists of α-1,4-linked glucose units, essentially linear amylose and α-1,6-branched high molecular weight amylopectin (Langlois & Wagoner, 1967). The granular structure of native starch is not considered in this context, as it is not relevant to the present work. Starch polymers are hydrophilic, with hydroxyl groups as their only functional groups. Therefore, starches and their hydrolysates are not surface active unless chemically modified (Figure 5). However, amylose and amylopectin, the linear and branched glucose polymers in starch, behave differently in water (Foster, 1965). The molecular ordering in amylose is due to strong secondary forces, causing interaction between neighbouring molecules. The interaction was proposed to be through hydrogen bonding. Miles et al. (Miles, Morris & Ring, 1985) showed that association behaviour in amylose systems was dependent on the concentration of the system studied, leading to gelation or precipitation. Gidley (1989) proposed that aggregation and gelation of amylose are dominated by the formation and aggregation of B-type double helices, which are hexagonal crystal units (Imberty & Perez, 1988). Amylose gels are still largely composed of an amorphous fraction (Leloup, Colonna, Ring, Roberts & Wells, 1992). Leloup et al. (1992) studied the structure of equilibrated and rapidly quenched frozen amylose gels, and found a structure of interconnected strands about 20 nm wide, which were proposed to consist of a large number of aggregated amylose chains. The width of the partially crystalline filaments was found to be independent of amylose concentration and only the porosity (Ø 100–1000 nm) was reduced due to more frequent occurrence of the filaments in concentrated systems. Co-existence of an amorphous structure with dangling chains of amylose in pores was suggested.

In film formation by drying, amylose has been found to crystallise to some extent depending on the drying temperature (Bader & Göritz, 1994) and plasticiser content (Myllärinen, Buléon, Lahtinen & Forssell, 2002a), and to be independent of the humidity during preparation (Rindlav-Westling, Stading, Hermansson & Gatenholm, 1998) and of storage time (Myllärinen et al., 2002a). Gelation of amylopectins from aqueous solutions was confirmed by Ring et al. (Ring, Colonna, l'Anson, Kalichevsky, Miles, Morris & Orford, 1987). Amylopectin chains were found to associate by crystallisation of branches with 15 glucose units. Association led to formation of a thermoreversible network, in which the level of B-type crystallinity was found to increase linearly with

increasing polymer concentration. Unlike films made of amylose, fresh amylopectin films without glycerol have been found to be amorphous (Rindlav-Westling et al., 1998; Myllärinen et al., 2002a). With glycerol as a plasticiser, crystallinity depended strongly on humidity during preparation (Rindlav-Westling et al., 1998) and to some extent on humidity during storage (Myllärinen et al., 2002a).

Solution and phase behaviour of hydrolysed starch, malto-oligomers have been studied in order to predict and interpret the behaviour of starch polymers (Sugiyama, Nitta, Horii, Motohashi, Sakai, Usui, Hisamichi & Ishiyama, 2000; Moates, Noel, Parker & Ring, 1997; Orford, Parker, Ring & Smith, 1989) and to investigate the effect of oligomers on crystallisation of polymers (Smits, Kruiskamp, van Soest & Vliegenhart, 2003). According to Moates et al. (1997), no crystal forms are known for maltotetraose, -hexaose, and -heptaose. Sugiyama et al. (2000) found evidence for helical coil conformation of oligomers in dimethyl sulphoxide, but in water conformation was suggested to be between that of maltose and that of amylose. Smits et al. (2003) reported that malto-oligosaccharides were able to reduce crystallisation of partly gelatinised starch.

1.1.2 Structure in whey protein matrices

The fundamental aspects of protein structure – primary, secondary and tertiary – are common variables for food and other proteins. Considering the structure formation in protein matrices huge variance exists due to differences in the above-mentioned factors and also due to the biochemical functions of proteins from different origins. For example the large colloidal casein particles (50–500 nm in size = micellar casein) in milk have been linked to the mechanism of calcium transport from mammary cells (Holt, 1992). The other major group of milk proteins is whey proteins of which globular beta (symbolina)-lactoglobulin is the most abundant (Fox & McSweeney, 1998). Association behaviour of milk proteins has been extensively studied in very different environments due to their importance in various food structures, such as oil-in-water (milk) and water-in-oil emulsions (butter), ripened and unripened cheese, yoghurt and curd (Fox & McSweeney, 1998).

In multi-phasic systems, the amphiphilic nature of proteins becomes important. Formation of a thick, viscoelastic layer is associated with adsorption of surface-active proteins to the interface. High interfacial shear viscosity is considered an

indication of strong forces acting on adsorbed globular proteins, which eventually lead to unfolding of the native structure (Kim, Cornec & Narsimhan, 2005). In β-lactoglobulin, disulphide-bond interchange is induced by adsorption and subsequent aging of oil-water interfaces.

As β-lactoglobulin is globular, most of the hydrophobic amino acid residues are buried inside the globule (Fox & McSweeney, 1998). β-Lactoglobulin can form quaternary structures up to octamers depending on pH. Further aggregation and gelation are possible through partial unfolding of the structure (van Vliet, Lakemond & Visschers, 2004). Unfolding can be accomplished through addition of chemicals, hydrostatic pressure, heating, cooling and enzymatic hydrolysis. The contributions of different types of bonds to the aggregation process are not fully understood, but the role of thiol-catalysed aggregation has been proposed (Roefs & de Kruif, 1994). Galani and Apenten (1999) demonstrated that aggregates are not only formed by disulphide bonds but also by hydrophobic interactions at temperatures above 90^{o}C, as the loss of compact structure exposes the hydrophobic residues to the aqueous environment.

1.2 Water-biopolymer relations in equilibrium

1.2.1 Water vapour sorption characteristics

At equilibrium, the chemical potential of water is the same in all phases of the system (Schmidt, 2004). Thus, the water content of a solid is linked to the water vapour pressure of the atmosphere surrounding it. At a constant temperature, a sorption isotherm can be drawn to describe this equilibrium relationship. In the present work, water vapour sorption is of crucial importance to the properties of powder and film systems. Typical sorption isotherms for amorphous food components are sigmoid curves (Roos, 1995). The association of water in crystalline structures of biopolymers may be characteristic to the crystals formed. For example, water vapour sorption occurs in crystalline starch with increasing environmental humidity, whereas in cellulose, only the amorphous regions sorb (van den Berg, 1981). In low molecular weight carbohydrates, water may have an essential part in crystal structure. Hysteresis between sorption and desorption curves is often observed, suggesting that a true equilibrium may not be reached. Van den Berg (1981) pointed out that the

capillary phenomenon, which is still today associated with hysteresis (Labuza & Altunakar, 2007), assumes rigid capillaries that do not exist in plasticised polymeric materials. Complex phenomenon has been explained by swelling, diffusion barriers, metastable local domains, activation energy and time-dependence of equilibria, which according to van den Berg (1981) may all contribute to it. Thus, hysteresis is affected by the microstructure of the system trying to reach equilibrium.

Sorption isotherms can be analysed with models, which have been used to determine the monolayer value for sorption (Labuza & Altunakar, 2007). The BET (Brunauer-Emmet-Teller) monolayer value closely correlates with the number of polar groups that are capable of forming hydrogen bonds with water (van den Berg, 1981). The importance of the monolayer value has been explained by the inability of water to act as a solvent below this limit (Bell & Labuza, 2000). Water interacts with other food constituents through weak interactions such as dipole-dipole forces, ionic bonds, van der Waals forces, and hydrogen bonding (Labuza & Altunakar, 2007). Even if these interactions change the properties of water from that of bulk water, their timescale is usually less than 10^{-11} seconds, unless water is strongly bound as in the case of crystal water. Furthermore, the values obtained are average values, whereas the properties of water probably change as a continuum (van den Berg, 1981). Thus very different interpretations regarding water structure and mobility arise from different techniques. It could be stated that *even if the effect of food components on water is still a field of confusion, much more is known about the effect of water on food components*.

1.2.2 Water as a plasticiser

Water acts as a plasticiser for hydrophilic biopolymers. The importance of the physical state of amorphous food matrices was emphasised by Slade and Levine (1985, reviewed in Schmidt, 2004; 1988; 1991), who applied the classical polymer science theory of glass transition to biopolymers and food systems. The glassy state of amorphous materials reflects their appearance as solid liquids, which have an extremely high viscosity, $\eta > 10^{12}$ Pas (Roos, 1995). Polymer movement in glassy matrices is restricted to vibrations, short-range rotational motions and movement of relatively short segments of the polymer chain.

According to Ruan & Chen (1998), segmental motions of polymer chains are activated when passing from the glassy to the rubbery state. The material starts to flow as the whole molecule starts moving when progressing to the liquid state. Both segmental and molecular motions are regarded as Brownian motions.

Since the first studies of Slade and Levine (1985), the effect of water on glass transition temperatures (T_g) of various food carbohydrates, proteins and their mixtures have been determined. The glass transition water content (w_g) at which the transition is detected at room temperature is often a matter of interest. For gelatinised starch, glass transition has been reported to occur at 22% water content (Zeleznak & Hoseney, 1987), whereas the T_g-values of dry amylose and amylopectin, the starch polymers, have been extrapolated to 227^{o}C (Orford et al., 1989). The effects of water on glass transition temperatures for many simple carbohydrates, such as mono- and disaccharides as well as sugar alcohols, have also been determined, the data also indicating that many of these systems are prone to rapid crystallisation (Roos, 1993).

Most of the studies on thermodynamic properties of proteins have been carried out in dilute solutions, in which T_g is below the freezing point of water (Sochava, 1997). This could explain why a recent review on glass transition in protein dynamics found a common change in dynamical properties of proteins at 200K (-73^{o}C) (Ringe & Petsko, 2003). Recently, Bengoechea et al. (Bengoechea, Arrachid, Guerrero, Hill & Mitchell, 2007) found in their studies with gluten, soya protein isolate and rennet casein that gluten had lower immobilising interactions in the rubbery state as compared to the other proteins. It was suggested that gluten was closer to an ideal synthetic polymer as compared with most other biopolymers. Noel et al. (Noel, Ring & Whittam, 1990) suggested that the glass transition behaviour in globular proteins might be different from that of other biopolymers, because it is not immediately apparent whether they have a disordered structure.

On the basis of dynamic neutron scattering experiments Doster and Settles (2005) reported that as the residues of compact proteins in the native state can only perform localised motions, they do not undergo glass transition. The freezing of a fraction of local motions was termed ‘protein-dynamical transition’. Sochava (1997) studied the thermal behaviour of a globular protein, legumin, at different water contents by DSC. It was found that T_g of the anhydrous protein

was 190°C. At 25–28% water content, T_g was below -30°C, but it could not be accurately determined. The author discussed the effect of denaturation during the first heating, which lowered the T_g from that of the native protein. This observation was explained by release of water in denaturation, which was suggested to plasticise the protein further in the second heating. The author concluded that due to denaturation, translational motions of chain segments are increased. Thus, a heat-capacity increment similar to that found in carbohydrates occurs in both globular and non-globular proteins, but its relation to functionality appears to be less well established.

Glass transition temperatures (T_g) of miscible compounds have been successfully predicted with the empirical Gordon and Taylor equation (Roos, 1995), which is based on glass transition temperatures (T_{g1}, T_{g2}) and weight fractions (x_1, x_2) of the pure compounds 1 and 2 (Gordon & Taylor, 1952) and a constant k:

$$T_g = \frac{x_1 T_{g1} + k x_2 T_{g2}}{x_1 + k x_2} \quad (1)$$

Although the effects of water and low-molecular weight compounds as plasticisers of amorphous carbohydrates are well-known, the molecular mechanism of plasticisation is not fully understood. Kilburn et al. (Kilburn, Claude, Mezzenga, Dlubek, Alam & Ubbink, 2004; Kilburn, Claude, Schweizer, Alam & Ubbink, 2005) studied plasticisation of maltodextrin at the nanolevel. Using positron annihilation lifetime spectroscopy (PALS), the authors were able to show that the average volume of the voids between the polymer chains increased with the water content, with simultaneous increase in density (Kilburn et al., 2004). Furthermore, it was found that the transition in water sorption properties between glassy and rubbery states was gradual (Kilburn et al., 2005). No sudden changes in slopes of swelling or density were detected due to transition. The volume and number density of holes were found to be independent of the molecular weight distribution of the carbohydrate in the matrix. Thus, it was concluded that the free volume of the carbohydrate matrix is essentially independent of the molecular weight.

Furthermore, a difference in plasticisation mechanism between water and simple carbohydrates was reported. The plasticisation effect of water was explained

through facilitation of reorientation and relaxation of carbohydrate chains even in glassy state, due to interference in hydrogen bonding of the polymer. Low molecular weight carbohydrates led to improved molecular packing, which was observed as a decrease in the size of holes and an increase in matrix density in the glassy state. The reduction in glass transition temperature due to low molecular weight carbohydrates was proposed to be due to reduced average number of entaglements between the polymers in the presence of low-molecular-weight compounds. This would allow molecular reorganisations under conditions in which the entangled polymer system would be frozen to glassy state. Kilburn et al. (2005) presented a model of the mechanism of water sorption by amorphous carbohydrates (Figure 2).

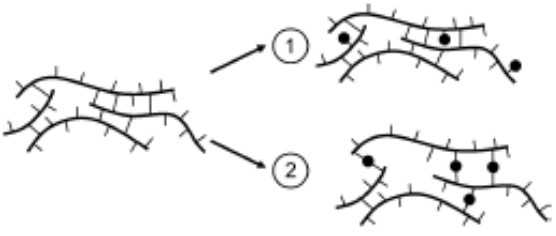

Figure 2. Schematic depiction of the mechanism of sorption of water by amorphous carbohydrates. Reproduced with permission from Kilburn et al. (2005). At low water concentrations all water molecules form hydrogen bonds with the hydroxyl groups of the carbohydrate, which are well dispersed in the matrix. 1. Water sorption occurs without extensive swelling of the matrix. This will lead to increased density and to a reduction in free volume of the matrix. 2. Water sorption leads to significant swelling, even in the glassy state. This will lead to an increase in the average size of the holes. For amorphous carbohydrate polymers, the actual mechanism is between the two limiting cases 1 and 2.

1.2.3 Challenges in measuring biopolymer-water interactions

The limitations of the simple techniques for studying biopolymer-water interactions and especially the complexity of data interpretation in more sophisticated techniques have frequently been discussed (Lillford, 1988; Hills, Takacs & Belton, 1990; Belton, 1997; Kilburn et al., 2005). Determination of

glass transition temperature and the extent of hydrogen-bonding are often of importance in these systems. Kilburn et al. (2005) discussed the lack of techniques by which hydrogen bonding in carbohydrate matrices could be determined, particularly in amorphous systems. It was suggested that computer simulations would be the most useful way of analysing the details of these interactions. Very different methods can be used to determine the glass transition temperature (T_g), e.g. mechanical, thermal and spectroscopic methods (Kalichevsky, Jaroszkiewicz, Ablett, Blanshard & Lillford, 1992). The time-scales of the measurements vary between the methods, which therefore do not all probe the same molecular motions. Less energy and thus lower temperature is needed for the short-range mobility to be activated as compared to long-range segmental motions. This can lead to substantial differences in the determined glass transition temperatures. Lillford (1988) discussed the methodology, with which hydration of biopolymers has been measured. Where gravimetric (sorption and desorption) methods result in the amount of water associated with polymer, the location and state of water remains unknown. Information obtained by calorimetric studies is limited to the amount of freezing water. Of the spectroscopic methods, infrared and Raman can identify different states of water, but suggest a short lifetime for these interactions. NMR can detect changes in molecular motions as induced by the interactions, but separation of various interactions is not possible unless the lifetime of the interactions is long ($> 10^{-6}$-10^{-3}s).

1.3 Instability of non-equilibrium biopolymer systems

The major quality defects in foods are caused by lipid oxidation, microbial growth and physical instability. All of these can be linked with water (Labuza et al., 1970, Slade & Levine, 1985). As water is one of the crucial determinants in food structure it cannot be significantly reduced or increased without consequences in sensory properties. Thus, in order to be able to control the quality, understanding of water-interactions is definitely needed. The relationship between water and microbial growth was not studied in this thesis and therefore it is not discussed in this context.

1.3.1 Mass transfer-induced instability

Free radicals or pro-oxidants capable of forming radicals are present in most real food systems (Orlien, Andersen, Sinkko & Skibsted, 2000). In the primary stages of lipid oxidation, a radicalised fatty acid reacts with molecular oxygen, forming a fatty acid hydroperoxide. In the reaction oxygen is consumed, causing a difference in chemical potential (Δμ) of oxygen at the surface and inside the matrix. This difference serves as a driving force for oxygen transfer in the system. Similarly to oxygen transfer conditions, change in water vapour pressure in the environment causes a chemical potential difference in the biopolymer matrix, which acts as a driving force for water vapour sorption or desorption. The non-equilibrium state i.e. Δμ is a prerequisite for the flux to occur, but kinetic considerations are needed to establish a transfer rate. A simplified mass transfer for oxygen across a multi-phase system is described in Figure 3. The reaction rate depends largely on temperature, as shown by the Arrhenius equation (2):

$$k = A \exp\left(-\frac{E_a}{RT}\right) \qquad (2)$$

where k is the rate constant at temperature T, A is a pre-exponential factor, R is the ideal gas constant and E_a is the activation energy. Under certain conditions, however, mass transfer may become rate limiting. According to Orlien et al. (Orlien, Risbo, Rantanen & Skibstead, 2006), hydroperoxide formation in glassy food matrices is controlled by different factors depending on temperature. At low temperatures (5^{o}C), the reaction rate and thus the oxygen consumption rate is slow, and the availability of oxygen is not rate limiting. At intermediate (25^{o}C) and high (up to 60^{o}C) temperatures, oxygen transfer becomes the rate-determining factor in the formation of lipid hydroperoxides.

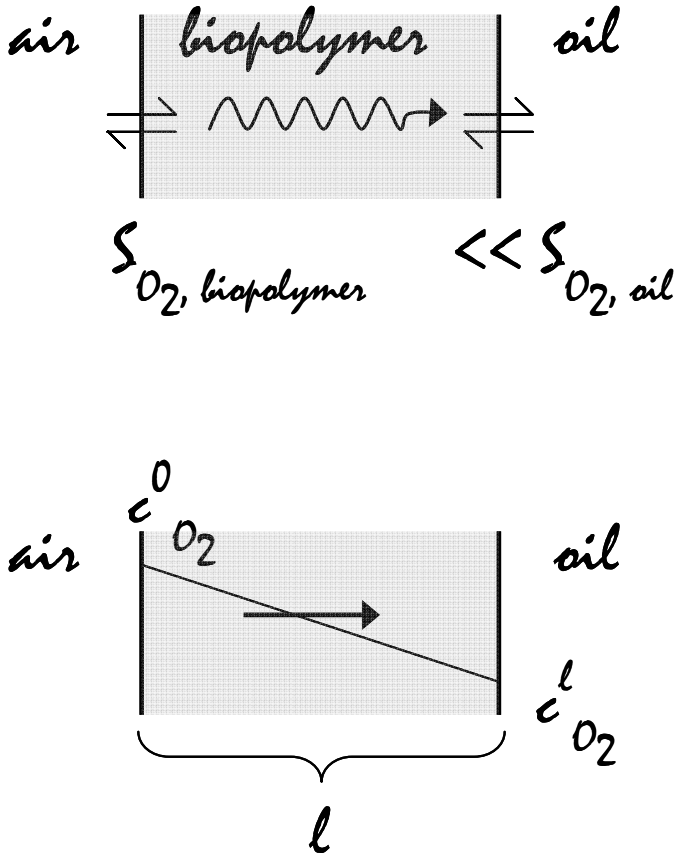

Figure 3. Oxygen transfer rate through a biopolymer matrix consists of three stages: dissolution into the matrix, diffusion across the matrix and dissolution into the oil. c^0_{O2} *is the stationary state concentration of oxygen at the matrix surface, which is approximated to be equal to oxygen solubility in the corresponding conditions. Since the solubility of non-polar oxygen in oil is expected to be much higher than its solubility in biopolymers, transfer at the polymer-oil interface is not expected to be rate-limiting. When the rate of oxidation is high, oxygen is consumed from oil as soon as it is dissolved, and thus* $c^l_{O2} \approx 0$.

The stationary (time-independent) conditions for flux in Fickian diffusion (Figure 3) are defined as (3) and permeability of permanent gases through matrix as (4):

$$\begin{cases} J_{O_2} = -\int_0^l D_{O_2} \dfrac{dc}{dx} = -D_{O_2} \dfrac{c_{O_2}^l - c_{O_2}^0}{l} = D_{O_2} \dfrac{c_{O_2}^0}{l} \\ D_{O_2} \neq D_{O_2}(x) \\ c_{O_2}^l = 0 \end{cases} \quad (3)$$

$$\begin{cases} c_{O_2}^0 \approx S_{O_2,biopolymer} \\ J_{O_2} l \approx P = D_{O_2} S_{O_2,biopolymer} \end{cases} \quad (4)$$

where J_{O2} is the oxygen flux, D_{O2} is the oxygen diffusion coefficient and c^0_{O2} and c^l_{O2} are the stationary phase concentrations of oxygen at the matrix surface and oil droplet surface, respectively. Furthermore, l is the distance for oxygen diffusion, and $S_{O2,\ biopolymer}$ is the solubility of oxygen in the biopolymer, which is approximated to be equal to c^0_{O2}. In polymer chemistry, however, the concentration of gases in the matrix has been divided into the gas dissolved in polymer and that sorbed by free volume holes (Lin, Shenogin & Nazarenko, 2002), suggesting that solubility alone may not be enough to determine the concentration, which could be further influenced by the nano-scale structure of the matrix. Permeability, P, is a parameter often used to describe gas transfer rate in polymer film systems, as it is relatively easy to measure.

The dependence of translational diffusion coefficient (D, comparable to that in equations (3) and (4)) on media viscosity (η) can be approximated on the basis of the Stokes-Einstein equation:

$$D = \frac{kT}{6\pi\eta\, a} \quad (5)$$

where k is the Boltzmann's constant, T is the absolute temperature and a is the hydrodynamic radius of the diffusing component. Based on the dependence of diffusion coefficient on viscosity, a dramatic reduction in diffusion rate would be expected with transition from rubbery to glassy state of amorphous biopolymers (Le Meste, Champion, Roudaut, Contreras-Lopez, Blond & Simatos, 1999). However, the Stokes-Einstein relationship describes diffusion in

homogeneous liquid media rather than in non-ideal amorphous biopolymer systems. Rather limited amount of data exist on diffusivity in amorphous biopolymer systems (Parker & Ring, 1995; Tromp, Parker & Ring, 1997; Gunning, Parker & Ring, 2000a; Karbowiak, Gougeon, Rigolet, Delmotte, Debeaufort & Voilley, 2008). Tromp et al. (1997) studied diffusion of water in glassy glucose syrup. The authors demonstrated that diffusion of water was not coupled with the macroscopic viscosity, as linear Arrhenius behaviour was found both below and above glass transition temperature. For maltose with more dense structure, some slowing of diffusion was found when approaching T_g (Parker & Ring, 1995), but the change was not pronounced enough to couple with viscosity. The changes in diffusion constants of water between the two matrices were explained by differences in jump distance in systems with more (glucose syrup) and less (maltose) random packing (Tromp et al., 1997).

Gunning et al. (2000a) studied the diffusion of short-chain alcohols in maltose-water systems close to glass transition temperature. Methanol, with the smallest diameter (0.478 nm) had the highest diffusion constant of the alcohols studied and propan-2-ol with the largest diameter (0.569 nm) had the lowest diffusion constant. Diffusion was found to increase with increasing water content, alcohol content and temperature, but it could not be coupled with viscosity as predicted by the Stokes-Eistein equation (5). It was suggested that the temperature difference between the actual temperature of the system and its T_g could be used as a predictor of diffusivity for the alcohols studied. Seuvre, Philippe, Rochard & Voilley (2006) studied the retention of aroma compounds in aqueous carbohydrate solution and in an emulsion with similar rheological characteristics. In equilibrium studies all the emulsions had higher aroma retention as compared to carbohydrate solutions. A pronounced effect was found for ethyl hexanoate, which had the lowest water solubility of the compounds studied. In kinetic studies, the release characteristics were found to differ within a chemical family. Again, ethyl hexanoate behaved differently in the two systems, as its release from the emulsion was considerably retarded as compared to the carbohydrate solution.

Gunning et al. (Gunning, Parker, Ring, Ringby, Wegg & Blake, 2000b) pointed out the influence of water on solubility of volatiles in carbohydrate matrix by re-interpreting the results of Flink and Karel (1970). Recently, Whitcombe et al. (2005) suggested that the good oxygen barrier properties of dry carbohydrates (Gaudin, Lourdin, Forssell & Colonna, 2000; Stading, Rindlav-Westling &

Gatenholm, 2001; Forssell, Lahtinen, Lahelin & Myllärinen, 2002) could be due to low solubility of oxygen in the matrix. The authors hypothesised that the gradual loss of barrier properties with increasing water content could be linked with the presence of more dissolved oxygen in the system due to water. The authors measured oxygen solubility in sugar alcohols and reported a decrease with decreasing water content. It was shown that with linear extrapolation, oxygen would be insoluble in sugar alcohols below 30% water content. As in many other aspects, it appears that biopolymers are not likely to conform with the behaviour of ideal systems, because various factors such as interactions between the matrix and permeants, as well as the structure and dynamics of the amorphous network are likely to make the system more complex.

1.3.2 Water-induced physical instability

Most amorphous biopolymer systems are not in equilibrium and therefore have a driving force towards a lower energy state. Typically this state can be reached by crystallisation, by minimising the interfacial area by phase separation or broadly speaking by increasing attraction in the net interactions at the expense of repulsion (or weaker attraction). Depending on the environmental conditions, amorphous systems can be metastable with very slow kinetics for changes, or unstable with changes occurring on a practical time-scale. As shown by the Arrhenius equation (2), the rate is largely determined by temperature, but according to glass transition theory, changes will only take place in the rubbery or liquid state ($T > T_g$) of these systems (Roos, 1995). As water plasticises i.e. decreases the T_g of the system, its amount is of crucial importance to physical stability.

Macroscopically, physical changes occurring during storage can be observed as caking of powders associated with loss of free-flowing properties and eventually as structural collapse (To & Flink, 1978; Barbosa-Cánovas, Ortega-Rivas, Juliano & Yan, 2005). Collapse has been found to occur in amorphous systems above T_g, where its rate is affected by the temperature difference ($T-T_g$) (Labrousse, Roos & Karel, 1992). It is not necessarily linked with crystallisation, since it has also been found in non-crystallising matrices. As a consequence of crystallisation a complete release of the lipids dispersed in the matrix has been found to take place. For an amorphous matrix, the collapsed state has been linked with partial release of the lipid phase or with re-encapsulation of the

lipids. Decrease of volatiles release (Whorton and Reineccius, 1995) and decrease in oxidation rate (Beristain, Azuara & Vernon-Carter, 2002) have also been reported due to collapse, but in these studies crystallisation of the matrix was not monitored.

Phase separation is found between incompatible polymers (Tolstoguzov, 1999), water and polymers in contracting gels (Lucey, Tamehana, Singh & Munro, 2001; van Vliet & Walstra, 1994) and in low water content polymer systems with excess of low molecular weight plasticisers (Lourdin, Ring & Colonna, 1998; Forssell, Mikkilä, Moates & Parker, 1997). The separation of low molecular weight plasticisers has been evidenced in the presence of water in starch systems (Lourdin et al., 1998; Forssell et al., 1997) and in the absence of water in starch (Moates, Noel, Parker & Ring, 2001) and protein systems (Gao, Stading, Wellner, Parker, Noel, Mills & Belton, 2006). The research has mainly focused on the consequences of such behaviour for the mechanical properties of the films and less on the dimension or evolution of these domains in time. Phase separation has mainly been studied by thermal and mechanical methods. Based on NMR relaxation time measurements, Gaudin, Lourdin, Le Botlan, Ilari & Colonna (1999) suggested that separation occurs as clustering of plasticiser molecules rather than formation of two distinct phases.

1.4 Starting point and aims of the present study

The work described in this thesis is composed of different aspects of structure-function relationships in biopolymer matrices as affected by water. The studies deal with formation of three-dimensional biopolymer structures in drying and the effect of re-introducing water on structure and stability. The major issues are water-induced molecular mobility in matrices and oxidation of matrix-embedded lipids. Where the published results are from separate topics with different goals, the summary focuses on understanding how carbohydrates and proteins control the stability of matrix-embedded compounds and transfer of compounds within these matrices. The state of current understanding in these issues is briefly summarised in Table 2 as a basis of this work. The references given are those directly dealing with specific phenomena; a more extensive review of the literature is presented in the Introduction.

Table 2. Summary of current knowledge and theories dealing with the topics most relevant to the present thesis.

	STATE-OF-THE-ART	
OBSERVATION	THEORY	UNCERTAINTIES / LIMITATIONS
WATER AND OXIDATION OF MATRIX-EMBEDDED LIPIDS		
a_w affects lipid oxidation rate[1]	monolayer theory – best stability at monolayer moisture[2]	assumption of equilibrium[3], sorption the only matrix property considered
oxygen is needed for hydroperoxide formation[1]	oxygen transfer is rate-limiting for oxidation[4]	shown for glassy system at ambient/high temperature[4]
diffusion of small molecules is facilitated in plasticised polymers[5, 6, 7]	glass transition theory[5]	diffusion not coupled with viscosity[6, 7]
dry starch is a good oxygen barrier[8, 9]	solubility of O_2 in dry carbohydrate limits permeation[10]	so far data only on solutions of sugar alcohols, extrapolated to low-water systems[10]
GLASS TRANSITION AND WATER IN BIOPOLYMER NETWORKS		
plasticisation with water and polyols affects functional properties[8, 9, 11]	glass transition theory[5]	oxygen permeability cannot be explained by Tg[9], films are semi-crystalline[12]
T_g observed at high and low temperatures[11, 13, 14]	phase separation theory – plasticiser-rich and polymer-rich phases[14]	the amount and dimensions of separated phase are not known
average volume of the voids between polymers increases gradually with increasing water content[15]	theory of glassy state swelling in carbohydrates, free volume not dependent on molecular weight[16]	more random packing of glucose syrup in comparison to maltose has been suggested based on jump distances[17]
properties of water in association with biopolymers differ from those of bulk water	theory of tightly bound, bound and weakly bound and bulk water[18]	time-scale of interactions is short and their strength is not easy to measure[19]

[1]Karel, 1986; [2]Labuza et al.,1970; [3]Schmidt, 2004; [4]Orlien et al., 2006; [5]Slade & Levine, 1991; [6]Parker & Ring, 1995; [7]Tromp et al., 1997; [8]Stading et al., 2001; [9]Forssell et al., 2002; [10]Whitcombe et al., 2005; [11]Myllärinen et al., 2002b; [12]Myllärinen et al., 2002a; [13]Moates et al., 2001; [14]Lourdin et al., 1998; [15]Kilburn et al., 2004; [16]Kilburn et al., 2005; [17]Tromp et al., 1997;[18] common in the past literature; [19] Lillford, 1988.

Water affects biopolymer matrices by several mechanisms: as a structural element, through the rheological properties and by facilitating the transfer of small molecules. The thesis was based on the following hypothesis derived from the literature:

- Glycerol and water are known plasticisers for starch films. They decrease glass transition temperature and increase oxygen permeation. At the same time their presence increases inhomogeneity by increasing crystallinity and, at high concentration, phase separation. Oxygen transfer may be slower in the crystalline domains. This would indicate that either the properties of the amorphous domains, the properties of the separated glycerol-rich domains and/or the extent of crystallinity may be responsible for oxygen permeability of the film.
- Oxygen transfer is a rate-controlling factor in non-enzymatic lipid oxidation in glassy food matrices. Both diffusivity and solubility may affect the transfer rate. Both of these probably depend on matrix structure and properties, which depend strongly on water activity and temperature in hydrophilic biopolymers.

The overall aim was to study the relationship between the consequences of water-induced plasticisation in biopolymer matrices: increased molecular mobility and more efficient transfer of small molecules, as demonstrated by changes in oxidative stability and loss of volatiles (Figure 4). The emphasis was on systems in which differences in molecular mobility were due to differences in water activity, but the effect of temperature was also studied where appropriate.

1. The aim of the amylose film study was to evaluate how the mobility of amorphous amylose increases with plasticisation by glycerol and water and to consider the relationship between chain mobility and oxygen permeation through the film matrix (determined previously by Forssell et al., 2002).
2. The aim of the oxidation studies was to determine to what extent the stability of matrix-embedded lipids is controlled by water-induced plasticisation of matrix forming carbohydrates and proteins. Carbohydrate and protein matrices were studied separately to determine whether the water-related factors leading to stability or instability differ between the two. Release of volatile compounds embedded in carbohydrate matrices was studied during heating and storage in order to gain information about matrix permeation to larger hydrophobic compounds.

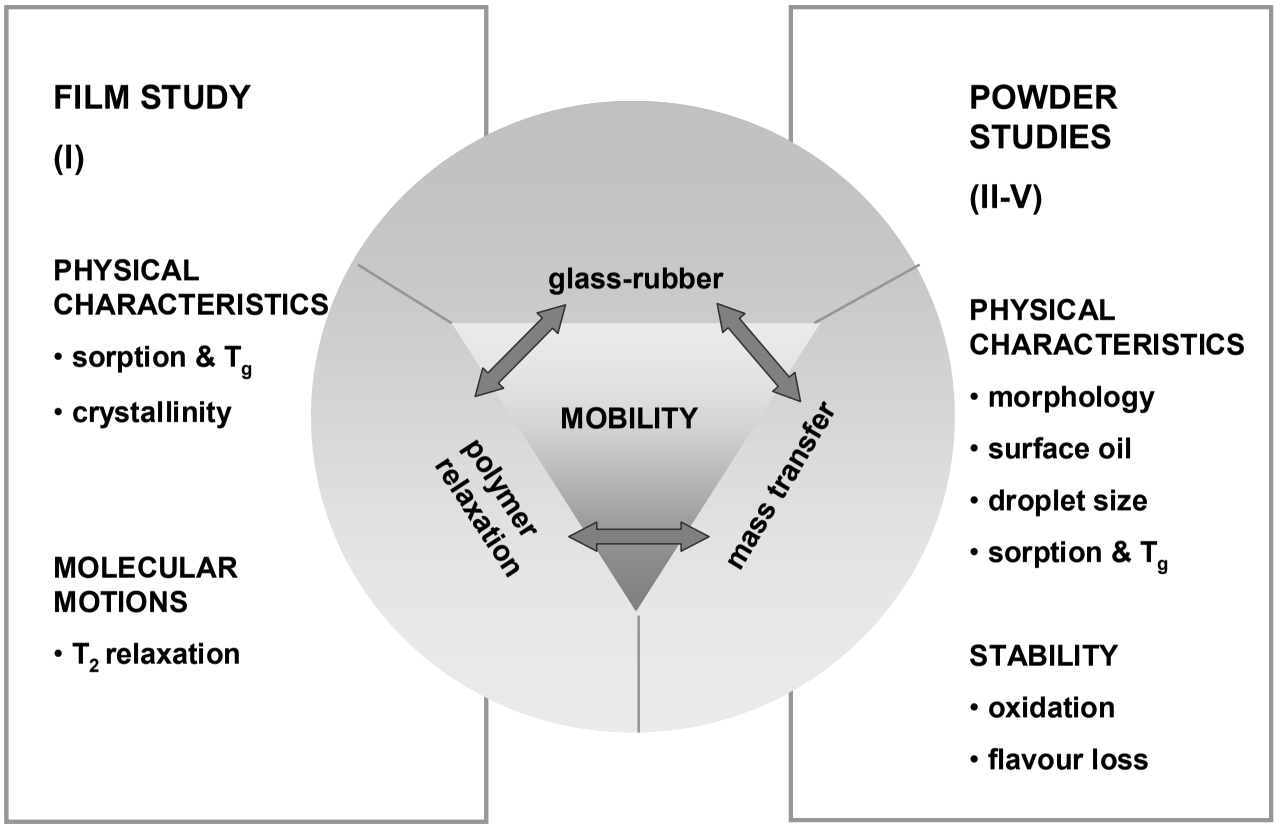

Figure 4. An overview of the study.

2. Materials and methods

The materials section focuses on the choice and composition of the materials used for *sample preparation*. Other materials and all the methods are described in detail in the original publications I–V. The summary of the methods focuses on introducing the principle and explaining the reasons for applying each specific method. These sections do not cover all the data presented in the original publications, but are limited to those discussed in the following sections.

2.1 Materials

The thesis is based on applied research. Therefore, the choice of materials demonstrates the diverse applications of biopolymers in the field where aqueous processing, non-toxicity and edibility are the key benefits, leaving the critical challenges in water-induced instability and feasibility. For flavour and oil carrier materials a high solid content in drying is needed due to high energy cost of water evaporation. Concentrated solutions can only be obtained by low-molecular-weight compounds with high water solubility. The material requirements for controlled release applications are somewhat different, as insolubility of the matrix is only one of the basic requirements. Whey proteins are potential for ingredient applications as a side-stream from cheese manufacturing. A potential material was chosen for each application. Table 3 presents a summary of the materials and the samples prepared from them.

As a consequence of these choices, there are certainly limitations in integrated interpretation of the results. However, each of these studies was aimed at filling a gap in a field where much has already been done and learned. Therefore, the results are mostly discussed in this context, giving less attention to the conclusions drawn concerning the material as a whole.

Table 3. Materials of the study and the samples prepared from those.

SAMPLE	MATERIALS	
	POLYMER	DISPERSED COMPONENT
POWDER HiCap-SBO 10 to 40% lipid HiCap-CAO 12% lipid MD-GA-SBO 10 to 40% lipid MD-GA-CAO 12% lipid WPI-FSO 0 and 40% lipid	– modified starch: octenyl succinate starch hydrolysate (HiCap)	– sea buckthorn oil: pulp and kernel oils (SBO) – caraway extract: volatiles 90 %, triglyceride 10 % (CAO)
	– hydrolysed starch: maltodextrin (MD) – gum arabic (GA) GA:MD (1:2, 1:7)	
	– whey protein isolate: free of lactose (WPI)	– flaxseed oil (FSO)
FILM AM-GLY 0 to 30% glycerol	– potato amylose (AM)	– glycerol (GLY)

2.1.1 Amylose

Amylose was a commercial (Sigma-Aldrich) potato starch isolate prepared by butanol complexation. The blue value of amylose was 0.5, as determined by the manufacturer for a crude measure of average chain length. Residual butanol content was below 4.7% as declared by the manufacturer.

Starch films have been successfully prepared both from granular starches including normal (ca. 20–25% amylose; Gaudin et al., 1999), waxy (amylopectin; Myllärinen et al., 2002a; Rindlav-Westling et al., 1998; Stading et al., 2001) and high amylose (up to 70% amylose; Bader & Göritz, 1994) starch, as well as from

purified amylose (Rindlav-Westling et al., 1998; Stading et al., 2001; Myllärinen et al., 2002a). Our previous studies have focused on the differences in functional properties of films made of purified potato amylose and granular amylopectin (waxy maize) (Forssell et al., 2002; Myllärinen et al., 2002a; 2002b). The present study focused on amylose, as its films are mechanically stronger (Myllärinen et al., 2002b) and more resistant to hydrolysis by acids and pancreatic α-amylase (Myllärinen et al., 2002a). The potential of amylose for controlled-release applications has been suggested, when used in combination with an insoluble polymer which controls the swelling of amylose in aqueous conditions (Milojevic, Newton, Cummings, Gibson, Botham, Ring, Stockham & Allwood, 1996a; 1996b).

2.1.2 Carriers for spray-drying

Maltodextrin was a commercial (Cerestar) corn starch hydrolysate with a dextrose equivalent of 18.5. Dextrose equivalent (DE) is a measure of degree of hydrolysis, i.e. mean percentage of cleaved bonds in native starch. Thus, DE 0 stands for native starch and DE 100 for glucose. A high DE maltodextrin was used to obtain a high solid content in the feed emulsion. Maltodextrin was used with gum Arabic (Sigma-Aldrich) as an emulsifier. Gum Arabic is a complex macromolecule (Figure 5a), in which separate hydrophilic carbohydrate blocks are attached to the backbone chain of hydrophobic protein (Dickinson, 2003). Once adsorbed to the interface, a film with high surface shear viscosity is formed. Gum Arabic is a traditional flavour carrier, but its relatively high price has led the industry for to search for alternatives. One of these is octenyl succinate starch derivative (Figure 5b), which at 0.02 degree of substitution (maximum) is permitted for food use (Trubiano & Lacourse, 1988). This emulsifying starch HiCap 100 (National Starch & Chemical) was chosen to be studied with the maltodextrin-gum Arabic matrix. The DE of HiCap was between 32 and 37, as declared by the manufacturer. Thus, the chosen materials differed considerably in their molecular weight distribution, which was likely to show in their physical stability with respect to relative humidity. Another difference in these matrices was in the composition of the surface active agents stabilising the oil-water interface in the emulsion.

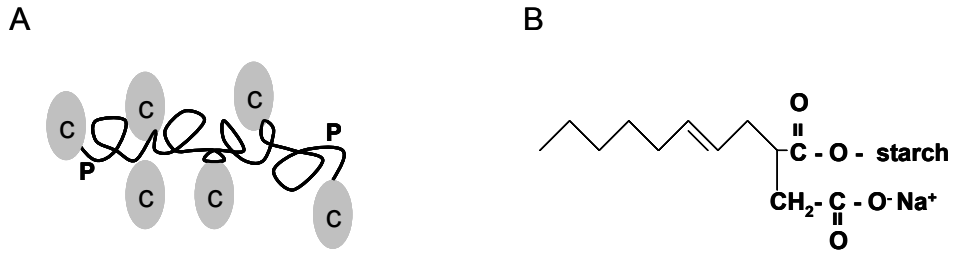

Figure 5. a) A schematic represention of the functionally active compound in gum Arabic (redrawn from Dickinson, 2003). Hydrophobic protein (P: ca. 2% in gum) is covalently linked to hydrophilic arabinogalactan units (C). b) The chemical structure of octenyl succinate starch derivative (redrawn after Trubiano & Lacourse, 1988).

Spray-drying of low molecular weight carbohydrates into amorphous solids has a major drawback in humid storage. Extensive caking and dissolution will occur in rubbery state. Increasing molecular weight increases the glass transition temperature and thus the amount of sorbed water needed to plasticise the matrix for caking. High molecular weight starch-derived compounds are poorly soluble in water and form highly viscous solutions at low concentrations. Thus, there was a need for a water-soluble, low-viscosity, higher molecular weight compound without contamination from low molecular weight species. Whey protein is a side-stream from cheese manufacturing. A commercial isolate (Arla Foods Ingredients) was chosen, which contained less than 0.5% lactose. The traces of lactose in the sample were not sufficient to interfere with the DSC-thermograms (Figure 3 in Publication IV). The main constituents of this protein mixture are β-lactoglobulin and α-lactalbumin, which are both globular proteins (Fox & McSweeney, 1998). The degree of denaturation of these proteins was assumed to be low on the basis of their denaturation enthalpy (7 J/g of dry protein in total) as determined by DSC using membrane-filtered (cut-off 3 kDa) native whey permeate as a reference (9 J/g of dry protein in total). Due to the low degree of denaturation of these proteins, they were highly soluble in water. Possibly due to their compact structures in solution, whey proteins could be dissolved at 10% without major increase in viscosity.

2.1.3 Matrix-embedded lipids

Matrix-embedded lipids were studied with respect to their stability against oil oxidation and volatiles release. Oils from two different origins were used in the oxidation studies. Sea buckthorn seed oil (Aromtech) was chosen due to the industrial interest in the oil of this health-promoting berry. The oil was a supercritical CO_2 extract. Flaxseed oil (Elixi oil) was prepared by cold-pressing in the absence of oxygen and it was chosen for its high content of linolenic acid. Triglycerides instead of pure fatty acids were used, as they were considered to represent the real system when incorporating polyunsaturated fatty acids in foods. In comparison to free fatty acids, triglyceride oils are non-polar and as such insoluble in the aqueous phase. In order to perform some comparison between the results of carbohydrate and whey protein matrices obtained with different oils, the oxidation of bulk oils was also followed. The fatty acid composition of the oils is presented in Table 4, which shows that although ca. 90% of both oils was composed of oleic (18:1), linoleic (18:2) and linolenic (18:3) acids, flaxseed oil was presumed to be more susceptible to oxidation due to its high content of linolenic acid.

Table 4. Fatty acid composition of the oils used in the oxidation studies.

OIL	**FATTY ACID COMPOSITION (%)**						
	16:0	*16:1*	*18:0*	*18:1*	*18:2*	*18:3*	*20:0*
SEA BUCKTHORN SEED OIL	7.4	1.5	2.5	20.7	36.2	31.3	0.4
FLAXSEED OIL	4	-	3	12	15	66	-

Volatiles release was studied with caraway oil (Flavex Naturextrakte GmbH), which had been produced by extraction with supercritical CO_2 from caraway fruit. This oil was chosen as a simple model for essential oil: 10% of the extract was composed of triglycerides, and the rest, 90% was essential oil consisting mainly of two volatiles, limonene (37%) and carvone (59%).

2.2 Sample preparation

2.2.1 Preparation of amylose films

Amylose was solubilised with glycerol (0, 10, 20 and 30% of dry weight) by heating in a pressure chamber to 140°C. In this way, a clear solution was obtained. After cooling to 90°C, the 2% solution was poured into pre-heated Teflon moulds and dried at 70°C at RH 50%. Film formation occurred typically in 4 hours. The films were then ground under liquid nitrogen to avoid heat damage. Amylose films prepared by this procedure are semi-crystalline, showing B-type crystallinity in x-ray diffraction diagrams (Myllärinen et al., 2002a).

2.2.2 Preparation of spray-dried powders

The powders with dispersed lipid phase (compositions in Table 2) were prepared by first dissolving the carrier polymer in water. Maltodextrin (DE 18.5) and HiCap were highly soluble and 40% w/w solutions could be prepared. Gum arabic and whey protein isolate (WPI) were used at 20% and 10% w/w solutions, respectively to limit the effect of viscosity in further processing. Carbohydrate carriers were homogenised with the lipid using a blender bar. Whey protein isolate was pre-homogenised with oil with a blender bar, and the droplet size was decreased further using a high-pressure homogeniser. The lipid content in carbohydrate carriers varied from 10 to 40% of the dry weight of the emulsion. The lipid content in protein emulsion was 40% of the dry weight. The fresh emulsions were spray-dried in a laboratory spray-dryer (0.8 m^3 chamber) with a rotary atomiser (for carbohydrates) or a two-fluid co-current nozzle (for WPI). The inlet and outlet temperatures of the dryer were 200°C and 80°C for carbohydrates and 180°C and 90°C for WPI, respectively. The differences in the methods of preparation may have influenced the properties of the dry powder particles, such as morphology (atomisation, drying temperature) and droplet size distribution (homogenisation, atomisation). These properties were characterised in order to distinguish the effect of matrix composition in these studies.

2.3 Analytical methods

2.3.1 ^{1}H NMR relaxometry

Proton relaxation behaviour was studied in amylose films with the aim of showing how plasticisers affect the mobility of amylose in solid state. There are number of thermal and mechanical techniques for detecting glass transition, in which the segmental motions of the polymer are activated. However, NMR was used to obtain direct information on the strength of dipolar interactions in the system. As a consequence of a decrease in these interactions, proton mobility increases. The spin-spin relaxation of rigid amylose protons occurs rapidly, and it can be measured as free induction decay (FID).

^{1}H NMR relaxometry is based on the orientations which ^{1}H protons adopt in a magnetic field (Schmidt, 2004). They exist either parallel (lower energy) or anti-parallel to the field. The equilibrium between the two states is determined by the Boltzmann distribution, and there is an excess of lower energy spins. The spins are precessing randomly with no phase coherence. Net magnetization along the z-axis is at maximum in equilibrium (Figure 6). When a resonance frequency pulse is applied for a given time (90^{o} pulse), the populations of parallel and anti-parallel positions are equalised and the spins precess in phase. The magnetization in the xy-plane is at maximum value (resonance). The spin-spin (T_2) relaxation process is a loss of phase coherence of the spins, whereas the spin-lattice relaxation (T_1) restores the equilibrium distribution of spins to the parallel and anti-parallel positions. The spin-spin relaxation after a 90^{o} pulse is called free induction decay (FID). By resolving the FID into multiple exponentials, components with different relaxation times can be distinguished.

Proton relaxation behaviour was determined using a Maran NMR (nuclear magnetic resonance) spectrometer operating at a resonance frequency of 23 MHz. Spin-spin relaxation parameters were obtained as follows: the second moment (M_2) from free induction decays (FID) after a 90^{o} pulse for low water system relaxations (amylose films). The relaxation parameters were calculated based on the equations presented in the original articles by fitting the data using MS Excel© Solver (in the case of amylose films).

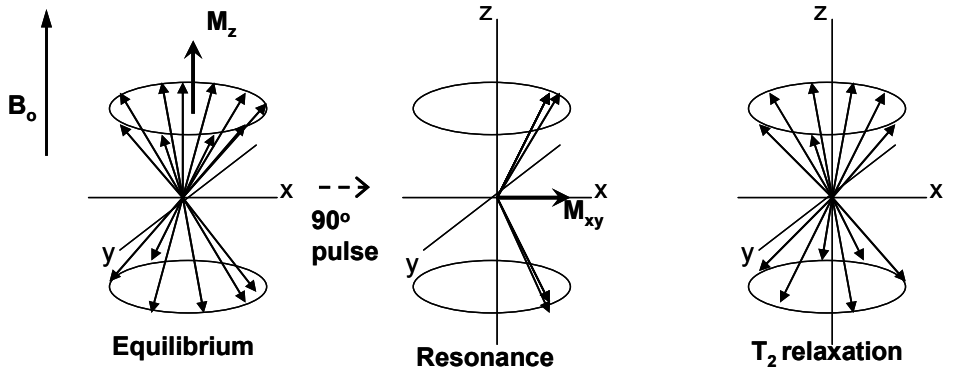

Figure 6. A schematic representation of spin-spin (T_2) relaxation in a magnetic field after a 90°pulse (modified from Schmidt (2004)).

The interpretation of relaxation measurements is complicated by the diversity of hydrogen protons in biomaterial. The carbon-bound protons are non-exchangeable, but protons from hydroxyl groups are in chemical exchange with the water molecules in the system. Therefore the observed relaxation times are often average values from exchanging populations. Furthermore, other processes such as diffusive exchange occur simultaneously, and their contribution must be considered in the time-scale of the measurement.

2.3.2 Microscopy

Scanning electron microscopy was used to obtain images beyond the resolution of conventional light or laser microscopy. Very little sample preparation is needed for this technique, which may exclude some artefacts. On the other hand, labels and dyes used in conventional microscopy allow the identification of specific compounds, which could not be achieved in this context. Scanning electron microscopy has been used as a high resolution technique to determine the morphology and internal structure of spray-dried particles with a high excitation voltage. Apart from this information, one step further was taken in detecting the structural changes in protein matrix during storage. To avoid destruction of the sample under the beam, a low excitation voltage was used.

Scanning electron microscopy is based on excitation of the sample with a beam of electrons, which interact with the sample material (Goldstein, Newbury, Echlin, Joy, Fiori & Lifshin, 1981). Subsequent emission of secondary electrons from specimen atoms is detected, from which the image is created. Conventional SEM requires samples to be imaged under vacuum, because a gas atmosphere rapidly spreads and attenuates electron beams (Danilatos, 1988). Environmental SEM (ESEM) allows samples to be observed in low-pressure gaseous environments and high relative humidity without coating.

The carbohydrate and protein-based powder particles were imaged with ESEM (Electroscan 2020) and SEM (Leo DSM982 Gemini), respectively. For cross-section images, particles were either crushed or an adhesive tape was attached on top of the powder on the stub which was then quickly ripped off. The samples were sputtered with Pt for SEM and imaged under high vacuum. With the low excitation voltage (0.7–1.0 kV) used in SEM, high magnifications (x 20 000) revealing the internal structure of the cross-section surfaces could be obtained.

2.3.3 Droplet size distribution

Droplet size analysis was performed to identify major differences in the surface area of embedded lipids in various matrices. The physical stability of the emulsion in drying was also determined by this method. Laser diffraction method was chosen as a rapid detection method for a wide distribution of sizes (40 nm – 2 mm), with the output of numerical values allowing the aimed comparisons. Two limitations of this technique should be kept in mind: the refractive indices of the specific system may not be known and all the samples are not stable to dilution. The lack of exact refractive indices of the emulsion droplets affected the correctness of the absolute values but was not considered major drawback due to the purpose of this measurement explained above. No indication of instability due to dilution was found in the two consequtive runs performed for each injected sample.

Particle size distribution can be analysed by monitoring the diffraction pattern of a monochromatic light directed through a flow of particles to be analysed (McClements, 1999). A Fourier lens is used to obtain similar diffraction from particles of the same size, shape and refractive indices irrespectively of their

position in the cuvette. Assuming a spherical shape of the particles, the diffraction pattern can be resolved to particle sizes. For this, an optical model defining the refractive indices of the particles and of the medium is needed. Laser diffraction can only be applied to particles of a certain minimum size. In the system of the present study, particles below 400 nm were detected by polarized intensity differential scattering of monochromatic light at three wavelengths.

Emulsion droplet size distributions were measured with a Coulter LS 230 instrument using a small volume module. Small droplets (40–400 nm) were determined by polarized intensity differential scattering of monochromatic light at three wavelengths (450, 600 and 900 nm) and large droplets (400 nm – 2 mm) by laser diffraction (750 nm). The geometric volume distributions were calculated on the basis of refractive indices of water (1.33) and particles (1.6) assuming a spherical shape.

2.3.4 Solvent-extracted 'surface oil'

A variety of extraction methods have been used in the past literature to quantify the amount of oil on particle surfaces in direct contact with air. This was also the aim in the present study, as it was initially assumed that the solvent-extractable oil would represent the oil fraction on particle surface, which however is not the case (Drusch & Berg, 2008). The results were included to demonstrate the non-specific nature of such extraction methods, which will be discussed in the following sections in more detail. The method used in this study was modified from Anker & Reineccius (1988). A dry sample was extracted in a filter for 3 hours in a Soxhlet tube with petroleum ether. Solvent phase was taken into a tared flask and petroleum ether was evaporated. Extracted oil was determined gravimetrically.

2.3.5 Oil oxidation

Lipid oxidation is a complex phenomenon, which can be analysed either by the reaction products or by quantitating the intact fatty acids (Karel, 1986). Generally, quantitation of reaction products is more sensitive (Fritsch, 1994).

The products can be determined from different stages of the reaction: the radical species initiating the reaction, conjugated dienes from the next step, peroxide value involving oxygen consumption (Figure 7) or volatiles, which already at low concentrations may make the product flavour inacceptable. For this study, analysis of peroxide value was chosen, as lipid oxidation rate has been linked with the availability of oxygen as controlled by oxygen transfer rate (Orlien et al., 2006).

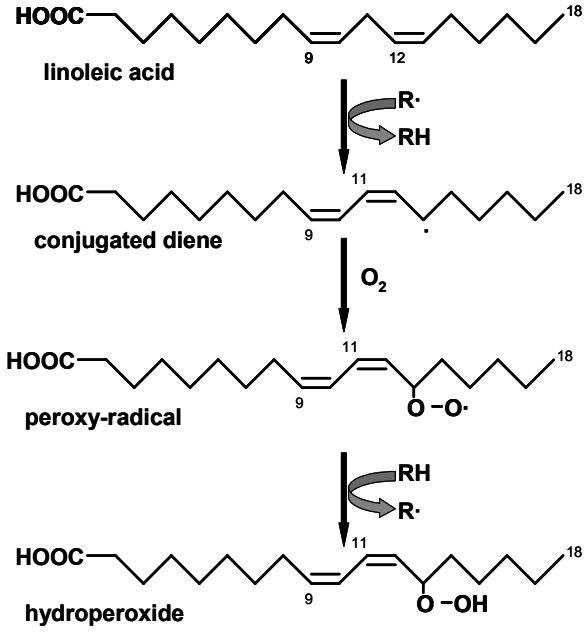

Figure 7. Hydroperoxide formation in linoleic acid (modified from Wheatley (2000)).

For peroxide value analysis of matrix-embedded oil, there are a few points to consider. Firstly, peroxides are not only formed but also simultaneously decompose in the system (Fritsch, 1994). Peroxide formation is favoured over decomposition in most domestic food oils. However, a possible effect of water activity on hydroperoxide decomposition rate cannot be ruled out. Generally, the decomposition of hydroperoxides is observed in the peroxide value during the late stages of oxidation, when formation of these compounds is slow. Another important factor is related to obtaining a representative sample of a matrix-embedded lipid with minimum processing. In the present study, only nearly

quantitative yields (ca. 90%) were considered representative. In this respect, gum Arabic was an extremely challenging component in the matrix, and extreme conditions had to be applied. Physical changes in matrices during storage also complicated the analysis of rubbery state samples.

HiCap and MD/GA samples. A weighed sample containing ca. 2 g of oil was hydrolysed with 8 M HCl in a boiling water bath in the presence of ethanol. Cooled sample was extracted in a separation funnel with a mixture of ethanol and ether:petroleum ether (1:1). Solvent was taken into a tared flask and water phase was re-extracted. The combined solvent phases were evaporated. The peroxide value of the extracted oil was determined based on the quantitative reaction of hydroperoxides with potassium iodide. The iodine produced was titrated against sodium thiosulphate in the presence of starch indicator (Wheatley, 2000).

WPI samples. A weighed sample containing ca. 0.2 g oil was suspended in water and shaken for 30 minutes to ensure dissolution of the powder. Oil was extracted with an iso-octane:isopropanol (2:1) mixture and the phases were separated by centrifugation as described by Kellerby et al. (2006). Longer times were applied in centrifugation if necessary. Peroxide value was determined spectrophotometrically according to IDF standard 74A:1991 (Anonymous, 1991) based on the reaction between hydroperoxide and iron(II)chloride, in which iron(II) is oxidised to iron(III), which forms a coloured complex with ammonium thiocyanate.

2.3.6 Release of volatiles

Analysis of volatiles release from powders aimed at understanding how the matrix controls vaporisation of embedded compounds. For this purpose, time and temperature-dependence of the release was studied. Two complementary techniques were chosen for the analysis. Gas phase FT-IR (Fourier transform infrared) spectrometry was used as a sensitive method to indicate the conditions in which vaporisation occurred continuously throughout the storage period and the temperature at which volatilisation becomes more pronounced. Headspace gas chromatography was used to obtain quantitative information on the release, which could not be obtained by gas phase FT-IR.

An infrared absorption spectrum is obtained by allowing an infrared beam to pass through the sample and by determining the fraction absorbed at each frequency of a particular range. The frequency at which a band appears in the spectrum is equal to the frequency of one of the vibrations occurring in the sample molecules. This frequency correlates with the bond strength and the atomic masses, allowing the sample molecules to be identified on the basis of a previously constructed library. The method for gas phase FT-IR analysis has been described in detail earlier (Ahro, Hakala, Kauppinen & Kallio, 2001). Briefly, the vacuumed sample was equilibrated at a constant temperature, after which the volatiles were allowed to flow into the sample cell of the spectrometer. The concentration of the volatile (mg/m^3) in the sample cell was determined and it was taken as a measure of the leakage from the powder. FT-IR measurement is similar to water activity measurement in the sense that it is only meaningful if the content of compounds in the solid sample is not affected by vaporisation to gas phase. The result therefore indicates how the matrix affects the vapour pressure of the compound. Total contents of carvone and limonene in powders were determined by headspace gas chromatography after overnight extraction in methanol using n-butanol as an internal standard in the storage test.

2.3.7 Water content and sorption isotherms

The equilibrium relation between water and carbohydrate matrices was determined as sorption isotherms. The aim was to relate the plasticisation of these matrices to water sorption. Residual water contents were determined by Karl Fischer titration. This method was chosen over oven-drying due to the possible instability of biomaterials at the high temperatures needed for quantitative evaporation of water. The residual water was extracted in methanol for 2 h with continuous stirring. The titrant was calibrated with water, and sodium tartrate dihydrate was used as a calibration control. The amount of solvent injected into the titrator was determined gravimetrically. Samples with determined water contents were weighed (100 mg) and placed in humidity chambers with different supersaturated salt solutions: LiCl (RH 11%), $MgCl_2$ (RH 33%), $Mg(NO_3)_2$ (RH 54%), NaCl (RH 75%) and $(NH_4)_2SO_4$ (RH 81%) and re-weighed after equilibration for 1 week, after which water sorption isotherms were determined at room temperature (23 ± 2^oC). The values of relative humidity are also used in connection with the storage test samples. Relative humidity is considered to give the most

accurate definition for these samples, as water activities were not determined. The values of relative humidity could not be transformed to water activity values, as equilibrium state could not be assumed throughout the whole storage period.

2.3.8 Glass transition temperature

One of the most important aims of the study was to relate water-induced plasticisation of biopolymer matrices to the oxidation of matrix-embedded lipids. In order to explore the stability of lipids with respect to glass-rubber transition in the matrix, the carbohydrate matrices were equilibrated under supersaturated salt solutions (see 2.3.7) and analysed by differential scanning calorimetry (DSC). Glass transition is detected as a baseline shift reflecting a change in heat capacity. Other methods for determination of glass transition temperature exist (see 1.2.3), but especially for samples in powder form, DSC was a practical approach.

The baseline shifts observed for carbohydrate matrices were considerably more pronounced as compared with those obtained earlier for amylose films (Myllärinen et al., 2002b). This was partly due to higher resolution of the sensor used in the present study, but may have been linked also with molecular weight distribution of the compounds studied. In the present amylose film study, T_g dependence on water content was calculated on the basis of the composition of the films, as explained in more detail in Publication I. T_g values were calculated assuming a homogeneous system. The crystalline fraction of amylose could be estimated based on Myllärinen et al. (2002a). However, the exact composition of the amorphous fraction could not be known (see 3.1). Due to this uncertainty, the validity of these values was evaluated by comparing them with the determined values. A satisfactory agreement was found between the calculated and measured values.

Glass transition temperatures were determined with a Mettler DSC820 differential scanning calorimeter equipped with a liquid nitrogen cooling system. Samples of 10 mg were weighed in aluminium pans, which were then sealed. The heating and cooling rate was 10^{o}C/min. Glass transition temperature was taken as a midpoint of the baseline shift in the second heating scan.

3. Results

3.1 Amylose films plasticised with glycerol (Publication I)

The amylose films with differing glycerol contents were all translucent, despite the B-type crystal pattern observed. In previous results (Myllärinen et al., 2002a), the degree of crystallinity was determined for amylose films produced by an identical method. In dry state, the crystallinity of fresh films varied between 6 and 14%, depending on glycerol concentration. With equilibration at more humid conditions, RH 54% and RH 91%, more crystallinity was found in amylose films (10% glycerol), 23% and 32%, respectively. Ageing of the films did not change the degree of crystallinity on a time scale relevant to the present study. In an earlier work (Myllärinen et al., 2002b) the glass transition temperatures for these films were also determined. With increasing amounts of glycerol and water, DSC thermograms showed two transition zones, at high and low temperatures, indicating partial phase separation.

Thus, three kinds of domains can be identified in the system: crystalline amylose, amorphous amylose mixed with plasticiser and glycerol-rich domains. The present study focuses on the plasticisation of the amorphous domains. In order to link the results on mobility with the glass-rubber transition of the system, the glass transition temperatures were calculated on the basis of the composition (see 2.3.8). The calculation was performed to overcome the deficiencies in the previously measured values. The extent of crystalline amylose can be evaluated from the previous study. Van den Berg (1981) concluded that the uptake of water into native starch crystals is without clear discontinuities up to around 20% (w/w). Thus, the amount of hydration water is also a variable in the *crystalline* domain (van den Berg, 1981). Above this 20% (w/w) hydration level, the amorphous fraction is responsible for water vapour sorption.

In the present study, we could therefore assume that the relative amount of water available for amylose plasticisation (below RH 94%) did not depend on the extent of crystallinity in the binary system (without glycerol). The presence of glycerol increases water sorption in the humid end (Myllärinen et al., 2002b), where 20% is reached at around RH 80%, giving an upper limit for water vapour sorption of the crystalline domain. Thus, at the highest relative humidity of the

present study (RH 94%) more water may have been associated with the amorphous fraction than was indicated in the assumed homogeneous distribution. The calculated T_g at high humidity would therefore be an overestimate. Another complication in the ternary system is the distribution of glycerol. Probably, glycerol is excluded from the crystalline domains, which would leave more in the amorphous domains. With 30% crystallinity, the amount of glycerol in the dry amorphous phase would be 38% instead of 30% and 14% instead of 10%. This would cause a 5–10°C decrease in the calculated T_g values. On the other hand, only part of the glycerol may interact with the amorphous parts due to phase separation. Therefore, the exact composition of the amorphous phase is not known. As a satisfactory correlation was found between the measured and calculated T_g values (data shown in Publication I), the calculated T_g values of the original Publication I were considered accurate enough for the aims of this study. In the subsequent results, all references to the sample composition should be understood as the amount of constituents in the film (as presented in Table 3) and not in the amorphous phase.

The free induction decays of amylose-glycerol-water systems consisted of a rapidly decaying rigid component and a slowly decaying component due to mobile protons (Figure 8). In between, centred at 50 μs, a strong beat pattern was observed. This pattern reflects strong dipolar interactions, which have previously been described in glassy carbohydrate systems (van den Dries, van Dusschoten & Hemminga, 1998; Kumagai, MacNaughtan, Farhat & Mitchell, 2002). The beat became less pronounced with increasing water and glycerol contents, but it did not disappear completely even at the highest plasticisation levels. This indicated that a fraction of amylose, most probably the crystalline fraction, was not significantly plasticised by either water or glycerol.

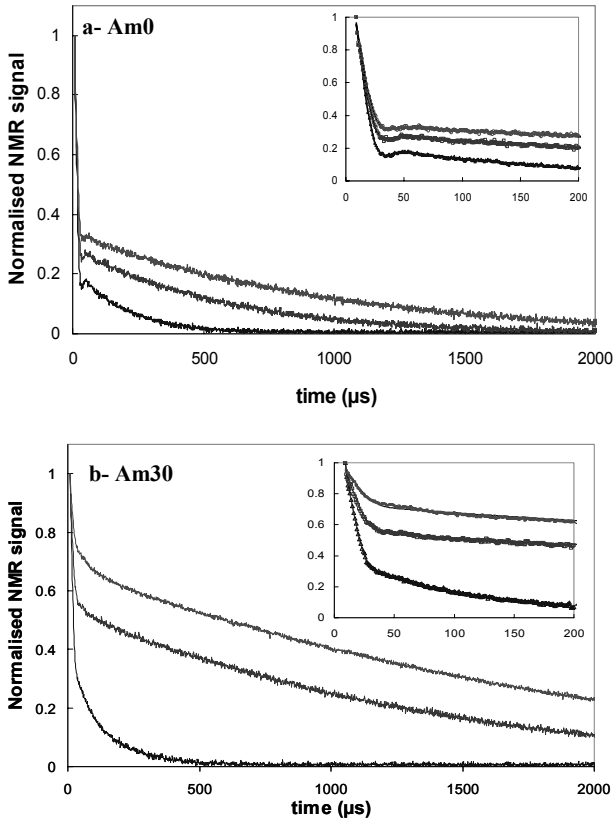

Figure 8. Typical FIDs of amylose films without glycerol Am 0 (a) and amylose films with 30% glycerol Am30 (b) equilibrated at RH 12% (bottom curve), RH 75% (middle curve) and RH 94% (top curve).

To evaluate the correlation between glass-rubber transition and the mobility of amylose, the free induction decays were fitted according to van den Dries et al. (1998) and a rigid lattice second moment (M_2) was calculated. M_2 is a measure of the strength of dipolar interactions: an increase in molecular mobility leads to decrease of M_2, as dipolar interactions are increasingly averaged out by rotational mobility. The M_2 was plotted as a function of temperature difference between measurement temperature and glass transition temperature (Figure 9). A master curve was found for amylose plasticisation in the glassy state, *irrespective of the composition of glycerol and water*. A steeper decrease in the second moment was found at the transition zone ($T-T_g \sim 0^oC$), most probably reflecting increased motional freedom in amylose. In the rubbery state, the

glycerol content affected the values of M_2. Thus, in addition to decreasing the glass transition temperature, increase in glycerol content increased the mobility of amylose in the rubbery state, which is somewhat surprising considering the phase separation. Van den Dries et al. (1998) determined M_2 values for maltose glasses, and reported an increased mobility in hydroxyl groups of maltose with increasing temperature at the glass transition region.

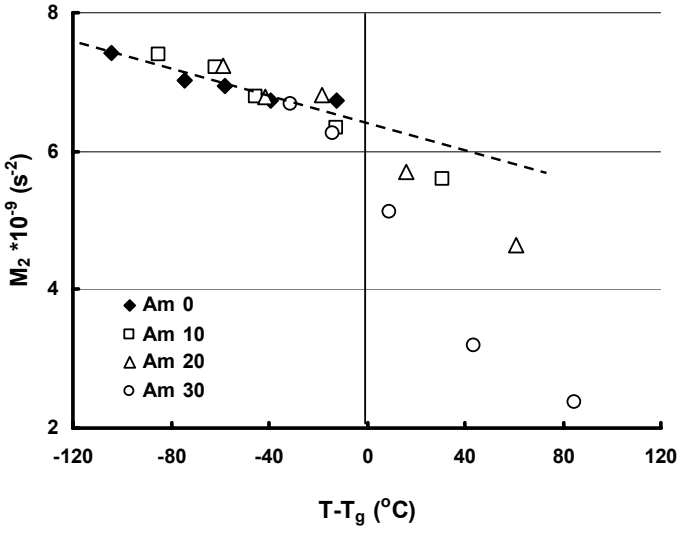

Figure 9. The second moment M_2 of the rigid component in amylose films at different glycerol contents (Am 0, 10, 20 and 30 representing 0%, 10%, 20% and 30% glycerol contents of the films per dry weight, respectively) as a function of temperature difference between measurement and glass transition temperature. Water content of each sample increases in the direction of the x-axis.

3.2 Powders with a dispersed lipid phase (Publications II–V)

3.2.1 Distribution of oil in powder particles

The distribution of oil in spray-dried particles was studied by determining oil droplet size before and after drying, by imaging the cross-sections of powder particles and by determining solvent-extractable oil from powders with carbohydrate matrices. In the fresh emulsions, the droplet sizes differed between the samples

(Figure 10). As the oil emulsions from octenyl succinate starch derivative (HiCap) and maltodextrin-gum Arabic (MD-GA) were prepared with the same homogenisation system, the observed difference was due to wall material. Starch hydrolysate derivatised with octenyl succinate has emulsifying capacity as such (Trubiano & Lacourse, 1988), but maltodextrin was mixed with gum Arabic to obtain a stable emulsion, which is a frequently used combination (Kenyon & Anderson, 1988). For an oil emulsion from whey protein isolate (WPI), a high-pressure homogeniser was used and sub-micron droplets were formed. Without high pressure, the droplet size of oil emulsions from WPI was much larger.

A small reduction in the size distribution of oil droplets in emulsion from HiCap was observed after spray-drying and re-dispersing in water. Soottitantawat et al. (Soottitantawat, Yoshii, Furuta, Ohkawara & Linko, 2003) suggested that the large droplets in the original emulsion may be sheared in atomisation. In their study, a size reduction was found for the MD/GA (3:1) emulsion at 30 000 rpm, and no further changes were detected due to drying. The opposite behaviour in the oil emulsion from MD/GA mixture of the present study might be explained by difference in the MD/GA ratios (7:1 in the present study). The speed of the atomiser wheel was 25 000 rpm in our study. The oil emulsion from WPI and the reference WPI solution were atomised with a two-fluid nozzle. The emulsion had already been subjected to high shear forces in the homogenisation step, where high pressure homogenisation was used. Some changes in the size distribution in emulsion from WPI were observed after spray-drying and re-dispersing in water. Similar observations with oil emulsions from whey protein were made by Millqvist-Fureby, Elofsson and Bergenståhl (2001), who assigned the phenomena to droplet coalescense. As the protein-oil ratio was rather high in the present study, the increased droplet size could also be explained by protein denaturation, which may induce aggregation and reduce the solubility of matrix protein. The solubility of the major whey proteins, β-lactoglobulin and α-lactalbumin, was recently determined after spray-drying at various outlet temperatures (Anandharamakrishnan, Rielly & Stapley, 2008). An increase in insoluble fraction was found above 80^{o}C for both proteins, representing approximately 5% of the total in both proteins at 90^{o}C (the outlet temperature of the present study) with 20% solids in the feed.

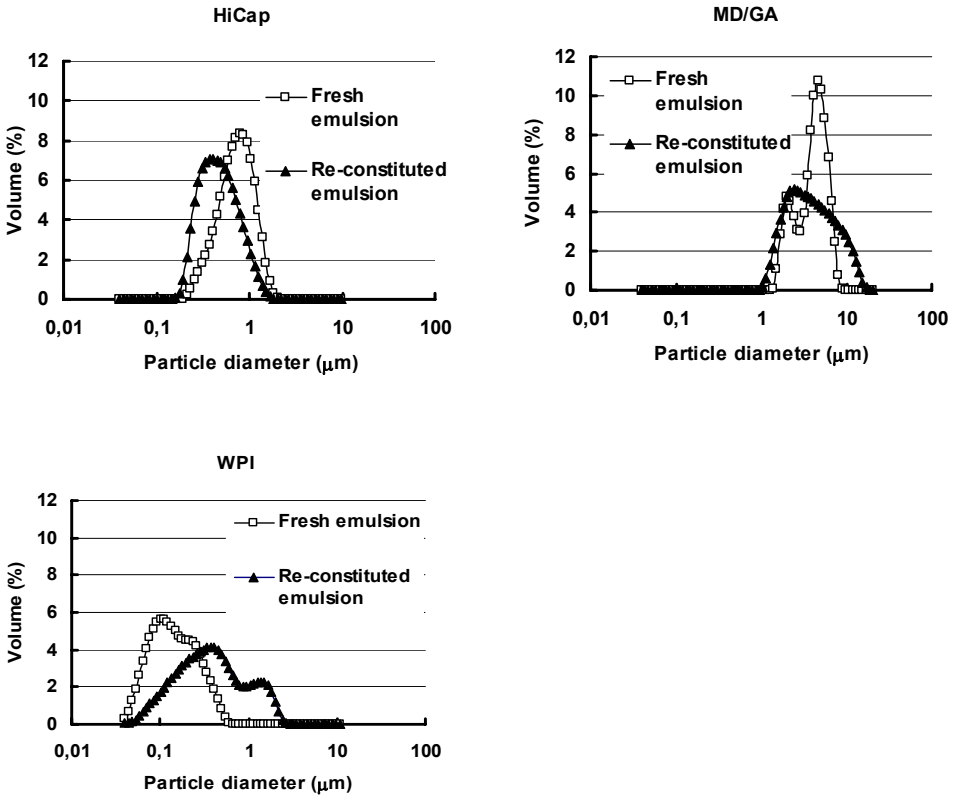

Figure 10. Size distributions of droplets from HiCap, MD-GA (7:1) and WPI emulsions before spray-drying (fresh emulsion) and after spray-drying and re-dispersing in water (re-constituted emulsion).

Particles produced by spray-drying are often hollow spheres. Formation of the central void is related to the expansion of particles during the later stages of drying (Ré, 1998). The internal structure of the powder particles was studied in detail by scanning electron microscopy. The cross-section surfaces of the particles showed clearly the size difference between the oil droplets embedded in MD-GA and HiCap matrices (Figure 11A–B: note the scale bars). The cross-section image of WPI powder with embedded oil was compared to that of a protein reference, which was dried in the same way but without the oil (Figure 11C–D). Small pores were observed in the reference, but spheres with a distribution similar to that determined by laser diffraction were found in all the samples with embedded oil. The droplet size distribution in microscopy may be easier to correlate with the number distribution (not shown) rather than with the volume distribution in which larger droplets are dominating.

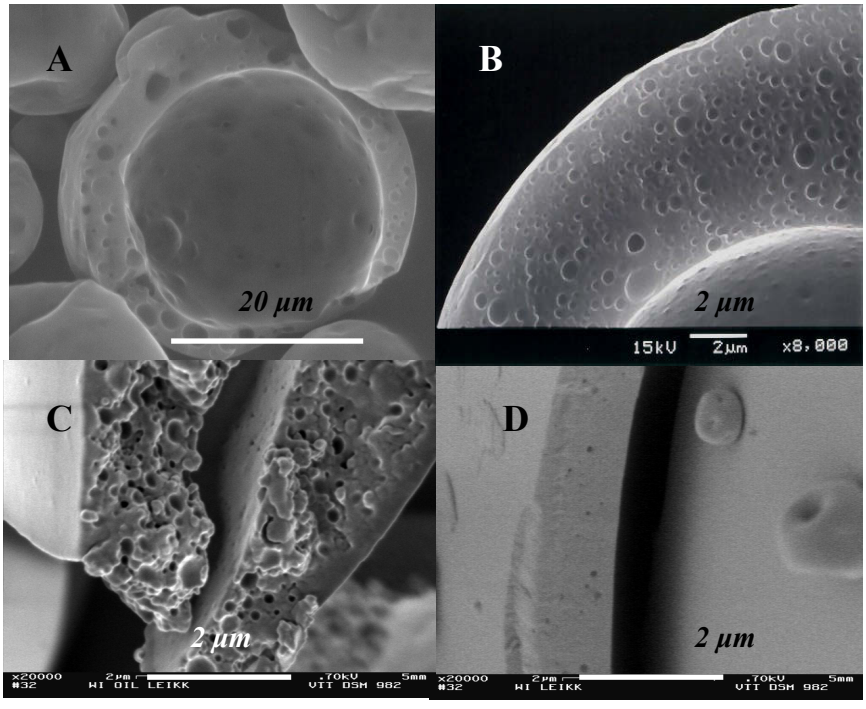

Figure 11. The cross-section images of A) MD-GA (7:1), B) HiCap and C) WPI samples all with embedded oil and D) WPI reference without the oil as imaged by ESEM (A, B) and by SEM (C, D).

The solvent extractable oil has been described as surface oil, and it has been claimed to be susceptible to oxidation (Hardas, Danviriyakul, Foley, Nawar & Chinachoti, 2002; Soottitantawat et al., 2003). The amount of oil which could be extracted by petroleum ether was determined in carbohydrate matrices with embedded oil. The extractable oil was determined as a function of total oil content in the powders (Figure 12). In HiCap powders with embedded oil, only a small amount of oil could be extracted despite the long extraction time (3 h). In MD-GA powders with embedded oil, the amount of extractable oil was significantly greater and increased with increasing oil content of the powder. It was also found (result not shown) that increase in MD-GA ratio had a major influence in increasing the amount of solvent-extractable oil. Oil embedded in pure gum Arabic matrix was distributed similarly to the oil in HiCap matrix. The results of the present study are not very comparable to those presented by Hardas et al. (2002) and Soottitantawat et al. (2003; Soottitantawat, Bigeard, Yoshii,

Furuta, Ohkawara & Linko, 2005), due to differences in the solvent and extraction times, which were very short in all these studies. Furthermore, Soottitantawat et al. (2003; 2005) studied extraction of volatile flavour in contrast to triglycerides in the present study. Despite the discrepancies between the methods, the solvent-extractable oil content of HiCap powder with embedded oil in the present study was similar to the values obtained by Soottitantawat et al. (2003; 2005). For MD-GA powder with embedded oil, the divergence in values was considerable, our values being five-fold compared to those with a similar droplet size obtained by Soottitantawat et al. (2003). Values comparable to the present study were obtained by Drusch et al. (Drusch, Serfert, Scampicchio, Schmidt-Hansberg & Schwarz, 2007), who reported 16% extractable oil for gum Arabic-glucose syrup system (Drusch, Serfert, Scampicchio, Schmidt-Hansberg & Schwarz, 2007). The authors also studied encapsulation with octenyl succinate starch derivatives, for which they reported extractable oil contents between 2 and 4%.

In the present study, the difference in the amount of extractable oil between oils embedded in HiCap and MD-GA matrices probably reflects the differences in particle morphology, the properties of the interfacial layer surrounding the droplet and the droplet size distribution of dispersed oil in these matrices. The median particle sizes differed in spray-dried powders. HiCap particles with embedded oil (48 μm) were larger than the corresponding MD-GA particles (31 μm). Even if the relative surface area is higher in smaller particles, this difference was not sufficient to account for the orders of magnitude difference in the amount of solvent-extractable oil. With long extraction time, it is assumed that the solvent flushes through the particles. In this respect, coarsening of oil emulsion from MD-GA after re-dispersing may be important. The amount of gum Arabic used as an emulsifier may limit the reduction of droplet size and the stability of formed interfaces. A nearly linear correlation of mean droplet size with the amount of solvent-extractable flavour was obtained by Soottitantawat et al. (2003). There is also a considerable difference in molecular size of the surface active compounds between the two wall materials used in the present study. Therefore, it would be interesting to compare the packing properties of these interfaces in dilute systems. These measurements might explain whether the solvent extracts triglycerides through the interface in the MD-GA system or by destroying it.

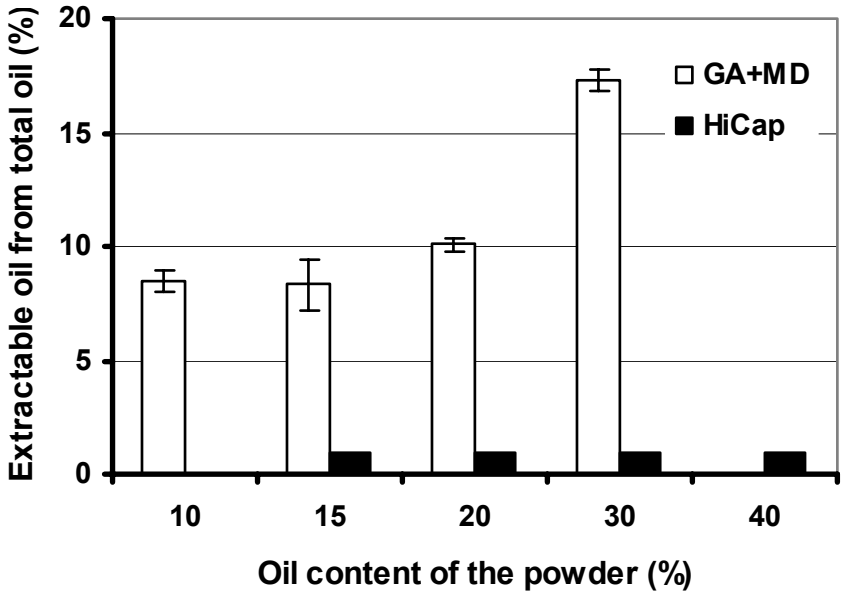

Figure 12. The amount of extractable oil in relation to the total oil content in maltodextrin-gum Arabic (2:1) and HiCap matrices using petroleum ether as a solvent. The samples with 10% and 40% oil were only analysed for the MD-GA and HiCap systems, respectively.

3.2.2 Glass transition and structural collapse

The glass transition temperatures (T_g) of the matrices were determined by DSC. In carbohydrate matrices, plasticisation of the components by water was observed as a reduction in T_g (Figure 13), and the values were in agreement with those obtained by Soottitantawat et al. (Soottitantawat, Yoshii, Furuta, Ohgawara, Forssell, Partanen, Poutanen & Linko, 2004). Sorption isotherms for the matrix compositions of the present study (Figure 11) are provided to elucidate the reduction in T_g as a function of relative humidity. In the storage stability test, caking and structural collapse in rubbery state eventually leads to dissolution of the powder at high humidities. For WPI matrix, an attempt to determine the T_g was only made at the highest humidity of the storage stability test. After equilibration at RH 91%, a weak baseline shift was observed in the second heating, from which the T_g of the protein matrix was determined to be 105 ± 1°C. There is little data on glass transition temperatures of globular

proteins, but by comparison with other biopolymers, such as starch (Myllärinen et al., 2002b) or sodium caseinate (consisting of non-globular proteins, unpublished results), the T_g at this high water activity was expected to be much closer to room temperature. Confusion with glass transition of lactose was not considered possible, as it is present in WPI only as a minor contaminant (< 0.5%). According to the determined T_g, the sample should have been well into the glassy state at the 37°C of the storage experiment. However, caking of the powder was observed at RH 91% (Figure 4a in Publication IV). Denaturation of the protein occurred in the first heating at DSC, and probably affected its mobility. Sochava (1997) discussed whether release of water occurs in protein denaturation, which would plasticise the protein further in the second heating. According to Sochava, segmental motions are increased due to denaturation. In our study with WPI, it should be noted that heat denaturation of this protein induces polymerisation through S-S bridges (Roefs & de Kruif, 1994). These complications may explain the lack of such data in the literature. The effect of water on the mobility of the compact native protein globules may not be evident, but the macroscopic effect of caking appeared to fit well with that observed for carbohydrates. Caking was studied further with electron microscopy (Figure 14), which showed a pronounced change in the structure of WPI reference matrix (without oil) from dry to humid conditions.

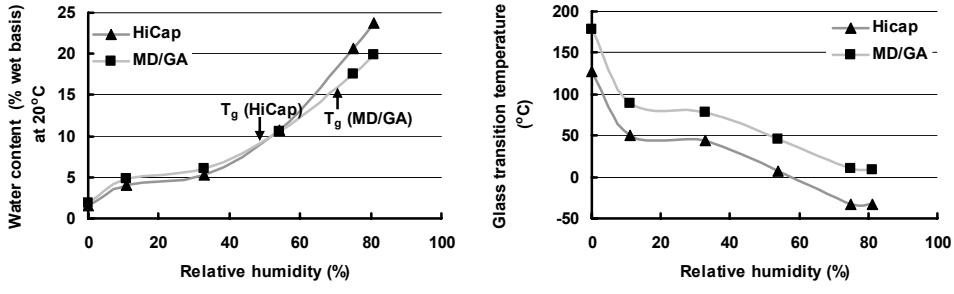

Figure 13. The sorption isotherms at 20°C and glass transition temperatures of carbohydrate matrices as a function of relative humidity.

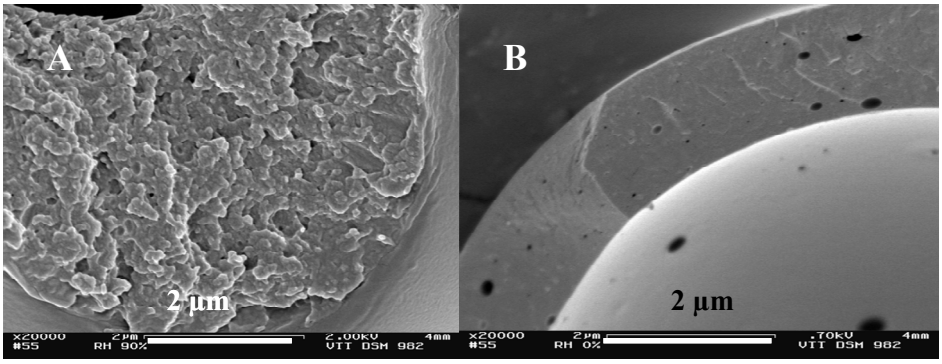

Figure 14. Cross-section images of spray-dried WPI reference (without oil) after 1 week of storage at 37°C and A) RH 91% or B) RH 0%.

3.2.3 Oxidative stability and volatile release

Oxidative stabilities of oils dispersed in carbohydrate and protein matrices were studied at 20°C and at differing relative humidities. Peroxide value (POV) quantifies the amount of fatty acid hydroperoxides in oil as a result of the primary, oxygen consuming stage after radical formation (see 2.3.4). POV was determined as a function of storage time for matrix-dispersed oil and for bulk oil reference.

Oxidation of sea buckthorn kernel oil embedded in MD-GA matrix was much less dependent on storage humidity than in HiCap matrix (Figure 15). Embedding in the MD-GA matrix retarded oil oxidation under both dry and wet conditions, but stability was improved more in dry conditions, in which the rate of bulk oil oxidation was high. In dry (RH 11%) conditions oxidation of matrix-embedded oil was much slower in the HiCap than in the MD-GA sample and even more reduced from the rate of bulk oil oxidation. At increased humidity, HiCap matrix was unable to protect the oil from oxidation, and in fact an increased oxidation rate as compared to bulk oil was observed. *This implies that oxygen concentration on the droplet surface is no longer rate-limiting for oxidation under these conditions.* Thus, matrix is no longer acting as a barrier. As compared to bulk oil, the oil in matrix has a considerably higher surface area, which could be one explaining factor for the higher oxidation rate in the matrix. As the trace metal contents of the materials were not determined, it can only be

speculated that catalysis of oxidation could be driven by trace metal contamination either directly from wall material or from processing, where the homogenisation step would be the most probable source of contamination.

A humidity effect similar, although much less pronounced, to that with HiCap was reported by Hardas et al. (2002), who studied the effect of relative humidity on encapsulated milk fat (40%). The main constituents of the powder were corn syrup solids (50%), milk fat (40%) and sodium caseinate (7.5%). The authors reported improved oxidative stability at RH 14% and RH 44% compared to that at RH 52%. For surface fat, an opposite behaviour was reported. The RH-dependence of oxidation rate found for surface fat is in agreement with that found for bulk oil oxidation in the present study. In the study of Soottitantawat et al. (2004), oxidation of limonene embedded in HiCap and MD-GA matrices was studied at 50°C. The initial rate of oxidation was highest at RH 51% in HiCap matrix, decreasing both above (RH 96%, RH 75%) and below (RH 23%) this value. Oxidation in MD-GA matrix followed the same trend, and the lowest oxidation rate was obtained at RH 75%. This was assigned to rehydration of the powder and to the onset of the particle adhesion, which was proposed to restrict the oxygen supply. A similar explanation of re-encapsulation at higher humidities was proposed by Labrousse et al. (1992). Results controversial to those of the present study have also been presented (Anker & Reineccius, 1988). The oxidative stability of limonene in orange oil embedded in gum Arabic was found to increase with increasing humidity from RH 0% to 54%. The possible factors explaining the controversy in these results are discussed in chapter 4.2.

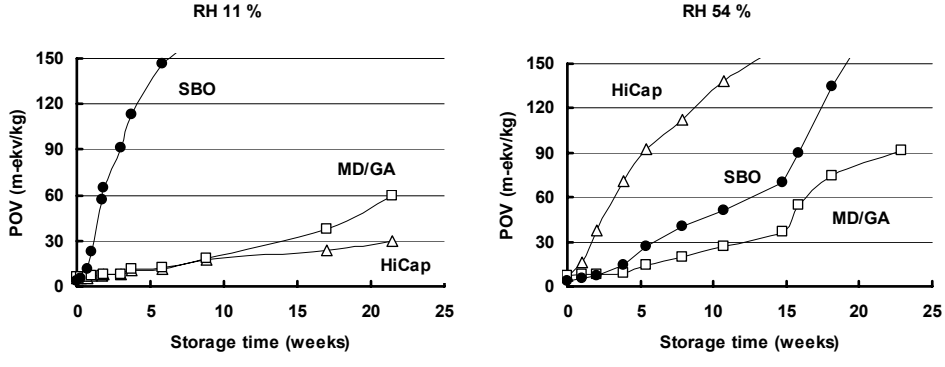

Figure 15. Oxidation of sea buckthorn kernel oil at 20°C and RH 11% and 54% embedded in MD-GA matrix and in HiCap matrix and as bulk oil (SBO).

Oxidation of flaxseed oil was studied in WPI matrix at 37°C and at RH 0%–91% (Figure 16), with bulk oil as a reference. Thus, the oil was different and the storage temperature was higher than that used in the study of oil oxidation in carbohydrate matrices. By increasing the temperature, physical instability of the powder could be induced in humid conditions. Due to differences in oil composition (Table 4), storage temperature and methods of peroxide value determination (2.3.5), the peroxide values are not directly comparable in these studies. The initial oxidation rate in flaxseed bulk oil did not depend on humidity, but after 9 weeks of storage the amount of hydroperoxides in bulk oil was lowest at the intermediate humidity (RH 49%), a result in agreement with that reported earlier for sea buckthorn oil. Hydroperoxide contents at low (RH 0% and RH 11%) and high (RH 91%) humidities were considerably higher. In all cases, the rate of oxidation was essentially the same in bulk and matrix-dispersed oils for the first three weeks of storage. After this initial phase, the amount of hydroperoxides decreased in all matrix-dispersed samples and started to increase again after 5 weeks of storage. For the sample stored at RH 75%, the lag time was even longer. Decrease in hydroperoxide content is possibly due to the fact that hydroperoxides are not only formed but also simultaneously decomposed in oxidation (Fritsch, 1994). At the end of the storage test, the highest amount of hydroperoxides was found in the very low humidity sample (RH 0%). A minimum amount was found at RH 75%.

The initial oxidation in the matrices was not limited by oxygen concentration, which would suggest the role of oil in direct contact with air. There are two surfaces in these hollow sphere particles, of which the composition of the outer surface has been a subject of discussion previously. Baik et al. (Baik, Suhendro, Nawar, McClements & Decker, 2004) observed a tenfold increase in fish oil hydroperoxides in surface oil compared to encapsulated oil during the first days of storage. The role of surface oil in the initial rate of oxidation was also found by Drusch et al. (Drusch, Serfert, Van Den Heuvel & Schwarz, 2006). In our study, the matrix was composed of surface-active protein, which was shown by ESCA (electron spectroscopy for chemical analysis) to concentrate on the surface during drying. The powder particles in our study were extremely thin-walled, leaving a large vacuole with a possible oxygen reservoir inside.

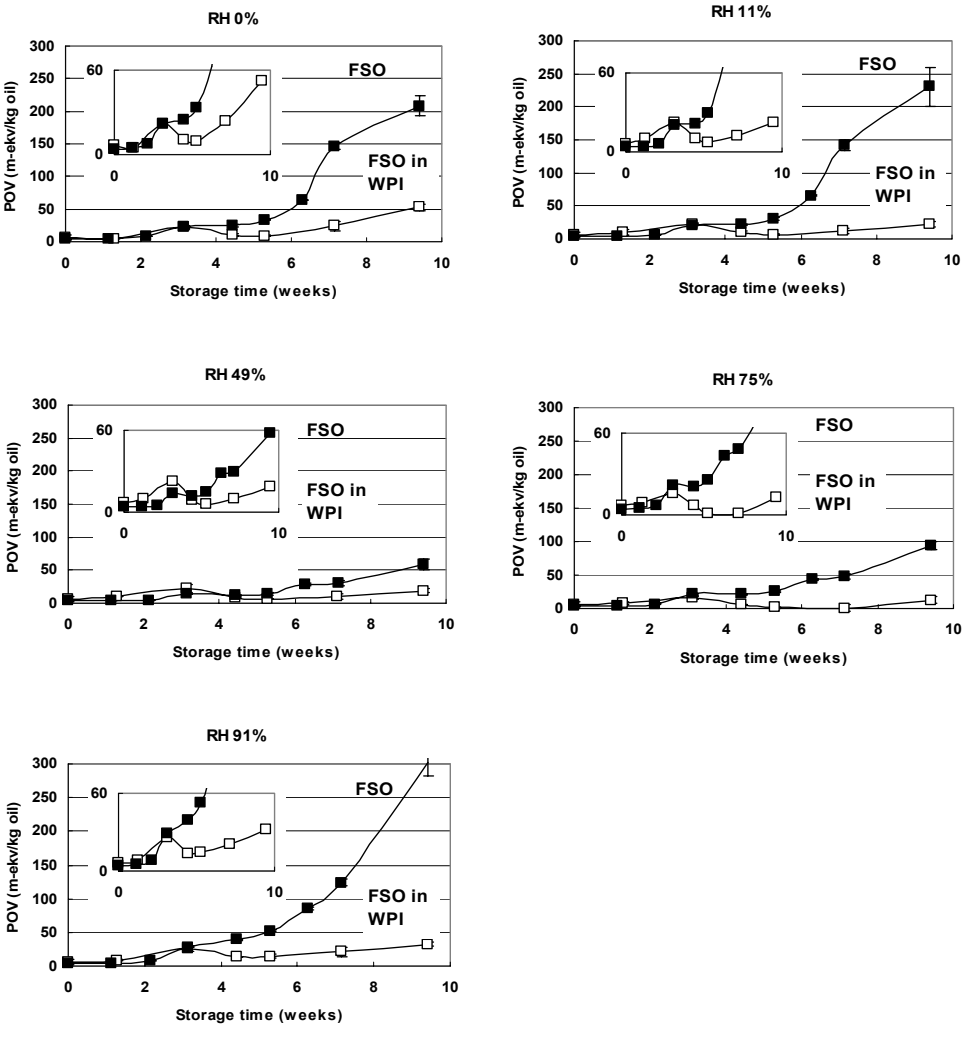

Figure 16. Oxidation of flaxseed oil (FSO) at 37°C and RH 0–91% embedded in WPI matrix and in bulk oil.

Release of volatiles from carbohydrate matrices was studied with caraway extract, of which 90% were volatiles with two main components: limonene and carvone. The powders with 12% embedded caraway extract were prepared with HiCap and with maltodextrin-gum Arabic mixture. Minor changes in carvone content were observed during storage of 2 months at elevated temperature (70°C). The release of carvone and limonene was studied as a function of temperature by equilibrating the dry samples under vacuum for 15 minutes (Figure 17).

The T_g values of the dry systems (< 2% water contents) were taken from Figure 12. The content of volatiles in the headspace of the test tube was low up to 120°C. At low temperatures the leak was rather constant, indicating that it represented the evaporation of free volatiles under high vacuum. Further heating caused a step-wise increase in vaporisation of carvone from MD-GA matrix already below matrix T_g, whereas limonene was quickly released at the transition zone. Carvone was also more easily released from HiCap matrix, from which a more pronounced release of both volatiles occurred above T_g. However, it was also associated with the decomposition of the matrix at 140°C, which was observed as browning of the sample. Temperature-dependence of volatiles release from sucrose-maltodextrin matrices was studied by Gunning et al. (Gunning, Gunning, Kemsley, Parker, Ring, Wilson & Blake, 1999). The authors found no evidence of matrix permeability to any of the flavour compounds studied and suggested that release occurred in the rubbery state due to sucrose crystallisation. Increasing release of aldehydes with increasing water activity was reported from vegetable oil dispersed in hydrolysed starches up to the point at which collapse of the matrix occurred (Whorton & Reineccius, 1995). Soottitantawat et al. (2004) linked limonene release to increased water activity in carbohydrate matrices. In this study, no triglyceride was present. Furthermore, physical instability of the matrices was found to affect release rates above T_g. Soottitantawat et al. (2005) also linked volatile release to the size of powder particles as well as to that of dispersed lipid droplets. The smaller relative surface area with larger particles and larger droplets was found to decrease the release rate, also depending on the carbohydrates comprising the matrix. This observation implies that comparison between the matrices of the present study is not straight forward.

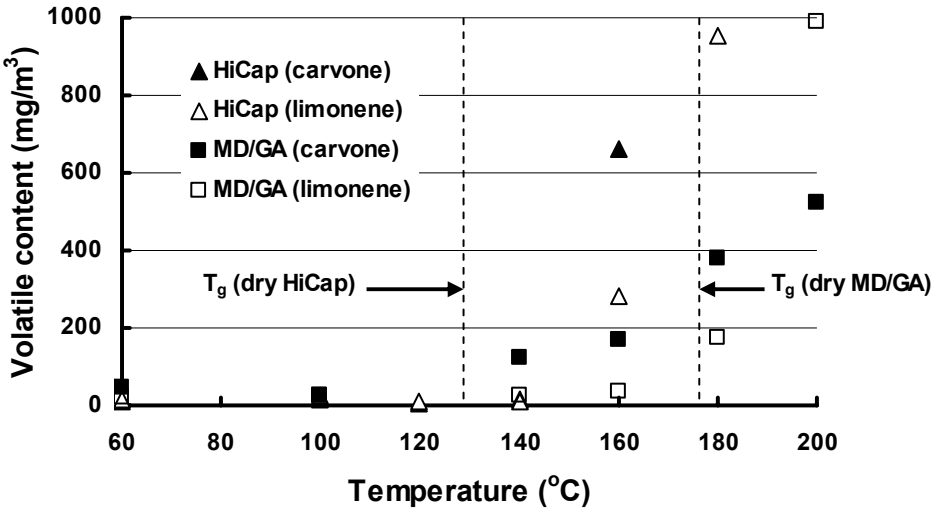

Figure 17. Release of limonene and carvone from HiCap and MD-GA matrices as a function of temperature as measured by FT-IR. The glass transition temperatures of the dry matrices are indicated as dashed lines.

4. Discussion

4.1 Plasticisation of amylose by water and glycerol (Publication I)

Three types of domains can be recognised in amylose films: crystalline polymer with sorbed water, amorphous polymer with glycerol and water as plasticisers and finally, separated glycerol-rich domains (3.1). Despite the water sorption in crystalline domains, the more significant plasticisation takes place in the amorphous domains. However, the addition of plasticisers increases the degree of crystallinity, which would be expected to show as an increase in the rigidity of the system. Thus, a critical question in the interpretation is the extent to which crystalline domains contribute to the observed relaxation behaviour. *It is very difficult to relate the crystalline domains to the values of second moment calculated from the relaxation experiments.* The second moment decreases both with increasing water and glycerol content (Figure 4 in Publication I). Considerably stronger dipolar interactions would be expected in the crystalline domains as compared with the amorphous domains due to denser packing of the former. The opposite result leads to the assumption that the spin-spin relaxation of crystalline amylose occurs very rapidly, during the deadtime of the spectrometer (ca. 10 μs). This behaviour has been recognised for ice crystals, in which context it has been used to quantify the amount of frozen water (Räsänen, Blanshard, Mitchell, Derbyshire & Autio, 1998). In the original publication I, the effect of crystallinity probably showed in the spin-lattice relaxation experiments, in which efficient cross-relaxation between rigid and mobile components was observed. In the results of the present study only the changes in the second moment have been summarised, as they are more representative of the changes in the amorphous domains.

The mobility of the binary amylose-water system was only studied in glassy state, but the system was close to T_g in the highest humidity conditions. The beat pattern became less pronounced with increasing water content, indicating a decrease in strong dipolar interactions. The strength of these interactions decreases with increasing distance. The intensity of the rigid signal (including the beat) corresponded to the proportion of amylose protons in the system at all levels of water plasticisation. The fast relaxing protons are expected to contribute to the intensity, which has been resolved from data extrapolated to the

zerotime of the experiment (the end of the 90^{o} pulse). Furthermore, it was shown that cross-relaxation between the rigid and mobile components was too slow to affect the free induction decays of the films, as also reported by van den Dries et al. (1998) for maltose glasses.

The second moment M_2 was found to decrease slightly with increasing water content. This could be explained by limited increase in rotational mobility. M_2 is affected by proton density, as the dipolar interactions decrease with the sixth power of the distance between protons (van den Dries et al., 1998). Van den Dries et al. (1998) showed this reduction in M_2 with deuteration, in which the distance between protons is increased due to the replacement of the exchangeable proton fraction with deuterium. In the present study, the mass fraction of protons in the system increases with increasing water content. The actual distance between the protons is also influenced by the packing of the system. This factor was recently pointed out in plasticised protein system by Gao et al. (2006). With increasing mass fraction of protons in the system, increased M_2 is expected unless the volume of the system changes. Therefore, the slight decrease in M_2 with increasing water content could well indicate that swelling occurred in glassy amylose. Glassy state swelling was also reported by Kilburn et al. (2005) for maltodextrin systems. Water was suggested to act as a plasticizer by interfering with the polymer-polymer hydrogen bonding.

The effect of glass transition on the mobility of amylose could not be reported on the basis of results of the present study, as the system was in glassy state even at the highest water content. Tanner, Hills & Parker (1991) reported a dramatic change in the free induction decay in native waxy maize starch at 30% water content, which was attributed to increase in starch mobility due to water.

The ternary amylose-glycerol-water systems were studied both in glassy and rubbery states. Glass transition temperatures were calculated over a wide range of water contents, for some of which T_g could not be determined by DSC. However, for low water contents, there was a relatively good correlation between the measured and calculated values. In glassy state, decrease in the M_2 of the rigid component by addition of plasticisers could be explained by the $T\text{-}T_g$ difference. In rubbery state, a steeper decrease was found for amylose with 30% glycerol. In this system, a fraction of amylose protons was also found to contribute to the mobile component of the free induction decay.

Moates et al. (2001) studied a binary amylose-glycerol system and found that glycerol and amylose were only partially miscible already at a glycerol content of only 7.5%. Similarly, phase separation has been reported for the ternary amylose-glycerol-water system (Myllärinen et al., 2002b). Therefore, it is not obvious why the mobility of amylose was increased by the high glycerol concentration in the present study. However, Gaudin et al. (1999) suggested that phase-separation in starch-sorbitol system is in fact due to formation of clusters of plasticiser molecules instead of true phase-separation. A rather similar explanation was given for protein films by Gao et al. (2006), who determined an upper limit for the size for glycerol domains (30 nm) based on transverse relaxation rates of the liquid components. The authors proposed that at low level of glycerol, few glycerol-glycerol interactions take place, but with increasing glycerol content the number of these interactions gradually increases eventually forming a bulk phase capable of undergoing a glass transition. At this point, the proteins are still diluted with glycerol, with reduction in protein-protein interactions. Thus, in our study, higher glycerol concentration might mean more clusters associated with higher amylose mobility, rather than increase in the volume of separated domains of plasticiser, which could to some extent explain the mechanism of plasticisation by a "phase-separated" compound.

The general dilemma of hydrophilic biopolymer films is that flexibility can only be attained at the expense of gas barrier properties by addition of plasticisers. The present discussion focuses on the loss of oxygen barrier properties of similarly prepared amylose films, which has been reported earlier (Forssell et al., 2002). In dry conditions, films were good barriers, but a dramatic increase in permeability was observed at 20% water content irrespectively of the glycerol content. In order to study whether the loss of barrier properties could be explained by increased amylose mobility, the data presented by Forssell et al. (2002) was plotted against the mobility data of the present study (Figure 18). Oxygen permeation generally increased with increasing chain mobility, but no master curve could be drawn to describe this relationship. The films with the highest glycerol content (30%) were associated with higher molecular mobility and less oxygen permeation as compared to the samples containing less glycerol. Thus, although more mobile chain segments of amylose may allow faster diffusion, mobility of the amorphous parts does not explain the oxygen transfer rate in all cases.

In the crystalline domains, retarded oxygen transfer would be expected. Increasing area of crystalline domains would decrease the effective surface area for "rapid" permeation. However, minor differences in the degree of crystallinity were expected between these samples, as it was previously found to be high (> 20%) already at intermediate relative humidity (RH 54%) and with low glycerol content (10%) in these films (Myllärinen et al., 2002a). Oxygen permeation data was measured from RH 50% to RH 90%. Furthermore, formation of glycerol clusters may affect oxygen permeation, as it was shown by Whitcombe et al. (2005) that two other polyols, sorbitol and maltitol are able to decrease the solubility of oxygen in water. Obviously, more separated domains of glycerol are expected in the samples with higher glycerol content. Based on these observations, it would seem logical that in both glycerol-rich domains and in the domains of amorphous amylose, water would increase the solubility of oxygen.

The effect of water content on oxygen permeation was already demonstrated in the original study by Forssell et al. (2002), in which a single mastercurve of oxygen permeability of samples with varying glycerol contents could be drawn as a function of water content. The importance of oxygen solubility in dry carbohydrate systems was proposed by Whitcombe et al. (2005). *The present study has demonstrated that the increase in molecular mobility of amylose may be only partly responsible for the loss of barrier properties of these films with increasing relative humidity.* The practical importance of this observation is that the flexibility of the film and its barrier properties are not mutually exclusive.

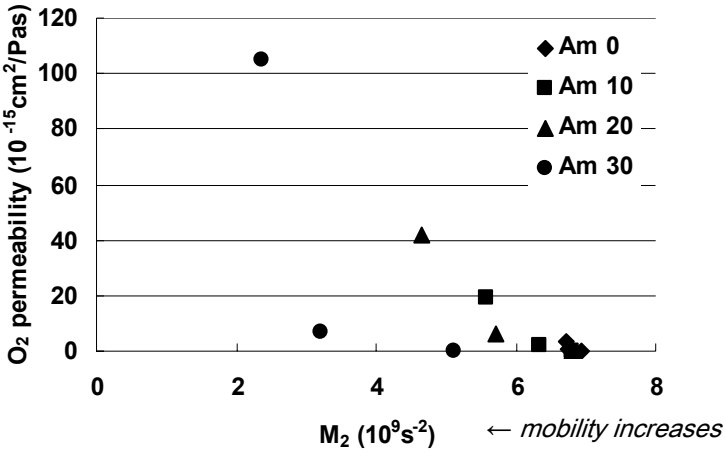

Figure 18. Oxygen permeability of glycerol-plasticised amylose films as a function of the second moment M_2 of the rigid component as a measure of mobility of the amorphous amylose. The permeability data was approximated from Forssell et al. (2002). Amylose films at different glycerol contents (Am 0, 10, 20 and 30) represent 0%, 10%, 20% and 30% glycerol contents of the films per dry weight, respectively. Lower values of M_2 indicate higher mobility.

4.2 The effect of water on the physical state of biopolymer matrices and on oxidation of matrix-dispersed oil (Publications II–IV)

For lipid oxidation in the glassy food model, availability of oxygen has been shown to be rate limiting for hydroperoxide formation at ambient temperature (Orlien et al., 2006). Some crucial factors affecting the presence of oxygen at the droplet surface in matrix-dispersed oils were identified in the present study: oxygen flux through the matrix and the fraction of oil in direct contact with air. Both factors are influenced by water. Oxygen flux is affected due to changes in solubility and diffusivity, as discussed in the previous section. Exposed oil is affected, as it is likely to follow the mechanisms of lipid oxidation in bulk, which have been linked with water. Oxidation of oils rich in polyunsaturated fatty acids was studied in carbohydrate and in protein matrices. The effect of relative humidity on oil oxidation will be discussed first in carbohydrate matrices, then in protein matrix, and finally an attempt will be made to recognise some distinct differences between the two.

Oxidation of oil embedded in carbohydrate matrices was studied at low (RH 11%) and intermediate (RH 54%) humidities. The larger droplet size of oil in MD-GA matrix was associated with a larger amount of solvent-extractable oil. The initial rate of oil oxidation in the matrices was essentially the same at RH 11%, and was thus not affected by the amount of solvent-extractable oil. This implies that *solvent-extractable oil is not a measure of surface oil*. Surface oil and its quantitation have been under considerable discussion in recent years, but as pointed out by Drusch and Berg (2008), low odour thresholds of many secondary oxidation products make surface oil an important determinant of sensory quality. However, these authors conclude that the amount of solvent extractable oil cannot be used to predict the shelf-life of matrix-embedded oils. In dry (RH 11%) carbohydrate matrices oxidation of embedded oil occurred at a slower rate as compared to oxidation of bulk oil. This could be attributed to the effect of matrix in limiting oxygen transfer.

In the case of HiCap at intermediate humidity (RH 54%), the matrix was found to accelerate oxidation of embedded oil from that of bulk oil. In addition to the matrix, the major difference between bulk and matrix-embedded oils is the larger surface area of the latter. The effect of surface area on oil oxidation in emulsions was pointed out by Coupland and McClements (1996). In the powder matrices of the present study, the higher surface area due to matrix was only important for oxidation under certain conditions. Therefore, as already pointed out earlier, the result may indicate that under these conditions, oxygen transfer was no longer rate limiting for oxidation. As the study of Orlien et al. (2006) relates oxygen transfer rate to oxidation in a *glassy state* system, the data from storage stability tests of the present study was plotted as a function of the temperature difference between storage and glass transition temperatures ($T-T_g$) (Figure 19). In order to compensate for the lack of data points, the data from an accelerated stability test (at 50^oC) was included. The storage time needed to exceed a limiting peroxide value (20 m-ekv/kg of oil) was used as a determinant for "shelf-life". Interestingly, in HiCap matrix, a steep decrease in stability was found to occur in the glass-rubber transition region. This observation appeared to fit the results of both 20^oC and 50^oC experiments.

On the basis of information from the literature, three possible factors responsible for increased availability of oxygen in rubbery state could be recognised: 1) increased amount of oil in direct contact with air due to physical instability of

the matrix, which leads to oil release (Labrousse et al., 1992; Drusch et al., 2006), or increased oxygen concentration at the matrix-oil interface due to 2) increased oxygen solubility in matrix with increasing water content (Whitcombe et al., 2005) and 3) increased rate of oxygen diffusion due to swelling of the matrix (Kilburn et al., 2005). Decreased stability was associated with high water content at room temperature and with high temperature already at low water content. No evident correlation between solubility of permanent gases and temperature can be found for synthetic polymers (Pauly, 2005). However, in the present study, derivatisation of starch introduces non-polar residues in the system, most probably also to the bulk matrix, which could facilitate the dissolution of oxygen. At RH 54% and at 20^{o}C, the powder with HiCap matrix caked in storage, but full collapse with dissolution in sorption water did not occur. Oil release was not determined, but it has earlier been connected with collapsed state and crystallisation of an amorphous matrix (Labrousse et al., 1992). Unfortunately, crystallisation of the samples was not followed in the present study. However, in the determination of sorption isotherms no discontinuities indicating crystallisation were observed, and therefore oil release was not the most likely cause for loss of stability. Instead, these observations would suggest the role of facilitated oxygen transfer due to increased molecular mobility and/or swelling of the matrix with possible contribution of increased oxygen solubility with increasing water content.

For oxidation of oil embedded in MD-GA matrix, a rather different behaviour was observed in glassy state. The stability was only slightly decreased by the approaching glass transition region in the 20^{o}C storage test. However, it was completely lost at 50^{o}C irrespective of humidity. The stability of oil could be explained by similar factors at 20^{o}C in both matrices, but at 50^{o}C the MD-GA matrix was unable to retard oxidation rate from that of bulk oil. The result could not be explained by the T_g of the matrix. The measured T_g values of multi-component systems are known to correlate with those predicted by the Gordon and Taylor equation (1) based on T_gs of the individual compounds. Possibly, for a matrix with a wide distribution of molecular weights, the "average T_g" may not always predict matrix performance. Another T_g-related factor was discussed by Gunning et al. (1999), who considered the role of water sorption rate in the matrix, which may affect the conditions experienced by the surface layers, possibly allowing oil release. In any case, for reliable conclusions concerning the T_g dependence of oxidation rate in carbohydrate matrix, more data points would be needed.

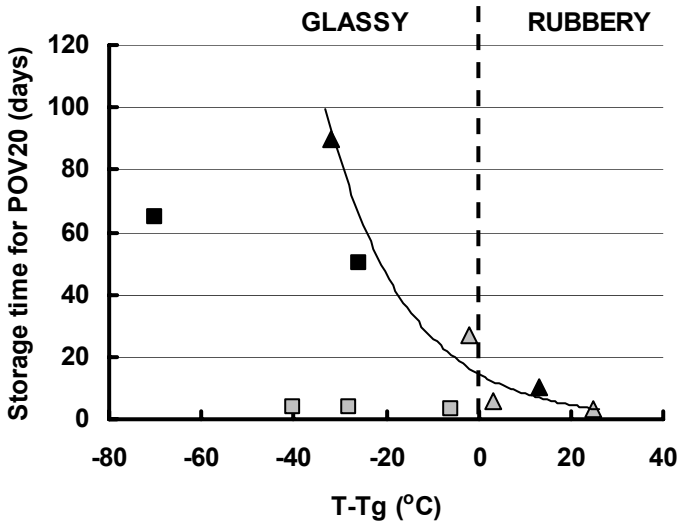

Figure 19. The storage time for HiCap (triangles) and MD-GA (squares) samples exceeding a peroxide value limit (POV 20) as a function of temperature difference between storage and glass transition temperatures ($T-T_g$). The black and grey labels are from 20°C and 50°C storage tests, respectively.

Oxidation of flaxseed oil embedded in whey protein isolate matrix was studied over a wide range of relative humidities, from RH 0% to RH 91%. Matrix retarded the oxidation rate from that of bulk oil at the end of storage test in all cases. The general effect of relative humidity was rather similar on bulk and embedded oils (Figure 6 in Publication IV). However, some observations deserve further attention. *Firstly,* the high rate of oxidation at RH 0% is consistent with the results of early studies on lipid oxidation (Karel, 1986). Oxidation of matrix-embedded oil was clearly affected by a small increase in humidity (RH 11%), but surprisingly, bulk oil was not. In this context, it may be informative to point out that the relative humidity in the chamber with P_2O_5 was measured, and it was very close to 0% (< 1%). Due to the observed difference between bulk and matrix-embedded oils, it appears that the ability of matrix to protect the oil is enhanced by the presence of water. Increasing stability with water has been associated with antioxidants formed in non-enzymatic browning, hydration of trace metals and free-radical interactions with other food components. On the other hand, extremely dry conditions could lead to formation of structural defects, such as cracking, in the matrix. Formation of microscopic cracks in freeze-drying of extruded carbohydrate matrix was demonstrated by Gray et al.

(Gray, Bowen, Farhat & Hill, 2008). Gunning et al. (1999) also found limited cracking on the surface of extruded rods in sucrose-maltodextrin system. These defects, if occurring in the present study, were below the resolution of electron microscopy and could not be observed visually.

The second interesting issue was the oxidation rate minimum at RH 75%. It may be that the matrix-forming protein oxidises under these conditions. Stapelfeldt et al. (1997) studied oxidative stability of whole milk powder. The authors associated better stability of milk fat with greater heat exposure during processing. The initial amount of reactive SH groups was found to be higher in the samples which were less susceptible to oxidation, but the reduction of these groups during storage was the same for all the samples. Recently, better stability of emulsions stabilised by β-lactoglobulin after heat treatment has also been shown (Elias, McClements & Decker, 2007). Peroxyl radical scavenging capacity of the protein was linked with inhibition of lipid oxidation, whereas iron chelating capacity and the amount of free sulfhydryls were found to be less important.

Finally, at high relative humidity (RH 91%) electron microscopy revealed a tremendous change in the internal structure of the matrix. Aggregation of partially denaturated proteins occurred when sufficiently high water content provided the mobility for rearrangements, most likely driven by hydrophobic interactions. A higher rate of oxidation was associated with this structural change, but the effect was minor as compared to the effect of collapse of low-molecular-weight carbohydrates associated with matrix crystallisation and oil release (Labrousse et al., 1992; Drusch et al., 2006). The minor effect on oxidation in the present study, despite the nano-scale cavities observed in protein matrix, may suggest that oxygen transfer is not determined by the bulk matrix but by the interfacial layer. This was also suggested by Moreau and Rosenberg (1998), who determined porosity in whey protein matrix, but were unable to correlate the observed changes in porosity with oxidation in similar particles (Moreau & Rosenberg, 1996). The packing of the interfacial protein layer is expected to be different from that of bulk protein matrix due to partial unfolding following adsorption. Immediately after adsorption, a β-lactoglobulin monolayer of densely packed particles is formed. With unfolding, a gel-like layer with increased physical interactions and slow formation of covalent disulphide bridges has been found (Dickinson, 2001).

The well-known food stability map (Figure 1, Labuza et al., 1970) suggests a high rate of oxidation at low and high humidities. Oxidation of bulk sea buckthorn and flaxseed oils was in agreement with this theory. However, these oils dispersed in carbohydrate and protein matrices behaved very differently with respect to environmental humidity. Whereas oil was most protected in low humidity conditions (RH 11%) in carbohydrate matrices, in protein matrix the best stability was observed at RH 75%, which is expected to be well above the monolayer moisture content. Monolayer moisture content is presently considered as a general indicator of oxidative stability in foods (Bell, 2007). Although the highest oxidative stability has been found at monolayer moisture content both for model (Maloney, Labuza, Wallace & Karel, 1965) and for real food systems (Quast & Karel, 1972), results in agreement with the present study have also been reported. Recently, a deviation from monolayer stability was reported for real food systems by Jensen & Risbo (2007), who studied oxidative stability of peanuts, pork scratchings, a mixture of rolled oats and wheat from muesli, and oatmeal. These authors reported a decrease in radical content with increasing humidity.

The results of the present study demonstrate the importance of the matrix properties, and thus the choice of materials in controlling the rate of oxidation in matrix-dispersed lipids. The focus was on clarifying the plasticising effect of water on the matrices studied, and its consequences for formation of fatty acid hydroperoxides, which is governed by the availability of oxygen. The theory of increasing oxygen transfer with increasing plasticisation with water fitted the oxidation in carbohydrate matrices, but for proteins the connection between plasticisation and loss of barrier properties was much less evident. The considerable reorganisation of the matrix which was observed at high humidity had only a minor effect on oxidation rate. The matrix consisting of globular protein "particles" may contain sufficient cavities for efficient oxygen transfer under all conditions. The dense interfacial layer would then be responsible for retardation of oxidation rate from that of bulk oil. A distinct role of oil-matrix interface in carbohydrate matrices with embedded oil cannot be ruled out. *The properties of the interface become especially important under conditions in which the ability of the bulk matrix to control oxygen transfer is limited.* A schematic representation of the effect of water on oxygen transfer in carbohydrate and globular protein matrices is depicted in Figure 20.

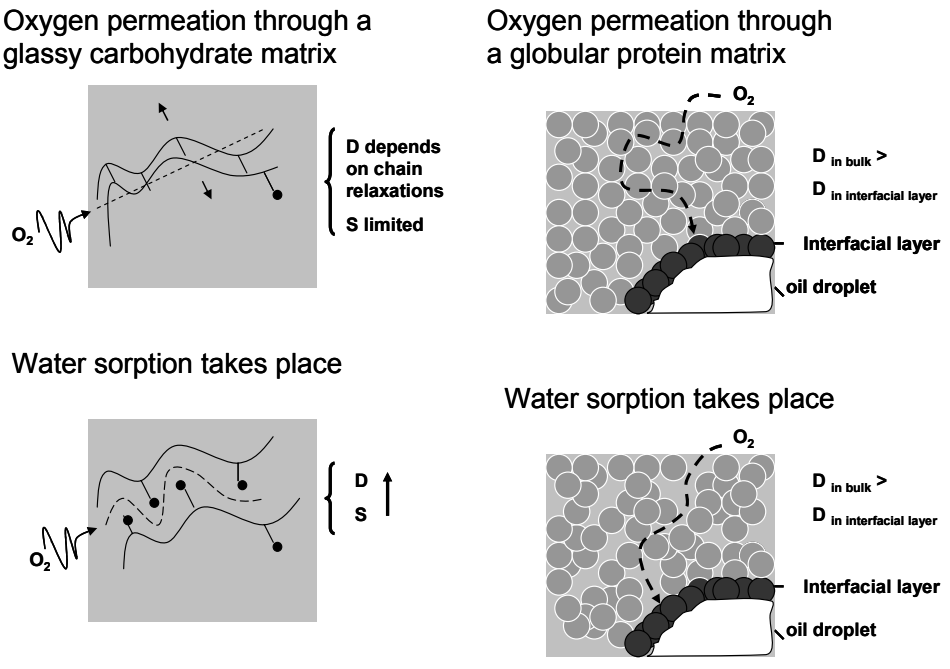

Figure 20. A schematic representation of the effect of water on permeation of oxygen in the matrices of the present study. For carbohydrate matrix swelling, increased rate of chain relaxation and increased oxygen solubility in system with water are suggested to affect oxygen flux depending on the specific composition of the matrix and the proximity of T_g. For a matrix formed of globular proteins, water-induced structural changes are of less importance, as the dense interfacial layer is proposed to control the oxygen transfer rather than the bulk matrix, where the reorganisation has been shown to take place.

4.3 Release of volatiles from carbohydrate matrices (Publication V)

A constant leak of volatiles from matrix-embedded oil was observed in storage from both carbohydrate systems. Although some changes in volatile content were found with storage, it is obvious that the systems were not in equilibrium. *In fact, steady state Fickian diffusion could be assumed.* Possible factors contributing to the release of matrix-embedded volatiles have been previously recognised: the proximity of matrix T_g was shown to increase the release in

glassy state (Whorton & Reineccius, 1995; Soottitantawat et al., 2004), when temperature difference was decreased by water-induced plasticisation. Gunning et al. (1999) showed that volatiles release from sucrose-maltodextrin matrix was due to sucrose crystallisation, and pointed out the low amount of flavour partitioning in low water content matrix. Solubility-based partitioning was studied further by Gunning et al. (2000b), who reported that the hydrophobicity of the major components of a liquid flavour system affected the retention of the minor components when the conditions allowed partitioning. Low water content amorphous carbohydrates were suggested to be poorer solvents for flavour components as compared to water (Gunning et al., 2000b).

In the present study, an increased release of volatiles in heating was observed above T_g in all cases, except for release of carvone from MD-GA matrix. The difference from the studies in which release already increased when approaching T_g may be explained by differences in sample composition and in the analytic techniques used. When comparing the results of the present study with the study by Soottitantawat et al. (2004), a considerable difference was the absence of triglycerides in the lipid phase of the latter. Thus, the presence of non-volatile hydrophobic compounds may have decreased the partitioning of the volatiles in the present study. Considering the role of matrix in controlling the release of volatiles, both diffusivity and solubility of these compounds are important. Diffusivity would be expected to increase with changes induced by both water and temperature (Kilburn et al., 2005; Parker & Ring, 1995). However, a mechanistic difference in approaching the T_g by water- or temperature-induced plasticisation may exist in solubility, i.e. flavour partitioning. The presence (Whorton & Reineccius, 1995; Soottitantawat et al., 2004) or absence (the present study) of water in carbohydrate matrix may affect flavour partitioning. Therefore, these results support the concept developed by Gunning et al. (1999) that volatiles release from solid carbohydrate carriers should be considered from the perspective of component partioning in a biphasic system.

5. Conclusions and future outlook

The general aim of this thesis was to test some of the current hypotheses (Table 2) on the role of biopolymer matrices in controlling mass transfer under various environmental conditions. In this section, the concluding remarks are focused on the effect of water on oxygen transfer and its consequences for the oxidative stability of matrix-embedded lipids.

A linear high molecular weight carbohydrate (amylose) is efficiently plasticised by water, as observed from the reduction in glass transition temperature. The present study demonstrates minor changes in the system with increasing water content in glassy state. The results may indicate that glassy state swelling occurred in the matrix with limited increase in rotational mobility until reaching the transition zone. It would seem likely that semi-crystalline linear high molecular weight carbohydrate behaves differently from the more branched and short-chained amorphous polymers. Furthermore, it has still to be confirmed whether the values of the second moment from NMR relaxometry can be used as an indication of matrix swelling. In future work, measuring the free volume in amylose films with increasing water content would provide further insight on this matter. Due to these constraints, the results of the present study cannot be used as a general indicator of barrier mechanism in starch-based systems. More swelling might be expected for amylopectin, which would make it a good reference for amylose in future studies. Unlike amylose, amylopectin crystallises in time. Therefore, the mobilities of systems with various degrees of crystallinity could be analysed.

The present study demonstrated that although increased mobility of amylose in the amorphous domains was generally linked with increased oxygen transfer through the film, under certain conditions amylose was effectively plasticised with minor losses in barrier properties. These observations indicate the importance of water (over glycerol) in controlling the transfer of oxygen in amylose films. In the systems where glassy state swelling is important, diffusion is probably affected. Solubility of oxygen may also be limiting the transfer rate. Possibly, diffusion controls the transfer rate under conditions of low humidity, but with swelling of the matrix, diffusion is facilitated and solubility may become limiting. When developing barriers from biomaterials, the choice of a plasticiser is of major importance. The efficiency of the phase-separated

plasticiser could suggest that less polar, partially or totally immiscible compounds could also have potential.

The hypothesis in the oxidation work was that water-induced changes in the matrix influence the rate of oxygen transfer, and therefore also the rate of hydroperoxide formation as the oxygen-consuming step of the reaction. The present study demonstrated the difference in carbohydrate and protein-based matrices in this respect. A comparison between oxidation of matrix-embedded oil and the corresponding bulk oil was performed. Several different oxygen transfer conditions can be recognised:

- glassy carbohydrate matrices: oxygen transfer (diffusivity and solubility) is rate-limiting to oxidation and depends on water
- rubbery carbohydrate matrices, not fully collapsed: oxygen transfer is not rate-limiting to oxidation and the interfacial area becomes important
- fully collapsed carbohydrates: oxygen transfer is dependent on the macroscopic geometry of the sample as oxygen is no longer transferred by rapid gas-phase diffusion to the particle surface (suggested from the literature data)
- globular protein matrices: oxygen transfer is rate-limiting to oxidation but it may not depend on water or the bulk matrix; it is rather limited by the dense interfacial layer between oil and bulk protein phases.

Thus, effects from both bulk matrix and interface have been recognised. Oxidation of oil embedded in glassy carbohydrate matrices has been shown to proceed at a rate with practical relevance. However, more data would be needed to derive the mechanisms of glassy state instability. Future research should assess the oxidative stability of oil embedded in carbohydrate matrix in the absence of water and also when approaching the T_g. Oxidative stability of systems containing a considerable amount of water is also of practical importance. Therefore, the proposed role of oil-matrix interface in controlling the rate of oxidation in protein matrix should be studied in more detail. The interface is probably important in all matrices where the bulk matrix is not acting as a barrier to oxygen.

The loss of sensory quality with oxidation is in most cases governed by formation of secondary oxidation products, which have low odour thresholds. The present study demonstrated a continuous leak of hydrophobic volatiles from glassy carbohydrate matrices, which however did not appreciably affect the flavour content. A more intense release was found above the glass transition region. The results indicate that although these matrices provided excellent stability as flavour carriers, they may not be expected to inhibit the release of secondary oxidation products even in dry, glassy state.

Finally, the outlines for developing a matrix providing high oxidative stability of oil could be drawn on the basis of the present study: the bulk should be composed of non-crystallising carbohydrate, which would retard oxygen transfer in dry conditions. Surface active protein component is located on both matrix-air and matrix-oil interfaces, maintaining structural integrity at the particle surface and forming a dense layer at the matrix-oil interface, thus controlling mass transfer at intermediate humidity.

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Title

Mobility and oxidative stability in plasticised food matrices
The role of water

Abstract

The importance of water in food structure and stability is well known, and the role of water as a plasticiser for biopolymers has been extensively studied during the last 25 years. Food stability is currently evaluated on the basis of water activity and the physical state of the matrix. It would be important to consider whether structures formed of starch-based carbohydrates and proteins, really act similarly with respect to water. In the present work, the role of water in system stability and biopolymer interactions was studied in two different systems: cast films with and without plasticiser and spray-dried particles with a dispersed lipid phase

Plasticisation of amylose films by glycerol and water was studied by proton NMR relaxometry. In glassy amylose the proximity of Tg did not strongly affect the amylose mobility. In rubbery state, high concentration (30%) of glycerol increased the mobility of amylose despite the phase separation that occurs in these systems already at much lower plasticiser content. The data on mobility of plasticised amylose was combined with results presented earlier on oxygen permeability of these films. Although increasing mobility generally resulted in increased permeability, conditions were found in which the plasticiser induced segmental motions in amorphous amylose without appreciable loss in oxygen barrier properties.

In powder particles, the stability of embedded lipid phase was studied in traditional carbohydrate carriers and modified starches, and in whey protein isolate. The powders were stored under controlled conditions, and the effect of relative humidity on the rate of oxidation was studied by following the increase in peroxide value during storage. Formation of hydroperoxides is linked with oxygen transfer in the system, as it is the oxygen consuming step of the reaction. The release of limonene and carvone from carbohydrate matrices was studied as a function of time and temperature. The starting hypothesis of this work was that a higher water vapour sorption at higher humidity would increase oxygen permeation in the matrices and lead to an increased rate of oxidation. This was in fact found to be the case in carbohydrate matrices during storage at 20°C. Intense release of volatiles was found in the proximity of the glass transition temperature in all the systems. In whey protein isolate matrix, oxidation of matrix-embedded oil was retarded compared to that of bulk oil at all humidities, but followed almost the same pattern as bulk oil with respect to humidity. The rate of oxidation was high at low humidities, was retarded at intermediate humidities and again increased at high humidity. Therefore, water did not have a similar role in the matrix formed of globular proteins as it had in a glassy carbohydrate matrix. The role of protein in controlling mass transfer is likely to be linked with the dense interfacial layer.

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Nimeke

Hapettumisstabiilisuus ja molekyylien liikkuvuus elintarvikematriisien pehmittyessä
Veden merkitys

Tiivistelmä

Veden merkitys elintarvikkeiden rakenteessa ja säilyvyydessä on suuri, ja veden pehmitinvaikutusta elintarvikkeiden rakennepolymeereihin on tutkittu runsaasti 25 viime vuoden aikana. Elintarvikkeiden säilyvyyttä arvioidaan pitkälti vedenaktiivisuuden ja matriisin fysikaalisen tilan perusteella. Olisi tärkeää ymmärtää, vaikuttaako vesi samalla tavoin tärkeimpien elintarvikepolymeerien, tärkkelyspohjaisten hiilihydraattien ja proteiinien muodostamiin rakenteisiin. Tässä väitöskirjatyössä veden merkitystä systeemin säilyvyydessä ja osuutta biopolymeerien vuorovaikutuksiin tutkittiin kahdessa eri matriisissa: valukalvoissa pehmittimen kanssa ja ilman pehmitintä sekä sellaisissa sumutuskuivatuissa partikkeleissa, jotka sisältävät dispergoidun lipidifaasin.

Amyloosikalvojen pehmittymistä veden ja glyserolin vaikutuksesta tutkittiin protoni-NMR-relaksaatiomittauksilla. Lasimaisen amyloosin liikkuvuus riippui lasisiirtymän etäisyydestä vain vähän. Kumitilassa suuri glyserolipitoisuus (30 %) lisäsi amyloosin liikkuvuutta huolimatta faasierottumisesta, joka tapahtuu jo huomattavasti alhaisemmassa pitoisuudessa. Mittaustulokset amorfisen amyloosin liikkuvuuden lisääntymisestä pehmittymisen tuloksena yhdistettiin aiempiin, kalvojen hapenläpäisymittauksista saatuihin tuloksiin. Polymeeriketjujen lisääntyvä liike johti myös läpäisyn lisääntymiseen, mutta suureiden välillä ei ollut yksiselitteistä riippuvuutta.

Dispergoidun lipidifaasin hapettumisstabiilisuutta tutkittiin perinteisissä hiilihydraattikantajissa – hydrolysoidussa ja muunnellussa tärkkelyksessä – sekä heraproteiini-isolaatissa. Jauheet säilytettiin säädetyissä olosuhteissa, ja ympäristön suhteellisen kosteuden (RH) vaikutusta hapettumisnopeuteen tutkittiin määrittämällä öljyn peroksidilukua säilytyksen aikana. Hydroperoksidien muodostuminen kytkeytyy hapen kulkuun matriisissa, koska se on pilaantumisreaktion happea kuluttava vaihe. Haihtuvien aineiden osalta tutkittiin limoneenin ja karvonin vapautumista säilytysajan ja lämpötilan funktiona. Työn lähtöoletuksena oli, että suurempi vesihöyryn sorptio suuremmassa kosteudessa lisää hapen kulkua matriisissa ja siten hapettumisnopeutta. Tämä piti paikkansa hiilihydraattimatriisien osalta 20 °C:ssa. Haihtuvien yhdisteiden voimakas vapautuminen havaittiin lähellä lasipistelämpötilaa molemmissa hiilihydraattimatriiseissa. Heraproteiinimatriisi hidasti öljyn hapettumista kaikissa olosuhteissa, mutta kosteuden vaikutus hapettumisnopeuteen oli sama kuin sellaisenaan tutkitussa öljyssä. Öljy hapettui nopeasti kuivissa olosuhteissa, hitaammin keskikosteuksissa ja jälleen nopeammin kosteissa olosuhteissa. Siten vedellä ei voitu havaita hapettumiseen tutkitussa proteiinimatriisissa vastaavaa vaikutusta kuin hiilihydraattimatriisissa. Proteiinin muodostamalla tiiviillä rajapintakerroksella (öljy-matriisi-rajapinta) on mahdollisesti muuta matriisia tärkeämpi rooli aineensiirron säätelijänä.

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