

# Preservation of oleic acid entrapped in a condensed matrix of high-methoxy pectin with glucose syrup



Vilia Darma Paramita, Anna Bannikova, Stefan Kasapis\*

School of Applied Sciences, RMIT University, Melbourne, Victoria 3000, Australia

## ARTICLE INFO

### Article history:

Received 31 May 2014

Accepted 11 August 2014

Available online 21 August 2014

### Keywords:

High-methoxy pectin

Glucose syrup

Oleic acid

Glass transition

Diffusion coefficient

## ABSTRACT

This investigation deals with the diffusional mobility of essential fatty acids in triglyceride form (oleic acid) from a condensed matrix comprising 3% (w/w) high-methoxy pectin and 81% (w/w) co-solute (glucose syrup) to absolute ethanol. Work utilises rheological measurements in shear, differential scanning calorimetry, ESEM, FTIR and WAX diffraction to identify the molecular properties of the composite system. Results showed that the amorphous carbohydrate matrix underwent vitrification at  $-15\text{ }^{\circ}\text{C}$  (mechanical  $T_g$ ) calculated mathematically using the Williams, Landel and Ferry (WLF) and modified Arrhenius equations. Diffusion kinetics of the fatty acid (monitored *via* UV-vis spectroscopy) was combined with the newly introduced concept of spectroscopic shift factor to demonstrate that, although the increment of oleic acid mobility appeared to respond to the predicted glass transition temperature, this is distinct from the structural relaxation of the matrix. Experimental observations were further treated with the concept of diffusion coefficient to provide an estimate of the transport rate of the triglyceride as a function of time or temperature of observation within the glass transition region.

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## 1. Introduction

It is known that essential fatty acids are susceptible to oxidation in the presence of oxygen, metal, light and heat (Gunstone, 1996). Therefore, recent molecular studies are mainly interested in the prevention of their oxidation during preparation and subsequent storage of foods. Common approaches for the delivery of essential ingredients like fatty acids in food and nutraceutical products include the addition of antioxidants, modification and control of packaging, and the technique of microencapsulation being used for more than 60 years. These techniques in delivery of nutrition and bioactivity remain largely empirical. However, recent innovation in the preservation of bioactive compounds has been pursued by utilising the “sophisticated synthetic polymer approach”, which relates to the capacity of condensed systems (>70% solids) to control the behaviour of food components near the glass transition temperature (Roos, 2013; Slade & Levine, 1991).

Vitrification or glass transition is the process occurring within liquid-like materials characterised by a disordered molecular arrangement during cooling at rates sufficient to avoid crystal

formation. In these conditions, molecules become immobilised as very high viscosity liquids, an outcome that limits their molecular mobility (Jiang & Kasapis, 2011). Glass transition temperature ( $T_g$ ) is then delineated as the reference temperature where amorphous materials change in structure from a hard solid to rubbery or melt consistency (Levine & Slade, 1992). Most biopolymers exhibit distinct glass transition temperatures, which are governed by their conformational characteristics, molecular weight distribution, amount of water in the system and composition of co-solute (Abiad, Carvajal, & Campanella, 2009).

Glass formation in condensed systems has received considerable attention due to its capacity to control chemical, biological and enzymatic reactions (Karel et al., 1994; Karmas, Buera, & Karel, 1992; Le Meste, Champion, Roudaut, Blond, & Simatos, 2002). At the glassy state, the rates of these molecular processes slow down considerably facilitating preservation of biomaterials and food-stuffs (Roos, 1998, 2005; Sablani, Kasapis, & Rahman, 2007). Recent studies have demonstrated the effect of  $T_g$  in controlling the enzymatic hydrolysis of  $\alpha$ -D-glucosidase in a high solid matrix comprising deacylated gellan and polydextrose (Chaudhary, Small, & Kasapis, 2013). Similarly, a limitation in deteriorative reactions including non-enzymatic browning and lipid oxidation below the vitrification temperature has been earlier reported (Aktas & Akkose, 2010; Karel & Saguy, 1991; Roudaut, Van Dusschoten, Van As, Hemminga, & Le Maste, 1998). Jiang and Kasapis (2011)

\* Corresponding author. Tel.: +61 3 992 55244; fax: +61 3 992 55241.

E-mail addresses: [stefan.kasapis@rmit.edu.au](mailto:stefan.kasapis@rmit.edu.au), [stefankasapis@hotmail.com](mailto:stefankasapis@hotmail.com) (S. Kasapis).

and Kasapis and Shrinivas (2010) reported on the importance of  $T_g$  in controlling the diffusion kinetics of a bioactive compound (caffeine) in high solid systems with industrial interest.

This work takes advantage of the glass transition process in high-solid carbohydrate matrices as a reference point to curb oxidation of essential fatty acids. Since lipid oxidation is dependent on the rate of diffusion and regulated with changes in temperature or time, we attempt to provide an understanding of the patterns of molecular diffusion with further expectations of building a foundation to control the delivery and preservation of lipid components. A combination of fundamental analytical techniques is utilised in combination with a mixture of high-methoxy pectin and high dextrose-equivalent glucose syrup owing to the capacity of this matrix to mimic confectionary, vegetarian and nutraceutical end products.

## 2. Materials and methods

### 2.1. Materials

High-methoxy pectin from citrus peel was purchased from Sigma Aldrich Co (Sydney, Australia) and contained 93.3% of polysaccharide on dry weight basis of which 86.3% was galacturonic acid (Gal-A) linked by  $\alpha$ -(1–4) glycosidic bonds with a degree of methyl esterification (DE) of about 65%.

Glucose syrup, as the co-solute, was a product of Edlyn Foods Pty Ltd (Victoria, Australia). The total level of solids was 81% with 40–45% of glucose residues present as reducing end groups (dextrose equivalent, DE is about 42). The material converts from a thick solution at ambient temperature to a transparent glass at subzero temperatures.

1-Oleoyl-*rac*-glycerol, as a source of oleic acid (OA) in its glyceride form, was obtained from Sigma Aldrich Co (Sydney, Australia). It contained 40% monoglyceride and 60% di- and triglyceride mixture (TLC) with an average molecular weight of 356.54 g/mol.

### 2.2. Sample preparation

The polysaccharide solution was prepared by dissolving the powder in Milli-Q water at 90 °C with constant stirring on the hot magnetic plate until the powder was fully dissolved and a clear solution was obtained. The temperature was then dropped gradually to 50 °C prior to addition of the required amount of glucose syrup. A weighed amount of 1-oleyl-*rac*-glycerol (1%, w/w) was then added to 3% (w/w) high-methoxy pectin with 81% (w/w) glucose syrup. The solution was maintained at 50 °C until ingredients were fully dispersed and the desired level of total solids, i.e. 85% (w/w), was obtained by evaporating slowly excess water using a rotary vacuum evaporator. The pH of the mixture was adjusted to 3.0 dropwise with 2 M HCl solution to form a three dimensional structure. Beakers with final preparations were wrapped in aluminium foil to reduce exposure to light. A similar experimental protocol of sample preparation was utilised for matrices of 85% (w/w) glucose syrup, and mixtures of 3% high-methoxy pectin with 82% glucose syrup (w/w) to provide the necessary baseline of behaviour in our studies.

### 2.3. Experimental analysis

#### 2.3.1. Modulated differential scanning calorimetry (MDSC)

Measurements were carried out using Q2000 (TA instruments, New Castle, DE) with a refrigerated cooling system (RCS90). Thermal properties of the polysaccharide and co-solute matrices with and without the addition of oleic acid were thus recorded. Cooling or heating profiles yielded an estimation of the enthalpic relaxation

in relation to vitrification processes. Ten milligrammes of sample were loaded to the  $T_{zero}$  aluminium pans, with a hermetically sealed empty pan serving as the reference. Samples were equilibrated at 20 °C for 1 min before cooling from 20 to –90 °C followed by heating to 20 °C. Nitrogen purged into the cell chamber at the rate of 50 mL/min. To calibrate the heat flow signals, traceable indium standards ( $\Delta H_f = 28.3$  J/g) were used and the heat capacity response was pinpointed with a sapphire standard. The temperature ramp was set at 1 °C/min with a modulation rate of 0.53 °C for every 40 s, and all measurements were performed in triplicate yielding overlapping traces in thermograms.

#### 2.3.2. Rheology measurements

These were performed using small deformation dynamic oscillation in shear with the Advanced Rheometer Generation 2 (AR-G2 from TA Instruments, New Castle, DE), which is a controlled strain rheometer with magnetic thrust bearing technology. They provide readings of storage ( $G'$ ) and loss ( $G''$ ) modulus for the elastic and viscous components of the network, respectively, with the variation of experimental time and temperature.

Molten preparations of 3% high-methoxy pectin and 81% glucose syrup with 1% oleic acid were loaded on the preheated Peltier plate at 90 °C using a 10 mm parallel plate measuring geometry, and edges were covered with silicone oil (BDH, 50 cS) to minimize moisture loss. Controlled cooling rate of 1 °C/min, oscillatory frequency of 1 rad/s and strain of 0.01% were applied throughout the experiment (maintaining a normal force of 0.08 N), which was within the linear viscoelastic region of the material. Materials were cooled to –30 °C, and frequency sweeps were then obtained from the low temperature end to 8 °C at intervals of 4 °C within the range of 0.1–100 rad/s. Mechanical spectra were utilised to produce the master curve of viscoelasticity based on the Time Temperature Superposition (TTS) principle for the estimation of the network glass transition temperature in these systems.

#### 2.3.3. Fourier transform infrared spectroscopy (FTIR)

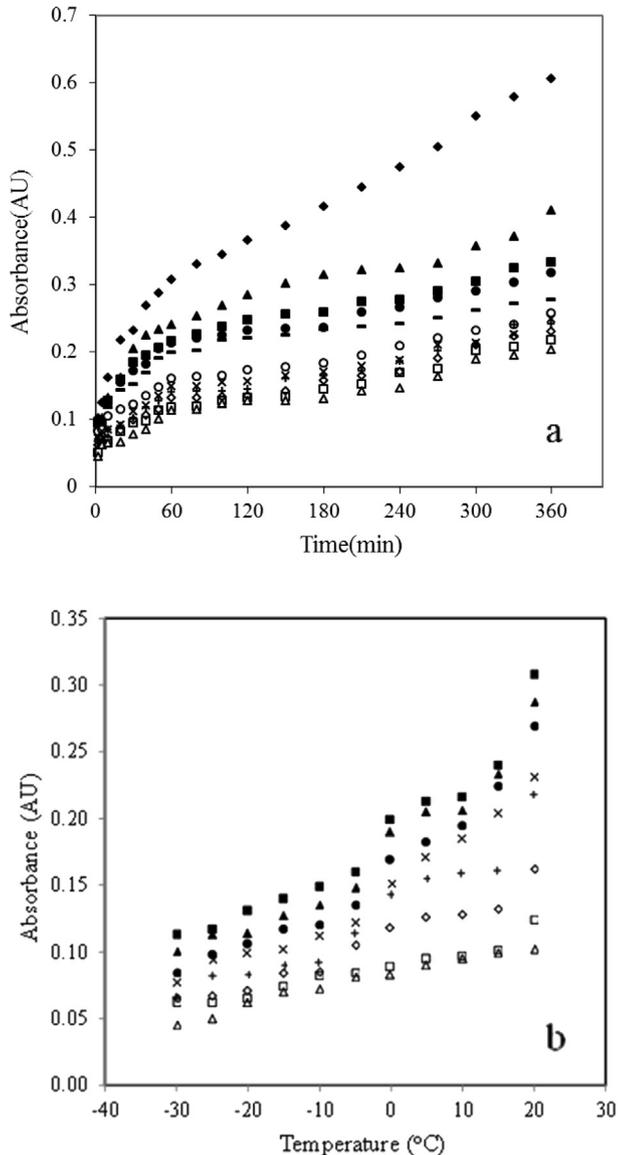
This type of work was carried out using a Perkin Elmer Spectrum 100 with MIRacle™ ZnSe single reflection ATRplate (Perkin Elmer, Norwalk, CT). Samples of high-methoxy pectin, glucose syrup, oleic acid and their combinations were analysed to identify potential molecular interactions amongst the polysaccharide, co-solute and essential fatty acid to further determine the nature of any alterations in molecular structure. Preparations were scanned within the range of 600–4000  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$  and averaged over eight scans. Each measurement was performed in triplicate.

#### 2.3.4. Wide angle X-ray diffraction (WAXD)

Diffraction patterns of single preparation of 3% high-methoxy pectin and 81% glucose syrup and their mixtures with or without oleic acid were obtained using a Bruker D4 Endeavour (Karlsruhe, Germany). All samples were freeze dried, placed in measuring compartments and exposed to the accelerating voltage and current of 40 kV and 40 mA, respectively. Raw data was obtained within the  $2\theta$  range of 5–90° in measuring intervals of 0.1° and converted using DIFFRAC<sup>plus</sup> Evaluation (Eva), version 10.0, revision 1. Results were used to assess the extent of amorphicity or crystallinity in the macromolecular structure of the carbohydrate network in the presence or absence of fatty acid. Measurements were performed in triplicate.

#### 2.3.5. Environmental scanning electron microscopy (ESEM)

Micrographs of high-methoxy pectin and glucose syrup with or without oleic acid were obtained using the FEI Quanta 200 ESEM (Hillsboro, Oregon, USA) in order to visualise the three dimensional



**Fig. 8.** Absorbance of 1% oleic acid diffused to absolute ethanol from 3% high-methoxy pectin with 81% glucose syrup: (a) as a function of time of observation (360 min) at  $-30$  ( $\Delta$ ),  $-25$  ( $\square$ ),  $-20$  ( $\diamond$ ),  $-15$  ( $+$ ),  $-10$  ( $\times$ ),  $-5$  ( $\circ$ ),  $0$  ( $-$ ),  $5$  ( $\bullet$ ),  $10$  ( $\blacksquare$ ),  $15$  ( $\blacktriangle$ ) and  $20$  ( $\blacklozenge$ ); (b) as a function of temperature of observation (60 min) at  $2$  ( $\Delta$ ),  $5$  ( $\square$ ),  $10$  ( $\bullet$ ),  $15$  ( $\blacktriangle$ ) and  $20$  ( $\blacklozenge$ ),  $-30$  ( $\times$ ),  $40$  ( $\bullet$ ),  $50$  ( $\blacksquare$ ) and  $60$  ( $\blacklozenge$ ) obtained at 525 nm.

unsaturated lipids with the carbonyl group of phospho-vanillin to form a coloured complex. The peak of UV-vis absorption at 525 nm has been confirmed experimentally scanning through a wide range of wavelengths from 700 to 200 nm. The relationship between oleic acid concentration and augmentation of the absorbance reading at  $\lambda_{\max} = 525$  nm resulted in a linear relationship that follows the Beer–Lambert Law from 0 to 1.4 AU (data not shown).

Absorbance data were recast as a function of temperature from  $-30$  to  $20$  °C in Fig. 8b. This temperature range covers the rubber-to-glass transition of the high solid HMP/glucose syrup matrix according to the thermomechanical observations in Figs. 1–5. Clearly, an increase in spectra intensity is recorded with raising the temperature and time of observation, whereas a relatively slow diffusion is recorded at short times within the mechanical glassy state, i.e. from  $-30$  to  $-15$  °C.

To rationalise the diffusion kinetics of oleic acid in the condensed HMP/glucose syrup matrix, we went back to the data in

Fig. 8a, which exhibit a good linear relationship ( $R^2 = 0.964$ ) within the first 60 min of observation. These spectra can be treated as the outcome of a zero-order reaction where the gradient provides the rate constant,  $k = dx/dt$ . Based on this, the spectroscopic shift factor was calculated for each experimental temperature to be (Kasapis & Shrinivas, 2010):

$$\log a_T = \log \frac{k_0}{k} \quad (4)$$

where,  $k_0$  is the rate constant at the reference temperature of  $-15$  °C.

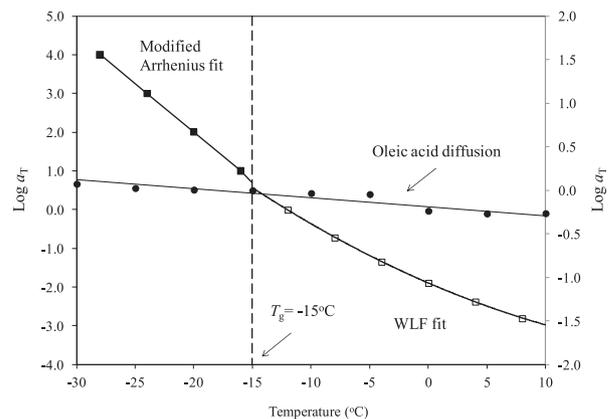
Fig. 9 reproduces the trend in spectroscopic factor as a function of experimental temperature, where it is plotted alongside the viscoelastic relaxation of the condensed matrix. Energies of activation for both systems were calculated from the modified Arrhenius Eq. (3) to produce values of about 24 and 251 kJ/mol, respectively, for the lipid and carbohydrate matrix. The relatively low energy of activation for oleic acid indicates a high free volume and, although, the vitrified network determines molecular mobility in the tertiary composite, the diffusion kinetics of oleic acid are distinct from the structural relaxation of the polymeric matrix.

To identify the transport rate of the lipid in the medium of this investigation, we considered the concept of Fickian diffusion. The theoretical framework builds on a power law equation, where the values of kinetic diffusion exponent,  $n$ , can be used to identify a wide range of experimental conditions leading to a variety of absorption/desorption mechanisms, i.e. less Fickian, ideal Fickian, non-Fickian (anomalous), Case II, and super-Case II diffusion (Crank, 1975; Singh & Chauhan, 2009):

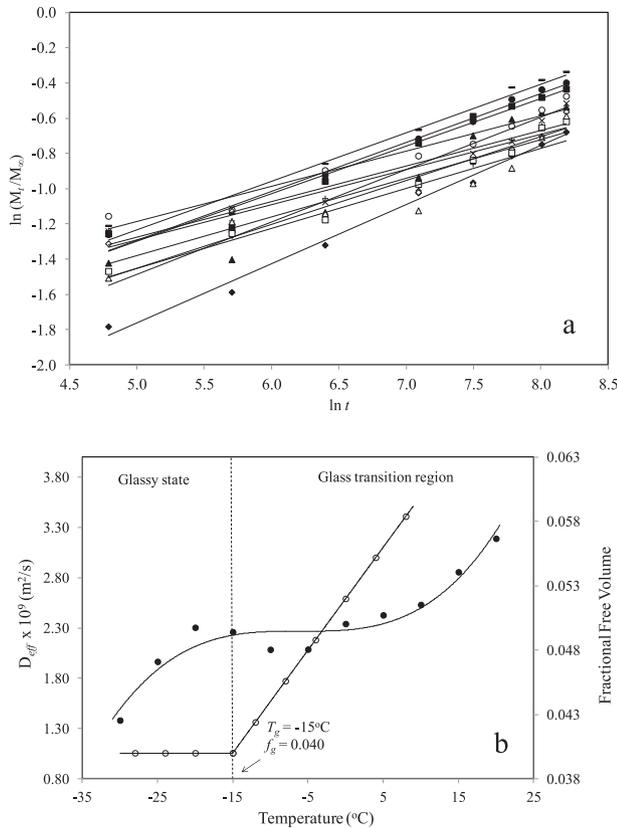
$$\frac{M_t}{M_\infty} = kt^n \quad (5)$$

where,  $M_t/M_\infty$  is the extent of bioactive compound release at experimental and equilibrium time,  $k$  is a constant characteristic of the bioactive compound-polymer system, and  $t$  is given in seconds.

Absorbance data from Fig. 8a were modelled within 60 min to produce relevant parameters from the slope and intercept in the plot of  $\ln M_t/M_\infty$  versus  $\ln t$  (Fig. 10a and Table 1). Literature proposes that for ideal Fickian kinetics the  $n$ -value should be equal to 0.5, whereas Case II transport is characterised by an  $n$ -value of 1.0, which makes the range of  $n$ -values between 0.5 and 1.0 a Non-Fickian or anomalous diffusion (Bajpai, Bajpai, & Shukla, 2001;



**Fig. 9.** Logarithmic shift factor  $a_T$  as a function of temperature within the glassy state (closed symbols) and glass transition region (open symbols) for 3% high-methoxy pectin with 81% glucose syrup and 1% oleic acid (left y-axis), and for the oleic acid diffusion from the carbohydrate matrix at the reference temperature of  $-15$  °C (right y-axis).



**Fig. 10.** (a) Absorbance  $\ln(M_t/M_\infty)$  with experimental time of observation ( $\ln t$ ) to obtain the diffusion exponent,  $n$ , and gel characteristic constant,  $k$ , for 3% high-methoxy pectin with 81% glucose syrup carbohydrate mixture of this investigation at  $-30$  ( $\Delta$ ),  $-25$  ( $\square$ ),  $-20$  ( $\diamond$ ),  $-15$  ( $+$ ),  $-10$  ( $\times$ ),  $-5$  ( $\circ$ ),  $0$  ( $-$ ),  $5$  ( $\bullet$ ),  $10$  ( $\blacksquare$ ),  $15$  ( $\blacktriangle$ ) and  $20$   $^\circ\text{C}$  ( $\blacklozenge$ ) within 60 min; (b) relative diffusion coefficient ( $D_{eff}$ ) of 1% oleic acid transported from the condensed carbohydrate system to absolute ethanol within 60 min ( $\bullet$ , left y-axis) and fractional free volume ( $f_v$ ) of the high solid matrix within the glassy state and glass transition region ( $\circ$ , right y-axis).

Wang, Wu, & Lin, 2008). Diffusion exponent values of this investigation were found to lie between 0.20 and 0.34, hence arguing for a less Fickian regime apparently due to the relatively slow mobility of the bulky triglyceride molecule within the high density carbohydrate network and the mainly hydrophobic or hydrophilic characteristics of the two systems.

This school of thought also allows estimation of the transport rate in the form of a diffusion coefficient,  $D_{eff}$ , in relation to the variable consistency of the polymeric matrix within the rubber-to-glass transformation. The diffusion coefficient can be readily

**Table 1**

Variation of the kinetic diffusion exponent ( $n$ ) and gel characteristic constant ( $k$ ) for oleic acid in high methoxy pectin and glucose syrup.

Temperature ( $^\circ\text{C}$ )	Kinetic diffusion exponent $n$	Gel characteristic constant $k \times 10^2$	Diffusion mechanism
20	0.34	7.54	Less Fickian
15	0.30	9.44	Less Fickian
10	0.27	4.45	Less Fickian
5	0.28	4.73	Less Fickian
0	0.28	4.08	Less Fickian
-5	0.20	1.53	Less Fickian
-10	0.20	1.94	Less Fickian
-15	0.20	1.93	Less Fickian
-20	0.22	2.97	Less Fickian
-25	0.25	4.93	Less Fickian
-30	0.23	3.89	Less Fickian

identified in Fick's second law, as follows (Busk & Labuza, 1979; Crank, 1975):

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (6)$$

where,  $c$  is the concentration of the diffusant molecule and  $x$  is the relative distance travelled.

Assuming that our sample is a finite slab with negligible edge effects, Fick's law can be solved in the form of an error function series (Siepmann & Peppas, 2011):

$$\frac{M_t}{M_\infty} = 4 \left( \frac{D_{eff} t}{L^2} \right)^{1/2} \left[ \frac{1}{\pi^{1/2}} + 2 \sum_{n=1}^{\infty} (-1)^n \text{ierfc} \frac{nL}{2\sqrt{D_{eff} t}} \right] \quad (7)$$

where, the term  $\text{ierfc } x$  represents the integrated complementary error function of  $x$ .

Moisture transfer studies in breakfast cereals has shown that only the first term in this series equation is significant, which, in the absence of the higher terms, yields the following relationship (Tutuncu & Labuza, 1996):

$$\frac{M_\infty - M_t}{M_\infty - M_i} = 4 \left( \frac{D_{eff} t}{\pi L^2} \right)^{1/2} \quad (8)$$

where,  $M_i$ ,  $M_t$ , and  $M_\infty$  denote the absolute amounts of the diffusant compound released at times zero, during experimentation and infinity/equilibrium, respectively, and  $L$  is the thickness of the slab.

Fig. 10b illustrates the outcome of employing Eq. (8) in the prediction of  $D_{eff}$  for the tertiary system of this investigation. It combines Fickian kinetics with the concept of free volume over the operational temperature of 20 to  $-30$   $^\circ\text{C}$ . There is a considerable drop in the values of diffusion coefficient from about  $3.3 \times 10^{-9}$  to  $1.3 \times 10^{-9}$   $\text{m}^2/\text{s}$  with controlled cooling and, in particular, below the mechanical glass transition temperature of  $-15$   $^\circ\text{C}$ . This is accompanied by a reduction in fractional free volume from about 0.06 at 20  $^\circ\text{C}$  to 0.04 in the glassy state of the carbohydrate matrix. Clearly, free volume is the molecular process that governs diffusional mobility of oleic acid in the condensed matrix of high methoxy pectin with glucose syrup. Thus the importance of network  $T_g$  and the structural relaxation of the polymeric matrix in determining the transport rate of entrapped lipid is documented.

#### 4. Conclusions

For some time now, work in this laboratory dealt with the interplay between free volume theory and the predictions of the reaction rate theory in shaping up the vitrification properties of high solid biomaterials. More recently, model food systems were designed by incorporating bioactive compounds and hydrolytic enzymes in the high solid preparations. However, basic understanding of the physics of vitrification needed a description of the time dependence for the mass transfer of diffusant molecules within a polymeric matrix. The present work addresses this gap in knowledge by examining the spread of oleic acid molecules, driven by a concentration gradient, within the condensed high methoxy pectin/glucose syrup mixtures. The mechanical transformation from the rubbery to glassy consistency leads to a reduction in free volume, which has a profound effect on the diffusion of oleic acid. Operational kinetics comply with less Fickian modelling, an outcome that makes sense considering the bulky nature of the triglyceride and its disparate polarity to the hydrophilic matrix. The low transport rate contrasts strongly with the rapid (non-Fickian)

diffusion of the relatively small molecule of vitamin C within the congruent polarity of a high-methoxy pectin and polydextrose environment reported elsewhere (Panyoyai, Bannikova, Small, & Kasapis, 2015).

## Acknowledgements

The Australian Awards scholarship provided to Vilia Darma Paramita is duly acknowledged.

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