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Fat hydrolysis in a food model system: effect of water activity and glass transition

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Abstract: The objective of this research was to study the effect of water activity and glass transition temperature on the fat hydrolysis in a food model system. The model system was prepared with tapioca starch, casein, palm oil and sugar as 58, 14, 16 and 12 g/100 g model matrix, respectively. Hydrolysis reaction was accelerated by commercial lipase at six levels of water content and water activity. Moisture sorption isotherm was obtained using isopiestic method while monolayer value was determined by BET equations. Glass transition temperature was determined from amorphous ingredients of starch and casein. Hydrolysis reaction showed a significant increase above the monolayer value at 3.55 g water/100 g solid and aw 0.19. Hydrolysis occurred even at the glassy state of the model system. The role of water in the hydrolysis reaction is more related to the water activity concept rather than glass transition concept.

Keywords: Water activity, glass transition, moisture sorption isotherm, fat hydrolysis, food model

Introduction

Water activity has been used for a long time as stability parameter for microbial growth, non enzymatic and enzymatic reactions, also lipid oxidation. Stability map from Labuza has been widely adopted as a reference map to design stable food product. In general, the rule of water activity concept is: Food products are most stable at their “BET-monolayer moisture content” or “BET-monolayer water activity” and unstable above or below BET monolayer (Rahman, 2006; 2009; 2010).

Another concept is the glass transition (Tg) introduced by Slade and Levine in the early 1990. Glass transition can be described as a change that occurs in amorphous materials from a high viscosity, “frozen” glassy state to a lower viscosity, rubbery state. This condition is assumed to play an important role in the stability of food. Rahman (2006; 2009) and Sablani *et al*. (2007) stated that the rules of glass transition concept are (i) food are most stabile at and bellow glass transition; and (ii) the higher T-Tg (i.e. above glass transition), the higher the deterioration or reaction rates. Many researchers showed evidences that non-enzymatic browning reaction was affected by Tg (Lievonen *et al*. 1998; Lievonen *et al*., 2002; Karmas et al., 1992; Miao and Ross, 2004; Adawiyah *et al*., 2005), vitamin degradation (Bell and White, 2000) and fat oxidation (Rahman *et al*., 2009). However some reactions such as the degradation kinetic of antocyanin (Gradinaru, 2003), hydrolysis rate of sucrose by invertase (Chen *et al*., 1999), activity of β-galactosidase enzyme (Burin, 2002) did not appear to be affected by Tg.

One of the chemical reactions that affect the quality of food during storage is fat hydrolysis reaction. Fat hydrolysis breaks down the acyl groups of triglycerides and produces free fatty acid. This reaction has negative effect on the quality of food and produces off-flavor. Hydrolysis reaction is strongly influenced by the presence of water in the system. Water acts as a controller of diffusion process, dissolution of solute, proteins and enzymes activation. Water also acts as the reactants and co-substrate. The aim of this research was to study the effect of water activity and glass transition temperature on the fat hydrolysis in a food model system.

Materials and methods

Materials

Food model system was prepared from tapioca starch, casein, palm oil and sugar, as 58, 14, 16 and 12 g/100 g model matrix, respectively. The food matrix was then dried with chemo-reaction absorption by placing it in a dessicator containing CaO for two weeks. Lipase enzyme (Lypozim TL100L) was obtained from Novozym.

Moisture sorption isotherm

Moisture sorption isotherm of food model was determined by isopiestic method (Rahman and Al-

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Belushi, 2006). Dried food model (around 2 g) was placed in small dessicator while maintaining its relative humidity (RH) using saturated salt solution (RH 7 – 97%) until steady state condition is achieved. The dessicators were placed in incubator controlled at 30°C. Steady state could be established if the weight difference was no more than 2 mg/g solid and high RH was no more than 10 mg/ g solid in three consecutive weighing (Lievonen and Ross, 2002).

Modelling of moisture sorption isotherm curve was carried out by GAB (*Guggenhaim-Anderson-deBoer*) (equation-1.), and BET (*Brunauer-Emmett-Teller*) (equation – 2.).

GAB equation is:

$$\frac{M}{M_g} = \frac{C_g k a_w}{\left[(1 - k a_w)(1 - k a_w + C_g k a_w)\right]} \qquad (1)$$

Where M_m = GAB monolayer moisture content (dry basis); C_g = monolayer heat sorption constant and k = correction factor related to heat of sorption of multilayer.

BET equation is :

$$\frac{a_w}{(1 - a_w)M} = \frac{1}{M_m C_b} + \frac{C_b - 1}{M_b C_b} a_w \qquad (2)$$

Where M_b = BET monolayer moisture content (dry basis, g water/g solid); C_b= a constant of heat sorption.

Glass transition temperature measurement

The glass transition temperature of equilibrated food models in various RH were measured by DSC (*Differential Scanning Calorimeter*) Perkin Elmer series 7 at material science center BATAN Serpong. DSC was calibrated by using indium (melting point 156.78°C). Ten mg of samples were put in aluminum pan and closed hermetically. Measurements were carried out from 20 – 200°C with heating rate 10°C/ minute. Tg was determined by plotting the first derivative of glass transition curve that showed a peak at the midpoint Tg value.

Hydrolysis experiment

Dried food models were mixed with water at six different levels of water content that was equivalent to a_w 0.11, 0.33, 0.57, 0.75, 0.84 and 0.91. Lypase was added 1% (v/w) from oil/fat content or 0.16% (v/w) from total dry solid. The mixture was then stored at 30°C and analyzed for free fatty acid content periodically. Free fatty acid content was analyzed using AOAC method (1995). Ten grams of food model samples were added with 50 ml ethanol before boiled. The samples were then filtered and titrated by NaOH 0.1N. Free fatty acid content was determined as palmitic acid (BM=262) using equation (3)

$$FFA = \frac{V \times N \times M}{10 \times MW} \qquad (3)$$

Where: FFA = free fatty acid content (%), V = volume of NaOH for titration, N = normality of standardized NaOH, M = weight of samples (g), MW= molecular weight of palmitic acid (262).

Results and discussion

Moisture sorption isotherm

Moisture sorption isotherm were plotted and fitted with BET and GAB models (Fig 1). The GAB model was fitted at the whole range of a_w 0.07 to 0.92. The GAB parameters C_g, K and M_g were 11.80, 0.93 and 4.09 gr water/100 g solid. As predicted, BET model only fitted in limited range (0.07 – 0.57). BET parameters C_b and M_b were 18.18 and 3.55 gr water/100 g solid. The M_b was BET-Monolayer moisture content equivalent to a_w 0.19. BET-monolayer was recomended for stability determination. According to Rahman (2006; 2009) BET monolayer estimation is an effective method for estimating the ammount of water molecules bound to specific polar sites in a food matrix and also stability of food product.

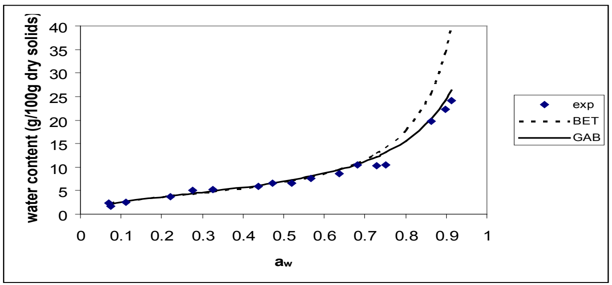

Figure 1. Moisture sorption isotherm of food model at 30°C from experimental data (exp), BET and GAB model

Glass transition

The results of DSC analysis of food model showed multi-peak diagram derived from the ingredients (tapioca starch, casein, sugar) (Figure was not presented). Therefore, glass transition temperature determined from the amorphous ingredients, tapioca starch and casein.

The glass transition temperature, Tg, usually was detected from the shift of baseline or change of heat capacity over a temperature range before melting endothermic peak. In fact, it was very difficult to detect Tg because the transition was not clearly identified. The approach was done by tracing the individual peaks at first derivative curve as mid point of predicted Tg (Figure 2). This approach was also performed by several previous researchers such as Schenz *et al.* (1991) on sucrose, Biliaderis *et al.* (1991) on starch, Mitsuiki *et al.* (1998) on galactan, Mizuno *et al.* (1999) on casein. Figure 3 showed predicted Tg values of tapioca starch and casein at different levels of moisture content.

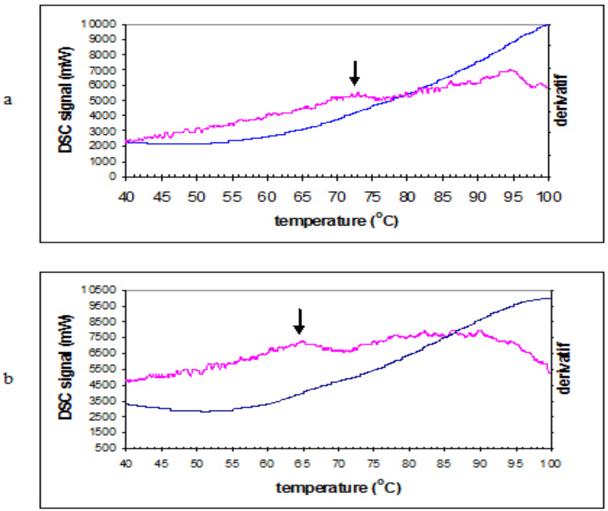

Figure 2. Determination of glass transtion of casein at moisture content 8% (a) and 12% (b)

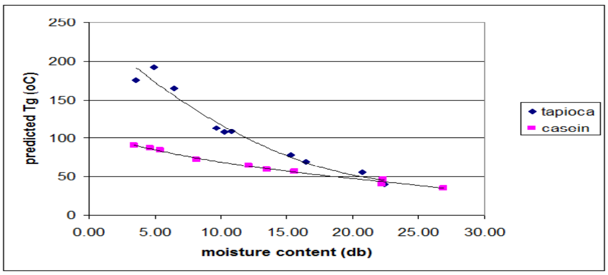

Figure 3. Predicted Tg values of tapioca starch and casein at different level of moisture content

Hydrolysis reaction

Figure 4 showed that the amount of free fatty acids (ffa) generated from enzymatic fat hydrolysis reaction was increasing with higher level of moisture content. At the lowest moisture content of 4.3%, the increase of free fatty acids did not seem significant. Water acts as co-substrate in a enzymatic fat hydrolysis reaction. Quantitative relationship of enzyme activity with the substrate is generally described using Michaelis Menten equation (equation-4) as the relationship between initial velocity (v) with the substrate concentration. Lipase enzyme initial velocity is determined using the rate or velocity of free fatty acids formation produced at the beginning of the reaction (slope of dotted the straight line in Figure 4).

$$V = \frac{V_{max}[S]}{K_m + [S]} \tag{4}$$

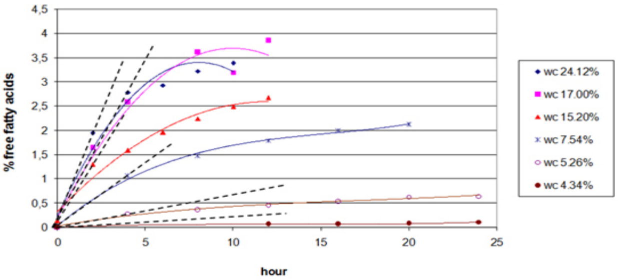

Figure 4. The effect of moisture content (wc) on free fatty acids formation from enzymatic fat hydrolysis in food model

The quantitative relationship of enzymatic reaction's initial velocity with substrate concentration according to Michael Menten's equation has a unique form that is hyperboled with two main parameters K_m (substrate concentration that produces half the maximum velocity) and V_{max} (maximum velocity) (Lehninger, 1993). Plotting of initial velocity (V) with the amount of water (as the substrate concentration or [S]) had sigmoid shape curves (Figure. 5). The pattern was similar to lipase enzyme activity with the substrate triacetin (Shahani, 1975) which explains that the substrate did not hydrolyze until saturation point of emulsion formation. Lipase showed unique kinetics since this enzyme had limited or no activity at low [S]. When [S] achieved the solubility limit reached, known as CMC (critical micellar concentration), it is also accelerated the lipase activity to formed oil-water interface (Hariyadi, 1998). The term oil-water interface is also revealed as lipid monolayer. Rao and Damodaran (2002) explained that the difference between the kinetics of lipase enzyme activity and Michaelis-Menten's pattern is mainly due to penetration stages of enzyme in lipid monolayer. Thus the role of water in the hydrolysis reaction was the formation of CMC or lipid monolayer. It will be activate lipase enzyme and initiate hydrolysis reaction process to break down acyl groups from triacylgliserol molecules.

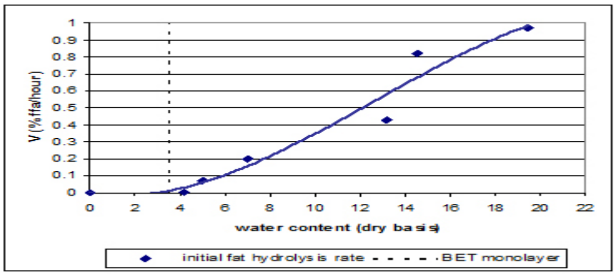

Figure 5. The effect of moisture content on initial fat hydrolysis rate in food model

Figure 5 also showed the effect of moisture content on initial fat hydrolysis rate in food model. Hydrolysis reaction did not occur (V~0) bellow monolayer value and tended to increase when it exceeded monolayer value. The water at monolayer was tightly bound to a specific side of the solid through hydrogen bonding or dipole interactions (Van den Berg, 1981; Schnepf, 1989). In food model, monolayer water was bound to the active polar groups of starch and protein components. Monolayer water could not be used for hydrolysis reactions to produce free fatty acids. Hydrolysis reaction rate was significantly increased in multilayer. Water in this region had lower degree of bound than monolayer that can be used for the hydrolysis reaction. According to Van den Berg (1981), dissolving process of solute and chemical/biochemical reactions were accelerated in this water region depending on the solid characteristics.

Another objective of this study was to study the effect of material state (glassy or rubbery) reflected in the value of the difference between storage temperature and glass transition temperature (T-Tg). In the condition where T-Tg value is less than zero (negative), the material is stored in glassy state while if the value of T-Tg is greater than zero (positive), the material is stored in rubbery state. According to Slade and Levine (1991), in the glassy system, the mobility of molecule and diffusion process was very slow or even non-exist due to the high viscosity of the matrix. Figure 6 showed that hydrolysis reactions occurred when conditions of main amorphous ingredients of food model i.e. tapioca starch and casein were in glassy state. The same results were also shown by the other researchers. Chen *et al.* (1999) did not see any dramatic changes of the reaction constants of sucrose hydrolysis by invertase enzyme around the glass transition temperature (T - Tg = 0). Sucrose hydrolysis is more affected by water activity than Tg.

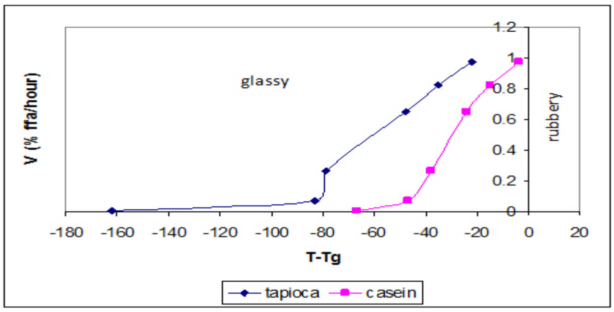

Figure 6. The effect of glass transition on fat hydrolysis rate in food model

Champion *et al.* (2000) stated that some enzymatic reactions occurred in low water levels or in frozen state such an enzymatic reaction by alkaline phosphatase, lipoksigenase, lipase or invertase. Chemical and physical changes related to glass transition temperature could not be use to explain enzymes activity. Changes at the molecular level could not be explained from supramoleculer level through the glass transition temperature parameters or structure collapse (Burin *et al.*, 2002).

Different mechanism was found in fat oxidation. Rahman (2009) reported that for fat oxidation, glass transition concept explained the process more adequately than the water activity concept. The glass transtion indicates breaks in the slope of fat oxidation rate constant. The experiment was carried out at two different levels of moisture content and five storage temperatures. The experiments of temparature dependence on enzymatic hydrolysis rate were limited by optimum temperature of enzyme activity. Optimum temperature for lipase was around 30-40°C. Decreasing rate of free fatty acid content in low temperature or high temperature was mostly affected by decreasing of enzyme activity.

Conclusion

The rate of enzymatic fat hydrolysis was affected by moisture content in food models. BET monolayer moisture content was 3.55 gr water/solids (a_w 0.19). Hydrolysis reaction rate increased significantly above BET monolayer. Hydrolysis reaction occurred when the two amorphous ingredients of food model i.e. tapioca starch and casein were in glassy state. Thus the enzymatic fat hydrolysis reaction in food model was more relevantly described by water activity concept at BET monolayer value than by glass transition.

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