2. LITERATURE REVIEW

2.1 Overview on Browning Reactions

The definition of “browning” in relation to food refers to several different processes which can be grouped as enzymatic and non-enzymatic browning reactions. Browning occurs when heat is applied to sugars (mono- and oligosaccharides). The effect of browning depends on a variety of factors such as the temperature, activity water, pH, concentration, and presence of other materials (Nursten and O’Reilley 1986; Lingnert 1990; Wijewickreme and Kitts 1997). In general, browning reaction is at first dehydrate, then leading to a further complex series of reactions and end-products. These whole reactions are not fully well understood, even though there had been many studies on some likely pathways and some frequently encountered intermediates.

As mentioned above, the effect of heat on simple sugars has generally been considered as two types of browning reactions, which are, enzymatic and non-enzymatic browning. Both lead to the development of brown color. This browning reaction has been fallen into three categories (Priestley 1979); First category is caramelisation of sugars, which occurs at high temperatures (above 100 °C) and under acidic or alkaline conditions. Caramelisation results in light to dark brown and new flavors in the product. Caramelisation plays an important role in roasting of coffee and commercial caramels are added as food colors or flavors. Second category is browning of vitamin C (ascorbic acid), the mechanism for browning via this method is speeded up by the presence of amino acids. The last category is Maillard reaction, which occurs between a compound with carbonyl group (usually reducing sugars) and compounds with free amino groups. Examples of a Maillard reaction are the flavors and colors produced during baking bread or frying meat.

2.2 Chemistry of Maillard Reaction

Maillard browning reaction is a type of non-enzymatic browning which involves the reaction of simple sugars (carbonyl groups) and amino acids (free amino groups). They begin to occur at lower temperatures and at higher dilutions than caramelisation. Hodge subdivides the Maillard reaction pathways as follows: 1) Initial stage with colorless products and without absorption in the ultraviolet.
This stage consisted of reaction A (Sugar-amine condensation) and reaction B (Amadori rearrangement); 2) Intermediate stage with colorless or yellow products and with strong absorption in the ultraviolet. This stage consisted of reaction C (Sugar dehydration), reaction D (Sugar fragmentation), and reaction E (Amino acid degradation (Strecker degradation); and (3) final stage which has highly colored products. This stage consisted of reaction F (Aldol condensation), reaction G (Aldehyde-amine condensation and formation of heterocyclic nitrogen compound).

The way these reactions fit together is outlined in Figure 1. Theoretically, the distinction is clear; however, in practice, it is very difficult to classify the dark brown products formed in foods, since they tend to be very complex mixtures and
are chemically relatively intractable. Reaction H has been inserted into Figure 1. It represents the much more recently discovered free radical breakdown of Maillard intermediates. Oxygen plays an essential role in enzymic browning, but is not essential for non-enzymic browning (Nursten 2005). It may help in fact, for example, in the formation of reductones, such as dehydroascorbic acid, but it may also hinder the progress of the reaction, for example, in oxidizing 2-oxopropanoic acid.

As mentioned before, the Maillard reaction can be divided into three stages:

*Initial stage*

The very first step of the Maillard reaction, involves a condensation between a carbonyl compound and an amino compound (Hodge 1953). The condensation takes place by attack of the sugar at the carbon of the carbonyl group by nucleophilic amino nitrogen. Protonation of the carbonyl group enhances the reactivity of the nucleophilic, while protonation of the amino group weakens the attack on the carbonyl group (Namiki 1988). This leads to the formation of an N-substituted glycosylamine via the reversible formation of the Schiff base by elimination of molecule water. The N-substituted aldosylamine formed undergoes subsequent rearrangement to the 1-amino-1-deoxy-2-ketose (Amadori rearrangement products, ARP) by the Amadori rearrangement and if ketoses sugar were involved, and 2-amino-2-deoxyaldoses (Heyns rearrangement product) by the Heyns rearrangement (Hodge 1953).

The first step of the Amadori rearrangement of an N-substituted glycosylamine is acid-catalysed and involves opening of the hemiacetal ring, followed by protonation of the nitrogen to form the cation of the Schiff base (iminium ion) (Hodge 1955). Rearrangement of the latter, with loss of a proton, gives the enol (1,2-enaminol) form of the ARP. This then tautomerises to yield the keto form. When the Maillard reaction involves free amino acids as the amino compound, the carbonyl group of the amino may act as a catalyst by providing the necessary proton (Wong 1989).
Intermediate stage

This step differs, depending on the isomer of the Amadori compound. Either the amino acid is removed, which results in reactive compounds that are finally degraded to the important flavor components furfural and hydroxymethyl furfural (HMF). The other reaction is called Amadori-rearrangement, which is the starting point of the main browning reactions. Overall, there are four main pathways involved in this stage; first step is 1,2-enolisation which are formed by 1,2-enolisation. The allyl hydroxyl group on carbon 3 of the 1,2-eneamio1 is removed, and a double bond is created between carbon 2 and 3. Hydrolytic scission of the amine residue, previously very tightly attached to the molecule, is promoted, giving rise to the formation of a 3-deoxyosone. Further dehydration of the 3-deoxyosone and translocation of the double bond finally yields 5-hydroxymethyl-2-furfuraldehyde (HMF), when the original aldose is a hexose (Hodge 1967; Baltes 1982).

Second step is 2,3-enolisation. Decomposition of the ARP via 2,3-enolisation initially involves the formation of the 2,3-enediol. The formation of a double bond between carbon 2 and 3 facilitates the elimination of the allyl amine residue, and accordingly a 1-deoxyosone is produced. Further decomposition of the 1-deoxyosone gives rise to the formation of reductones and scission products, such as diacetyl and pyruvaldehyde (Hodge 1967; Baltes 1982).

Third step is 1-amino-1,4-dideoxyosone formation. 1-amino-1,4-dideoxyosone have not been isolated; however, the formation of certain Maillard reaction products can only be explained by their formation as intermediates. 2-aminoacetylfuran and aminoreductone are likely to form via 1-amino-1,4-dideoxyosones as intermediates (Ledl and Schleicher 1990).

Finally, the last step is Strecker degradation. Initially, an oxidative degradation of the amino acids occurs to form a Schiff base. This then enolises to an amino acid derivative, which is easily decarboxylated. Subsequent hydrolysis yields an α-aminoketone and the Strecker aldehyde of the amino acid (Hodge 1967; Mauron 1981; Wong 1989). The Strecker aldehydes produced condense with each other, furfurals and other dehydration products, with ultimate formation.
of brown pigments (Hodge 1953). The α-aminoketone may dimerise to yield pyrazines.

**Final stage**

In this stage, many of the compounds formed during the intermediate stage, such as enamino derivatives, low molecular weight sugar analogues and unsaturated carbonyl products, undergo further reactions, including polymerization, to yield, ultimately, brown polymers or melanoidins, the final products of the reaction. The main reactions involved are thought to be aldol condensation, aldehyde-amine reactions and the formation of heterocyclic nitrogen compounds (Hodge 1953; Namiki 1989).

### 2.3 Parameters Influencing the Maillard Reaction

The maillard reactions is affected by several factors which influence the difference chemical reaction involved. These factors are temperature, time, pH, water activity (a_w), and reactant source and concentration (Jing and Kitts 2002).

#### 2.3.1 Time and Temperature

The effect of heating duration on browning was actually studied by Maillard, who reported that the rate of browning increases with time and temperature. This report has been confirmed several times by other researcher (Kato and Fujimaki 1968, Lee and Nagy 1988). Time of heating which referred to length of heating is important as the formation of melanoidins usually occurs at longer time of heating at any given temperature (Mauron 1981) and different flavors are formed depending on the extent of reaction (Rizzi 1997).

#### 2.3.2 Reactant source and Concentration

One of the reactant source in Maillard reaction is reducing sugars which will condensed with a free amino group from amino acids or protein. This reducing sugars have different reactivity from one to another. There had been many studies who focused on sugars reactivity towards amino acids in maillard reaction, especially in aqueous system. O’Brien and Morrissey (1989) reported that aldoses sugar are more reactive than ketoses. This report were denied by other researcher (Brands et al. 2000; Ashoor and Zent 1984; Suarez et al. 1995) who convinced that ketose sugar fructose browned more fast than the aldose isomer glucose due to its higher proportion of acyclic forms in the solution. This
conflicting reports may be related to the great diversity of conditions used in the heating treatment, the indicators which used to measure the reaction, and most of all, amine sources such as amino acids or proteins used in the reaction. In general, pentoses sugar are more reactive than hexoses in sugar-amino acid models (Ashoor and Zent 1984). Furthermore, from the data result which used casein-sugar as a model system, confirmed that the pentose sugar, ribose, is more reactive than hexoses, such as glucose and fructose in browning intensity (Jing and Kitts 2000).

Table 1 Percentage of acyclic as function of pH

<table>
<thead>
<tr>
<th>Type of Sugar</th>
<th>pH 6.5</th>
<th>pH 7.0</th>
<th>pH 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.012</td>
<td>0.022</td>
<td>0.040</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.040</td>
<td>0.062</td>
<td>0.110</td>
</tr>
<tr>
<td>Ribose</td>
<td>10</td>
<td>18.5</td>
<td>30</td>
</tr>
</tbody>
</table>

Source: Cantor and Peniston (1940)

As we can see from the table above (Table 1), the amount of acyclic form is much higher for pentoses such as ribose than hexose. In that condition, we can say that pentose sugars react more rapidly than hexoses. Beside acyclic sugar concentration, the extent to which the reaction mixture is buffered also effect the relative rate of browning of glucose and fructose. In general, the rate of browning of fructose with amino acids is greater than that of glucose under unbuffered media. Sources of amino groups have a great effect on the rate of maillard reaction. Many studies has been carried out on the effect of free amino acids to maillard reaction rate. Ashor and Zent (1984) stated that lysine are the most reactive amino acids when compared to glycine, tryptophan and tyrosine. Lysine appears to be the most reactive amino acid because it has two available amino groups.

2.4 Color Formation in Maillard Reaction

There had been many studies who investigated the color formation of Maillard browning reaction (Ames 1987; Ledl and Schleicher 1990; Hofmann 1998a; Hofmann 1998b). However, there are only few studies on the maillard browning’s compound which suspected to cause the color formation. Most of the journal that has been citied, stated that the color formation in maillard occurs due
to the formation of high molecular weight (up to 12,000 Daltons) known as melanoidins which are formed by the reaction of the Amadori product or with other dicarboxyls with amino acids or protein (Labuza and Davis 1998). Moreover, conflict exists as to whether color formation are caused only by the formation of high molecular weight or low molecular weight.

Hofmann (1998a & 1998b) elucidated a new theory about compounds that formed in the maillard reaction and mainly responsible on color formation. He hypothesised that low molecular weight chromophores rather than high molecular weight melanoidins are in the main responsible for color formation in foods and that coloured low molecular weight compounds can cross-link proteins. There is evidence that color formation in food system are depends on the intensity of thermal treatment such as time and temperature (Fogliano et al. 1999). They reported that in wet gluten-glucose model system, color formation is poor and not many differences were detectable for temperatures up to 120° C but were increasing dramatically as the temperature raised until 150° C (seven times higher that at 120° C, from zero minutes after 45 minutes). Similar result were also reported but in different model systems (Glucose and free amino acids) (Monti et al. 1998). In fact, the main differences between those systems is the higher temperature required by the former to observe the browning formation, which is 150° C for gluten-glucose versus 100° C for single amino acids-glucose model systems.

2.5 Reactivity of Reducing Sugar in Maillard Reaction

The major factors that influence the rate and extent of color browning development in Maillard reaction are temperature and water activity (Labuza and Saltmarch 1981; Davies et al. 1998) and the nature and amount of reducing sugar. The initial kinetics of non-enzymic color browning is dependent on the proportion of the reducing sugar existing in the acyclic or active form under the reaction conditions (Yaylayan et al. 1993; Labuza and Basier 1992) and on the electrophilicity of the sugar carbonyl group (Bunn and Higgins 1981). The reactivity of reducing sugars was reported to decrease in the following order: aldopentoses > aldohexoses > aldoketoses > disaccharides (Spark 1969). Kato et al. (1990) also reported that a terminal pyranose group at the C-4 position of the
reducing end of disaccharides retarded further reactions of protein disaccharide adducts. Although it is accepted that, in general, aldoses are in (Labuza & Saltmarch 1981) stringently more reactive than ketoses, there have been conflicted reports on the reactivities of glucose and fructose. Several researchers (Suarez et al. 1995; Walton et al. 1989; Mauron 1981; Kato et al. 1969) have reported glucose to be more reactive. It has also been reported that fructose is more effective in causing protein crosslinking and in generating protein-bound Maillard florescence than glucose (Sakai et al. 1990; Suarez et al. 1991; Walton et al. 1989). The discrepancies in the literature may be related to differences in the conditions under which the Maillard reactions were conducted and the methods used to monitor the reaction.

A study by Rizzi (2007) on “the effect of tetraborate ions in the generation of colored products in thermally processed glycine-carbohydrate solution”, stated that, in Maillard reaction, the free (or hydrated) carbonyl form of sugars is believed to be a key reactive species. They observed that, the degree of Maillard browning in a series of aldose sugars increased significantly with a higher free aldehyde content reported in sugar equilibria. Aldohexoses are considered to be more reactive than ketohexoses, because of their more electrophilic carbonyl groups (Bunn and Higgins 1981).

2.6 Role of Glycerol in Maillard Reaction

This paper highlights that we need to move further from the water activity related reaction rate theory since physic-chemical reaction can take place in absence of water if the non-water liquid component is able to dissolve the solid components in the mixture at a given temperature condition, this non-water liquid can be polyols (for example, glycerol). Glycerol is found in all natural oils and fats, and is an important intermediate in the metabolism of all living beings. It is naturally present in many foods, and is used as a food additive, example, as a conditioner and moisturizer, as well as a solvent for flavor extracts. Glycerol influences the Maillard reaction of reducing sugars and amino acids (Cerny and Guntz-Dubini 2006). Bhandari and Yap (2007) have found that common food sugars (lactose, sucrose, fructose, glucose, and maltose) are all soluble in pure glycerol at various levels (Table 2). Obviously the solubility is a function of
temperature of solvent and it was found that the equilibrium time taken was longer than in water due to the high viscosity of glycerol which limits diffusivity/dispersibility of solvated molecules across the solution (results not shown) and relatively lower polarity than water. The solubility of various pharmaceuticals in glycerol has also been reported (Schieberle et al. 2007). The solubility of glycerol will certainly be enhanced by the presence of small amount of strongly polar water.

Table 2  Sugars Solubility in Glycerol. Approximate solubility of some sugars in glycerol at 60°C after equilibration period of 10 days (Bhandari and Yap 2007).

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Solubility (g/100 g glycerol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>50</td>
</tr>
<tr>
<td>Glucose</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15</td>
</tr>
<tr>
<td>Maltose</td>
<td>40</td>
</tr>
<tr>
<td>Lactose</td>
<td>10</td>
</tr>
</tbody>
</table>

In some earlier and also recent studies, it has been indicated that the presence of glycerol could promote the Maillard reaction (Warmbier et al. 1976; Mustapha et al. 1998; Cerny and Guntz-Dubini 2006). Some of these studies have been done in the presence of some amount of water (glycerol was a co-solvent). However, Mustapha et al. (1998) presented some Maillard reaction results at near zero moisture content (aw=0.01) in glycerol. They found that the Maillard reaction between lysine and zylose system was higher in glycerol than in water medium. However, an addition of water in glycerol enhanced the reaction rate presumably due to the increased solubility of reactants. Our preliminary work found that sugars and amino acids are dissolved in glycerol and there was Maillard reaction taking place in anhydrous glycerol-sugar-glycine system. The data clearly indicated that there is a Maillard reaction and the rate is dependent on the temperature and duration.

2.7 Mobility of Water in Glycerol System of Maillard Reaction

Among the most familiar expressions of Maillard browning is the bell-shaped curve which relates the rate of the reaction to water activity. Maximum browning reaction occurs in most foods between water activities \( (a_w) \) 0.3 and 0.7. The position of this maximum depends on the type of food. Therefore, water
activity, though it reflects the effect of water being bound to specific polar groups in the food and other factors limiting the availability of water molecules for chemical reaction, cannot be used to predict optimum browning conditions. At higher water activities the browning reaction rate decrease has generally been attributed to dilution of the reaction partners. The decreased reaction rate at lower water activities when the amount of mobile water lowers has been ascribed to an increasing diffusion resistance which lowers the mobility of the reaction partners (Labuza et al. 1970).

Since the earliest investigations, an accepted interpretation for the bell-shaped curved in Maillard browning was that, on the low moisture side, the rate is limited by diffusion resistance, which lowers the mobility of reactants and reaction products (Labuza et al. 1970; Loncin 1975). It was considered that diffusion, but also solubilization of reactants, can only occur at a water content above the monomolecular on the sorption isotherm, although browning could also develop, to some extent, even below this moisture level. At high water contents and water activities, the rate decreases due to the effect of dilution of the reactants (Maltini et al. 2003).

In systems with different reactant concentrations and different ratios of viscosity to \( a_w \), the browning maximum will increase with increasing concentration and shift toward higher \( a_w \) in systems with high ratio viscosity/\( a_w \), for example, with higher molecular complexity (or glass transition temperatures) (Maltini and Anese 1994). Similar trends can be recognized in many experimental data, such as those from Karel and Eichner (1972). Based on their finding, the browning rate is increased by a decrease of water content in a glucose-glycine-glycerol-water system. This finding implies that water is slowing the net reaction in some important steps of browning by laws of mass action (water being formed during the browning reaction). Moreover, they concluded that the observed decrease in the browning rate is due both to the product-inhibition of the forward reaction by water and the diluting influence of water and glycerol, decreasing the concentration of the reactants. Therefore, with increasing amounts of glycerol, two opposite effects are causing a maximum in the rate of browning. First, in the presence of relatively low water contents, the plasticizing affect of glycerol...
improves the mobility of the reactants, thus increasing the reaction rate. Second, the increasing amount of water and glycerol lowers the reaction rate due to the product-inhibition of the forward reaction by water and the diluting influence of water and glycerol which resulted in decreasing the concentration of the reactants.

Table 3 Some physical, physico-chemical and structural factors influencing the rate of non-enzymatic browning (Maltini et al. 2003)

<table>
<thead>
<tr>
<th>Rate enhancing</th>
<th>Rate limiting</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reactants concentration (law of mass action)</td>
<td>• Reactant solubility</td>
</tr>
<tr>
<td>• Diffusivity within the matrix</td>
<td>• Water content as a diluent</td>
</tr>
<tr>
<td>• Water content as a matrix plasticizer (enhances diffusivity)</td>
<td>• High viscosity (affects mobility and diffusivity)</td>
</tr>
<tr>
<td>• Solubility in the matrix (affects diffusion paths for reactants)</td>
<td>• Water content as a product of the reaction (shifts backwards the equilibrium of the reaction)</td>
</tr>
<tr>
<td>• Porosity (increases the potential for shirinkage)</td>
<td></td>
</tr>
<tr>
<td>• Glass-viscous transition (allows reagents to diffuse)</td>
<td></td>
</tr>
<tr>
<td>• Crystallisation of the matrix (increases reactants concentration in the non-crystalline volume)</td>
<td></td>
</tr>
<tr>
<td>• Phase separation in non-crystalline systems (increases reactant concentration in the liquid volume)</td>
<td></td>
</tr>
<tr>
<td>• Water non-miscible components (increases reactant concentration in the water phase)</td>
<td></td>
</tr>
<tr>
<td>• Possibly, water binding ability of the substrate (subtracts water from the systems)</td>
<td></td>
</tr>
</tbody>
</table>

Even if reactant concentration and diffusivity may be the main controlling parameters, in real foods many additional physical, physico-chemical and structural factors can have an influence on the dynamic of browning in real foods. Some of these parameters are presented in Table 3 among these are different roles of water as a solvent, as a plasticizer, as a diluents and as a product of the reaction.

2.8 Overview Studies on Kinetic Maillard Reaction

Maillard browning reaction is a complex cascade reaction which leads to the production of several important odor and colour in foods. In the past few
decades, hundreds of research who studied on the kinetics of maillard browning in both authentic foods and model system had been conducted in order to provide the knowledge which eventually needed for controlling and designing food processing. Most of them focused on the effects of various concentration and ratio of amino acid/protein and sugar, water activity, moisture content, pH, and temperature on the kinetics modelling in different system (Baisier 1992; Labuza 1992; Ibarz et al. 1999; Carabasa-Giribet 2000; Martins 2003). These studies were prominently done in a conventionally heated system. General kinetics of maillard reaction can be divided into two main phase: one is the rate of formation of maillard product from either Amadori Rearrangement Product (ARP) or melanoidsins as the final maillard product; the other phase is the rate of loss of both sugar and amino acid which involved in the reaction. In addition, the side reactions of amino acid and sugar was to be the main factor that complicate the kinetic analysis.

Maillard reaction has contribution on developing flavor compounds in heated foods. There have been many studies on flavor formation via the maillard reaction (Reineccius 2006; Tressl et al. 1998). Unfortunately, only few studies which have focused on reaction kinetics of formation of flavor compounds via maillard reaction. Thus, if we understand the reaction kinetics of flavor compounds then we can better manage the production of both desirable and undesirable flavors via the maillard reaction. Flavor compound which produced from maillard reaction had been classified by Nu (Nursten 1980) into three groups: first group is simple sugar dehydration products; which includes compounds that are formed by the breakdown of glycosylamine in the early stages of reaction, such as furans, pyrones, cyclopentenes, carbonyl compounds, and acids. Second group is simple amino acid degradation product; which are consists mostly of compounds from the Strecker degradation of amino acids and dicarbonyl compounds, such as aldehydes, sulfur compounds (example, hydrogen sulfide, methanethiol), and nitrogen compounds (example, ammonia, amine). Third group is volatile compounds which are produce by futher interactions from group 1 and 2, such as pyroles, pyridines, pyrazines, immidazoles, oxazoles, furanthiols, compounds from aldol condensation, thiazoles, thiophenes, di-/tri-thiolanes, and di-/tri-thianes.
2.9 Collision Theory in Related with Molecular Mobility

In order to obtain collision between atoms or molecules there are three necessary requirements in order for a reaction to take place (Wright 2004). First requirement is that molecules must collide to react, this means if two molecules simply collide, however, they will not always react; therefore, the occurrence of a collision is not enough. Second, there must be enough energy (energy of activation) for the two molecules to react. This is the idea of a transition state; if two slow molecules collide, they might bounce off one another because they do not contain enough energy to reach the energy of activation and overcome the transition state (the highest energy point). The last requirement is, molecules must be orientated with respect to each other correctly. For the reaction to occur between two colliding molecules, they must collide in the correct orientation, and possess a certain, minimum, amount of energy. As the molecules approach each other, their electron clouds repel each other. Overcoming this repulsion requires energy (activation energy), which is typically provided by the heat of the system; i.e., the translational, vibrational, and rotational energy of each molecule, although sometimes by light (photochemistry) or electrical fields (electrochemistry). If there is enough energy available, the repulsion is overcome and the molecules get close enough for attractions between the molecules to cause a rearrangement of bonds.

At low temperatures for a particular reaction, most (but not all) molecules will not have enough energy to react. However there will nearly always be a certain number with enough energy at any temperature because temperature is a measure of the average energy of the system-individual molecules can have more or less energy than the average. Increasing the temperature increases the proportion of molecules with more energy than the activation energy, and consequently the rate of reaction increases. Typically the activation energy is given as the energy in kilojoules needed for one mole of reactants to react (Wright 2004).
Figure 2 Reaction coordinates showing the relationship between energy and progress of reaction

2.9.1 Reaction Kinetic

There is a vast amount of literature on the Maillard reaction, and it becomes increasingly incomprehensible to review the state of the art. However, one aspect is somewhat neglected, namely the study of kinetic aspects of the reaction, even though kinetic information is badly needed with respect to control of the reaction. The reason for this relative lack of information is undoubtedly the complexity of the reaction, consisting of many parallel and consecutive reactions (Martins et al. 2001). It is also very difficult to compare literature results because the experimental conditions are usually quite variable. Based on van Boekel (2001) the reaction rates depend on many conditions, the most important ones being the following:

**Temperature range**

Since the Maillard reaction consists of several reaction steps, each with possibly different temperature sensitivity, it strongly depends on temperature which reaction route prevails. Thus, kinetic results obtained at rather low temperature, say 20-60 °C, are not immediately comparable to that ones obtained at 100-150 °C. Furthermore, temperature affects the activities of the reactants. The active form of the sugar is considered to be the open chain, the concentration of which increases with temperature. The rate of mutarotation is also of importance in this respect, and changes with temperature.

**Type of sugar**

It is known for a long time that there is a big difference in the browning capacity of sugars. The generally accepted view is that these differences can be
ascribed to the differences in open chain form: the sugars with a higher concentration in the open chain form brown faster and more intense.

**Water activity (A_w)**

At a certain water activity, however, the system becomes so concentrated that diffusion becomes increasingly difficult, reactants do not meet so easily anymore and the rate decreases, as is the case in powders and glassy foods. This explains, qualitatively at least, the maximum observed when the rate of Maillard reaction is plotted as a function of water activity. Apart from that, water participates also in the reaction itself, for instance when the glucosylamine is rearranged in the Amadori product. Water thus has a dual role in the Maillard reaction: as a solvent and as a reactant.

Several authors have tried to study the kinetics of the Maillard reaction by fitting simple kinetic models (like a zero-, first, or second-order reaction) to one selected reaction, for instance, formation of color or the degradation of reducing sugar (Martins & van Boekel 2005). Bell *et al.* (1998) reported that glycine loss followed a second order reaction model in a glucose/glycine mixture in a glassy state at 25 °C. However, no real distinction could be made between the first- and the second-order plot (Martin *et al.* 2001). Also, Carabasa and Ibarz (2000) reported that both zero- and first-order reaction models gave acceptable fits for brown color formation in glucose and amino acid systems heated at four different temperature (85, 90, 95 and 100 °C).

The most studied aspect of the Maillard reaction is of course, color formation, in most cases measured as absorption at 280-420 nm (depends on the Maillard products which were formed), but also rather frequently as a tristimulus measurement according to the CIE L*, a*, b* scale. As for kinetics, most authors tend to model color formation as a zero-order reaction, after some induction period. The induction period is of course due to the fact that melanoids are end products and so it takes time before the precursors are formed. The length of an induction period depends on experimental conditions, notably the time scale and temperature (Lievonen *et al.* 1998; Morales and van Boekel 1999; Song *et al.* 1966).
Van Boekel and Walastra (1995) have given a detailed explanation on the use of kinetics in food applications (Table 4). Based on the general rate law, the disappearance of a compound (in a closed system with only one compound reacting) is:

$$-\frac{d[A]}{dt} = k[A]^n$$  \[2.1\]

Where $[A]$ stands for the molar concentration of $A$ and $k$ is the rate constant and $n$ (usually $0 \leq n \leq 2$) the reaction order. Integrating the equation to a chosen order, with respect to time, a zero order reaction would be:

$$[A] = [A]_0 - kt$$  \[2.2\]

A first-order reaction would be

$$[A] = [A]_0 \exp(-kt)$$  \[2.3\]

For a second-order reaction would be

$$\frac{1}{[A]} = \frac{1}{[A]_0} + \frac{t}{k}$$  \[2.4\]
2.9.2 Mathematical formulation

The activation energy for a reaction is experimentally determined through the Arrhenius equation and the Eyring equation. The Arrhenius equation gives the quantitative basis of the relationship between the activation energy and the rate at which a reaction proceeds (Lowrey and Kathleen 1976). From the Arrhenius equation, the activation energy can be expressed as:

\[ k = A e^{-\frac{E_A}{RT}} \]  [2.5]

Where \( A \) is the frequency factor for the reaction, \( R \) is the universal gas constant, and \( T \) is the temperature (in Kelvin). The higher the temperature, the more likely the reaction will be able to overcome the energy of activation. \( A \) is a steric factor, which expresses the probability that the molecules contain a favorable orientation and will be able to proceed in a collision. In order for the reaction to proceed and overcome the activation energy, the temperature, orientation, and energy of the molecules must be substantial; this equation manages to sum up all of these things.

Lee et al. (1984) who investigated the kinetics of the formation of Amadori compound and brown pigment under closed and isothermal condition at temperature 90-110 °C, found out the system was fitted at first-order reaction. Activation energies were reported between 23 and 70 kJ/mol. In whey system studied by Labuza and Saltmarch (1981) with different condition of water activity with temperature between 25-45 °C, resulted in activation energy between 29-34 kJ/mol. Another research related to the color formation has been done by Lievonen et al. (1998) in a glucose-lysine system with temperature 50-100 °C, they resulted in activation energy between 125-147 kJ/mol. Most of the kinetic studies color formations have been limited to aqueous model system. Knowing the diversities of food processing in industry, it is also necessary to study the kinetics of color formation in non-aqueous system since the kinetic parameters and the analytical procedures can be quite different between non-aqueous and aqueous solutions.

2.9.3 Transition states

The figure below (Figure 3) demonstrates the relationship between activation energy \( (E_a) \) and enthalpy of formation \( (\Delta H) \) (Wright 2004). The highest
energy position (peak position) represents the transition state. If there is catalyst, the energy required to enter transition state decreases, thereby decreasing the energy required to initiate the reaction. The transition state along a reaction coordinate is the point of maximum free energy, where bond-making and bond-breaking are balanced. Transition states are only in existence for extremely brief \((10^{-15} \text{ s})\) periods of time. The energy required to reach the transition state is equal to the activation energy for that reaction. Multi-stage reactions involve a number of transition points; here the activation energy is equal to the one requiring the most energy. After this time either the molecules move apart again with original bonds reforming or the bonds break and new products form. This is possible because both possibilities result in the release of energy.

Figure 3 Relationship between \(E_a\) and enthalpy of formation without a catalyst (Wright 2004)