

The Maillard Reaction
Application to Confectionery Products

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Introduction

The reaction between sugars and amino groups was first described in 1908 by two Englishmen, Ling & Malting, who considered color formation in beer. In 1912 Louis-Camille Maillard described a browning reaction between reducing sugars and amino groups. Despite not being the first to report the reaction, Maillard was the first to realize the significance of the reaction in areas as diverse as plant pathology, geology and medicine. It is interesting to note that despite there having been six international symposium on the Maillard reaction, not one of the papers was a direct study on the Maillard reaction in confectionery (e.g. Waller & Feather, 1983; Finot et al., 1990, Labuza et al., 1994). In fact, it seems that recent symposia were as much concerned with the medical aspects as food chemistry of the Maillard reaction. The medical world has begun to realize that there may be a role for the Maillard reaction in the formation of complications of diabetes and aging, in which the blood glucose becomes bound to proteins in the body in a similar way to food proteins and glucose. Despite the lack of direct research on the Maillard reaction in confectionery, it can be seen that the Maillard reaction will play an important role in the formation of flavors and colors of some confectionery products since the main Maillard reactants are present. This paper is particularly concerned with the Maillard reaction in non-chocolate confectionery.

Non-enzymatic browning reactions pathways

The Maillard reaction is one of four nonenzymatic browning reactions which occur in food. The other three are:

- 1) the degradation of ascorbic acid,
- 2) lipid peroxidation
- 3) sugar-sugar caramelization.

The chemistry of these reactions is related to the Maillard reaction. Ascorbic acid (AsA) undergoes a reaction chemically similar to that of sugars except that amino acids are not necessary for browning. Since AsA is very reactive, it degrades by two pathways, both of which lead to the formation of dicarbonyl intermediates and subsequently to form browning compounds (Davies & Wedzicha, 1992).

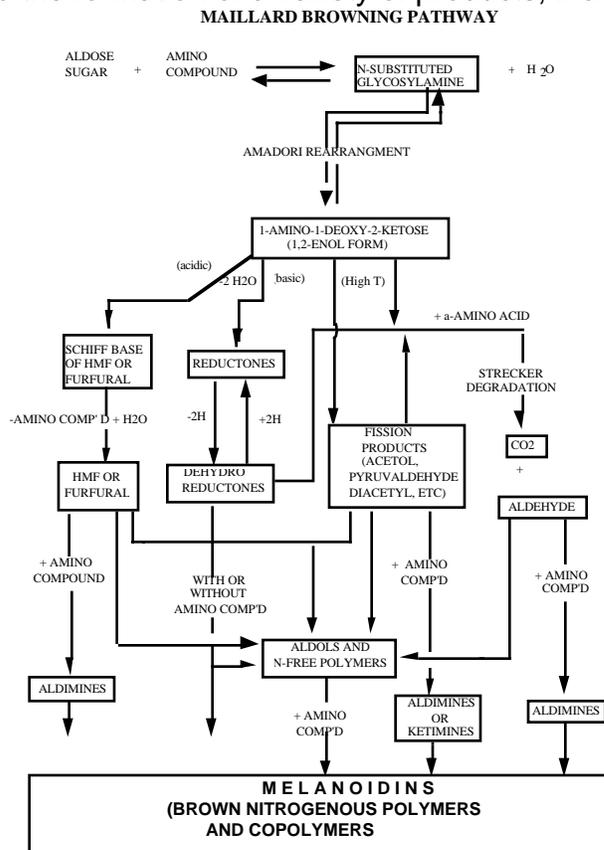
Lipid peroxidation occurs by the action of oxygen and reactive oxygen species on the fatty acids, especially unsaturated fatty acids. These are oxidized to form aldehydes

and ketones which then react with amino acids to form brown pigments, as in the Maillard reaction. It is possible that peroxidation products induce the browning reaction of the Amadori products (Hermosin *et al.*, 1992).

At high temperatures (> 80 °C) sugar-sugar interactions or the caramelization reaction occurs. This is a complex series of reactions but many of the intermediate flavor compounds and products are similar to those observed for the Maillard reaction.

Mechanism of the Maillard reaction

The chemistry of the Maillard reaction is known as a complex series of reactions leading to the formation of a variety of products, including the flavors, aromas and colors



considered important in food science today.

The classical scheme of the chemical reaction is that of Hodge as shown in Figure 1 (Hodge, 1953) This is still used today to describe the reaction. The Maillard reaction was first described as between reducing sugars with amino acids, but now is extended to include many other carbohydrate and amine groups. Sugar sources include dextrose, fructose, high fructose corn syrup, sucrose, corn starches and maltodextrins. Protein (-NH₂) sources for candy may include milk solids, cream, egg solids, nuts and nut fragments, cocoa solids, butter (small source of nitrogen), fruits and fruit juices provide free amino acids, gelatin, whey proteins and emulsifiers such as lecithin.

Figure 1. Modified Hodge scheme for nonenzymatic browning

The mechanism of the Maillard reaction as shown in Figure 1 is very complicated. However, it is generally divided into three stages (Lee, 1983; Mauron, 1981):

- (1) The first stage involves the sugar-amine condensation and the Amadori rearrangement. The reaction steps have been well-defined and no browning occurs at this stage
- (2) The second stage involves sugar dehydration and fragmentation, and amino acid degradation via the Strecker reaction especially at high temperatures as used in candy manufacture. At the end of stage two there is a beginning of flavor formation - depending on which flavor is studied.
- (3) formation of heterocyclic nitrogen compounds. Browning occurs at this stage.

Stage One

The Maillard reaction is initiated by a condensation reaction between the carbonyl group of the aldose and the free amino group of an amino acid to give an N-substituted aldosylamine. This is the result of a nucleophilic attack group by the NH_2 group of the amino acid on the electrophilic carbonyl groups of sugar. It is basically an amine-assisted dehydration reaction of sugar. The condensation product rapidly loses water as a product and is converted into a Schiff base. This reaction is reversible and acid-base catalyzed. The Schiff base then cyclizes into the aldosylamine. The Amadori rearrangement follows (for aldoses; Heyns rearrangement for ketoses) to form a ketosamine. If the aldose is glucose and amino acid glycine, then the Amadori product is 1-amino-1-deoxy-2-fructose (monofructoseglycine, MFG). The Amadori rearrangement is considered to be the key step in the formation of major intermediates for the browning reaction.

Ketoses, such as fructose, react with amines to form aminoaldoses, this is called the Heyns reaction. The intermediates to this reaction are imines. Aminoaldoses are not very stable and readily react forming the Amadori compound (Ledl & Schleicher, 1991).

Stage 2

The Amadori product degrades by one of three main pathways depending on the conditions (Fig 1).

- 1) The free hydrogen of the amino group of the ketosamine may react with a second molecule of aldose to form a diketosamine. This compound is less stable than the monoketosamine and decomposes to give a monofructoseamine and nitrogen-free carbonyl compounds (Anet, 1959), e.g. 3-deoxyosuloses (Anet, 1964) and the cis- and the trans- forms of 3,4-dideoxyosulos-3-ene (Anet, 1962) which are probably the most important intermediates in the Maillard reaction (Wedzicha & McWeeny, 1974). The decomposition of the diketosamine has a maximum rate at pH 5.5 (Anet, 1959).

- 2) In acidic media an enolization of the Amadori product gives the 1,2 enediol form, which is followed by the elimination of the hydroxyl group at C₃ and subsequent deamination at C₁ yields the 3-deoxyosulose after water addition (Anet, 1964). This compound then degrades to 3,4-deoxyosulos-3-ene which degrades to form colored products (Reynolds, 1963) and flavor compounds. In neutral and weakly alkaline media the 2,3-enolization pathway is favored (Feather, 1981).

- 3) The Strecker degradation of amino acids involves their oxidative degradation by α -carbonyl compounds, which arise from the degradation of ketosamines. In this degradation reaction, amino acids first react to form Schiff bases and then undergo acid-catalyzed decarboxylation. The new Schiff base is easily hydrolyzed to form an amine and aldehyde. Strecker degradation is characterized by the production of CO₂ and the net result is a transamination reaction which may be important for the incorporation of nitrogen into melanoidins (Mauron, 1981). The aldehydes formed may be important as auxiliary flavor compounds (Hodge *et al.*, 1972) and they also contribute to melanoidin formation. Strecker degradation products are implicated in storage related off flavors.

Stage 3

This stage is characterized by the formation of brown pigments and roasted and toasted aromas and tastes. The formation of melanoidins is the result of the polymerization of the highly reactive intermediates that are formed during the advanced Maillard reaction (Mauron, 1981). The chemistry of these compounds is not well-known and their

formation mechanism also remains obscure. The molecular weight of these compounds increases as browning proceeds, until eventually they become insoluble high molecular weight species.

Color formation

Most of the studies investigating the mechanism and rate of the Maillard reaction are concerned with the formation of brown color. Thus, the mechanism of the Maillard reaction given above would appear to be synonymous with that of the browning reaction. However, there are only a few studies on the final browning stage of the Maillard reaction (Wedzicha & Kaputo, 1992). Color occurs due to the formation of high molecular weight (>12, 000 Daltons) polymeric compounds also known as melanoidins. These are generally formed by the reaction of the Amadori product or/and with other dicarbonyls, i.e. the deoxyosuloses, with amino acids.

In general, in a given reaction mixture, there is present a heterogeneous mixture of melanoidins, each of which has a different maximum absorbance. The observable color is the result of a highly concentrated mix of UV active species. There are many suggestions as to the structure of melanoidins and in particular the structure of the chromophoric moiety (Heyns & Huber, 1970; Nursten & O'Reilly, 1986; Kato & Tsucida, 1981; Rubinzstain *et al.*, 1984) but this also seems to depend not only on the reactants but also on the method of isolation of the melanoidin. The nature of the melanoidin mixture makes it hard to separate individual compounds and determine individual structures. There is evidence of heterocyclic compounds present in melanoidin structure. Different structures have been suggested for the melanoidins. In a food system, the composition of the melanoidin is also influenced by the fact that the amine group is probably the N- H₂ of lysine bound to proteins.

The composition of the melanoidin mixture is has been elucidated for mixtures containing glucose or arabinose and glycine. In general the ratio of sugar to amino acid is 1:1 for pentoses and 1.5:1 for glucose. For glucose-glycine melanoidins it is thought that formaldehyde, formed by the Strecker degradation of glycine also becomes part of the melanoidin structure (Wedzicha & Kaputo, 1992; Davies & Wedzicha, 1994; Vlasiliauskaite, R. & Wedzicha, 1994).

Fluorescence

The use of fluorescence for chemistry studies reached a peak during the 1940s, after which the measurement of absorbance became more prominent. Today, fluorescence measurements are generally used only for *in vivo* studies on the Maillard reaction where absorbance measurements are affected by the presence of naturally occurring interfering colors. Thus, fluorescence may be an important measure of nonenzymic browning in food that is already colored such as chocolate or colored candies.

It is generally accepted that the fluorescent-species are precursors to the melanoidins (Freidman & Kline, 1950) as the peak in fluorescence precedes the increase in pigment formation. Also, temperature and pH influence the formation of fluorescent species and browning species in the same way (Pearce & Bryce, 1947).

An overall kinetic scheme of product flow in the Maillard reaction is represented in Figure 2. It can be seen that the Maillard reaction represents a typical multi-step

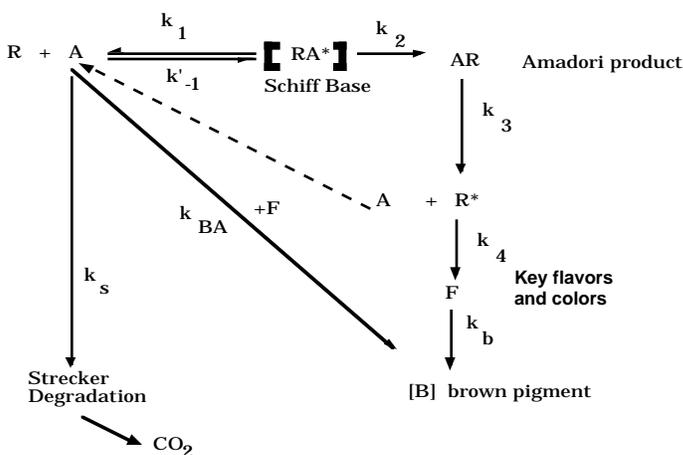


Figure 2 Simplified reaction scheme for the Maillard reaction

reaction, where the level of intermediates increases with time and then decreases again; whereas the products increase only after an extensive induction period.

Interestingly, it shows that the amine is partially recycled at a early stage, indicating that the first stage of the Maillard reaction is probably catalyzed by amine which is then released. This scheme shows that the fluorescing compounds probably

are intermediates for the browning pigments. Flavor formation occurs slightly earlier to brown pigment formation but is otherwise parallel.

The major factors which influence the rate of color and flavor development are the time temperature sequence, water activity-water content profile, concentration versus time profile, type of reactants, solution state (dilute to supersaturated solution) and whether the final solid is in a crystal, glass or rubber state. This can be affected by the ingredients and the processes used to make the candy. However, there is a problem

when trying to interpret the Maillard reaction in relation to confectionery products because there are over 2000 kinds of candy, with hundreds of different recipes for each candy. For example, there are at least 500 different recipes for nougat and over 1000 for marshmallows. None of these recipes have been defined and few of the processing techniques are published (Alkonis, 1977).

Considerations for confectionery products

The main ingredient of non-chocolate candies is a sweetener(s) which includes crystalline cane and beet sugar (sucrose), brown sugar, various liquid sugars, various corn syrups, dextrose, fructose, lactose, honey, molasses, maple syrup, sugar blends, sorbitol and non-nutritive sweeteners. All of these compounds, except for sucrose (unless it undergoes hydrolysis) and sorbitol, have Maillard reactivity. For processing of most candies, most are heated to above 100 °C, i.e., the boiling process needed to reduce moisture content. The major differences between candy confectioneries other than specific ingredients, are a result of the way they are cooked to reduce the water content, the ingredients used to prevent or control the formation of crystals and the rate of cooling. Hard caramels, for example, owe their hardness to the removal of all but 1% of the water by heating and vacuum processing, with the resulting mass being in a non-crystalline rubbery or glassy state depending on the final water content. These sweeteners have different properties and hence, influence the final candy in a variety of ways. They are often used in combination. Manufacturers choose the sweetener on the basis of its chemical and physical properties, generally considered to be in this order:

- . relative sweetness of sweetener
- . solubility and crystallization characteristics
- . density of liquid sweeteners and moisture content of solid sweeteners
- . hygroscopicity upon storage
- . flavor and color development
- . fermentation or preservative properties
- . osmotic pressure and depression of freezing point

The most common sweetener in candy is sucrose refined from either cane or beet sugar

Sucrose is not a Maillard reactive sweetener unless it hydrolyzes to form equimolar quantities of glucose and fructose which as noted have significant Maillard reactivity. At low temperatures sucrose hydrolysis occurs at $\text{pH} < 3$, but at temperatures above $70\text{ }^{\circ}\text{C}$, hydrolysis occurs even at $\text{pH} 7$ during typical processing times. Lactose, which is the sugar found in milk, is used because it has a low sweetness value, provides moisture and flavor retaining characteristics and controls graininess. Lactose also has a higher caramelization temperature than the other sugars and so the use of lactose will ensure the development of optimal color of caramels. Corn syrups provide sweetness, control graining, improve texture and extend keeping quality. These sweeteners may also retard or prevent crystallization of the sugar, retain moisture and add body.

Basic to the manufacture of candies is the melting and/or the solubilization of the ingredients followed by a reduction of the moisture content. Cooking the mixture is the main step responsible for the dissolving, melting, stabilizing and caramelization of the mixture. The finer the crystals of the sugar used, the more rapidly it dissolves into solution. During cooking, as noted above, sucrose hydrolyzes to form invert which is more hygroscopic and is very Maillard reactive. Color formation in candies made from sucrose depends upon the extent of sugar inversion and the pH of the syrup. The lower the pH , the faster the reaction. There are many different ways that candies are cooked (batch, under pressure, under vacuum, continuous and semi-continuous) and all require a temperature of $> 100\text{ }^{\circ}\text{C}$. After cooking, the resulting mass may be treated to various processes such as whipping, extrusion, foaming and pulling.

Another color and flavor reaction in candy making is caramelization, which involves sugar pyrolysis and then the formation of sugar-sugar polymers. For browning to develop, no amine needs to be present, but if amines are present the intermediates from the caramelization reaction may interact forming similar products to those isolated from Maillard reaction systems. Caramelization occurs above $80\text{ }^{\circ}\text{C}$, taking place in both a liquid and a dry mixture.

Undesirable consequences

Some changes caused by the Maillard reaction are unwelcome and reduce the quality of a food product, making them both unpleasant to eat and in appearance, e.g. the browning of milk powders, apricots. (Ames, 1990). As proteins react with reducing compounds to form Amadori products and other intermediates, these undergo further

reaction and polymerization, reducing the solubility of the protein. The most likely reactive site on a protein for the Maillard reaction is the α -amino group of lysine with a loss of nutritional value, although this latter point is not that important in candies. Table 1 shows the percentage of lysine amino groups that reacted during different processing procedures for dried milk products (Mauron, personal communication).

Table 1
Nutritional Consequences of the Maillard Reaction

Product	% blocked lysine
raw milk	0
freeze dried milk	0
pasteurized milk 74°C 40 sec	0
UHT pasteurized 140°C 3 sec	0-2
spray dried milk	0-3
sweetened condensed milk	8-12
roller dried (no preconcentration)	10-15
evaporated milk	15-20
drum dried milk	20-30

Recent studies have shown that processed meat products contain heterocyclic amino compounds (HAs) which have been shown to have mutagenic effects (Knize et al, 1994). These compounds are also formed in model systems on the reaction of amino acids, creatinine and sugars. There is a possibility that these products may also have a carcinogenic effect. How important this is in confectionery is unknown but a mutagenic effect of caramel has been reported (Barnes et al., 1983) and the carcinogenic effect of thermolyzed sucrose and casein has been reported for rats (Corpet et al., 1990).

Desirable Consequences

The most important influence the Maillard reaction has on confectionery is the formation color, aroma and taste, mainly during processing, e.g. the flavor and color of many chocolate, candy and combination baked products. Thus, the Maillard reaction gives rise to the colored and the flavored compounds which are part of the food acceptance.

Flavor production is quantitatively an extremely minor pathway in the Maillard reaction since the vast majority of reactions form carbon dioxide, melanoidins (color) or the

numerous intermediates. The compounds generally responsible for the characteristic smells of food are usually volatile heterocyclic compounds such as pyrazines, pyrroles, pyridines, oxazoles and oxalines. Maillard derived aromas are extremely complex and many of the compounds are formed in trace amounts by obscure side reactions and pathways (Baltes *et al.*, 1989). The heating of carbohydrates with amino acids leads to the formation reactive compounds which, in turn, react to form heterocyclic compounds. Aromas are generally formed from a mixture of volatile compounds each present in trace amounts. These volatile compounds have low sensory thresholds and so a little goes a long way.

The most important steps of thermal aroma formation via the Maillard reaction are (Baltes *et al.*, 1989):

- 1) Condensation of amino acids to the C-1 of an aldose or a C-2 of a ketose, followed by the rearrangement to the keto/aldo sugar (Amadori or Heyns rearrangement). The intermediate aldol structures give rise to a facile sugar decomposition by which different aliphatic or cyclic mono- or di-carbonyls are formed. For Amadori products two pathways are known: The 1-deoxy or the 3-deoxyosulose pathways.
- 2) α -Dicarbonyls react with amino acids to yield the Strecker aldehydes and aminoketones which are converted via dimerization to yield pyrazines.
- 3) Sugars are the main precursors of α -dicarbonyls. Thus, the amino acid to sugar ratio is decisive for the proportion of pyrazines, furans, furanones, pyranones in the volatile fraction. In general the sugar has less of an influence on the sensory properties of the final reacted mixture than the amino acids. The sugar choice, however, has been shown to strongly influence the total amount of flavor formed (Reineccius, 1990).
- 4) Sugars as well as amino acids are decomposed by heat treatment. The final reaction products from sugars are often identical with the products formed by the Maillard reaction.
- 5) Temperature is the most influencing factor in the composition of thermal aromas. Time and temperature are known to strongly influence the sensory properties of foods. The effect of time can be summarized as low temperatures (i.e. storage) produces gluey and stale aromas, intermediate temperatures results in cooked notes and high temperatures results in roasted or toasted flavors (Reineccius, 1990).

There is an assumption that Maillard reaction flavor formation responds to formulation pH, temperature and water activity like the browning reaction. This might not be the case since the formation of a specific flavor may require, in correct proportions, the simultaneous generation of 100 or more individual compounds. As each of these compounds arises from an individual reaction, each will be influenced by its own reactant concentration, environment and processing conditions especially temperature. Thus, recreating flavors from batch to batch is extremely difficult

Most α -amino acid-sugar model systems form similar compounds at the same temperature. In fact, reaction with ammonia yields a great variety of pyrroles, pyrazines and pyridines. However, a great number of the special aromas that can be found in candies are formed by the reaction of secondary amino acids with sugars, or when sulfur-containing amino acids react with sugars to give rise to thiazoles and thiophenes and other aromas associated with meat product. It has been established that during the roasting of cocoa, peptides and amino acids act synergistically to form flavor compounds. The removal of either the peptide or the amino acids can be carried out without altering the final flavor of the system (de Kok & Rosing, 1994). Kinetics of the Maillard reaction

The measurement of the kinetics of a reaction provides the most general method of determining its mechanism and can help a food scientist in making decisions on how to optimize a process. At a fixed temperature, the reaction rate is a function of the concentration of some or all of the components of the system. The effect of reactant concentration is usually considered to be the most important.

The relationship between the rate of a reaction and the concentration of its reactants is called the rate expression. A general form of the rate expression is:

$$\text{Rate} = k [R] [A]$$

where k = rate constant
 [R] = concentration of reducing compounds
 [A] = concentration of reacting amines

Many different models of the kinetics of the Maillard reaction have been suggested (Haugaard *et al.*, 1951; Kato *et al.*, 1969; Song *et al.*, 1966; Labuza, 1984; Wedzicha,

1984b; Labuza et al., 1994 and Davies et al., 1997). Problems with interpreting the kinetic data arise from the fact that many different reaction conditions and measurements are made. The extent of the Maillard reaction is often taken as a comparison between the concentration at zero time and at the end of the reaction and then the rate is extrapolated. Also the level of browning is often measured using one absorbance wavelength between 420-490 nm. This gives satisfactory results which can be interpreted but the melanoidins do not have an absorbance peak in this range. Maillard studies are also carried out at different temperatures, at different pH and with different solvents. All of these have an important influence the rate of the Maillard reaction which may then change the kinetics.

Parameters influencing the Maillard reaction

The course of the Maillard reaction is strongly affected by factors which influence the different chemical reactions involved. These are temperature and duration of heating, pH and presence of weak acids and bases, water content, type of reactant, amino acid to sugar ratio and oxygen.

Reactant type

Maillard reactants are not just the reducing sugars and amino acids. The common reactive reducing sugars are xylose, ribose, glucose, fructose, lactose and maltose and sucrose under acidic conditions or high temperature conditions where it forms invert sugar. Other reactive reducing moieties include carbonyl compounds formed during lipid peroxidation (glyoxal, methylglyoxal, formaldehyde), those added from flavoring ingredients and natural orthophenolics. The amines may be comprised of free amino acids, the N-terminal amine of proteins or peptides, the α -amine of lysine in proteins, aspartame (a non-nutritive sweetener) and the amine moiety in lecithin.

Typical sources of reducing compounds in candies are dextrose, fructose, high fructose corn syrup, sucrose (if it has undergone hydrolysis), corn starches, maltodextrins, lipid oxidation products in butter creams. and from added butter in caramels or oxidation of cocoa butter in chocolates, aldehydes and ketones added as flavors (e.g. cinnamic aldehyde). For sugars, the rate of the reaction depends on the rate at which the sugar ring opens to the reducible, open-chained form and this increases with increasing pH

% acyclic as function of pH			
Sugar	pH 6.5	pH 7.0	pH 7.5
glucose	0.012	0.022	0.040
mannose	0.040	0.062	0.110
ribose	10	18.5	30

from Cantor and Penniston (1940)

(Burton & McWeeny, 1963) . Table 2 shows that the amount of acyclic form is much higher for pentoses such as ribose than hexoses. Thus, pentose sugars react more rapidly than hexoses (Reynolds, 1963).

However, as Table 3 shows the relative concentration of acyclic sugar present does not completely explain the differences between the reactions observed for the different sugars. The relative rates of browning of glucose and fructose also depend on the extent to which the reaction mixture is buffered. In unbuffered media, the rate of browning of fructose with amino acids is greater than that of glucose. However, in buffered media such as when whey or egg albumin is added to a candy, fructose browns more slowly than glucose (Wedzicha, 1984a). For hexoses, the order of reactivity is D-galactose>D-mannose>D-glucose. Reducing disaccharides are considerably less reactive than their corresponding monomers.

The rate of the Maillard reaction is also dependent on the type of amine. Typical sources of amino groups present in candy are milk solids whey proteins, gelatin, cream, egg solids, nuts and nut fragments, cocoa solids, butter (contains a small

Table 3

Comparison between amount of reaction and % acyclic state for the glucose - hemoglobin reaction (from Bunn and Higgins 1981)

sugar	k ($10^{-3} \text{ mM}^{-1}\text{h}^{-1}$)	rate relative to glucose	%acyclic	ratio to glucose
glucose	0.6	1	0.002	1
mannose	3.6	6	0.005	2.5
galactose	2.8	4.7	0.02	10
ribose	10	16.7	0.05	25
fructose	4.5	7.5	0.7	350

note: sugar:hemoglobin 5:1 @ pH 7.3 and 37°C

amount of nitrogen containing compounds), fruit and fruit juices (contain free amino acids), and emulsifiers such as lecithin which has an amino group.

Most studies have been carried out on the relative reactivities of free amino acids. For example, Kroh & Westphal (1989) found that basic amino acids are more reactive than neutral or acid amino acids. Ashor & Zent (1984) suggested a classification of amino acids into three groups depending on the extent of browning when reacted with glucose at different pH, and 121 °C for 10 minutes. The most reactive were lysine, glycine, tryptophan and tyrosine. These results should be of applicability to candy because this is a typical processing range. Other results show that comparisons can only be used if the same pH and buffering conditions are used. For example, the effect of pH is especially significant due to the different pK_a values of the amino acids. Lysine appears to be the most reactive amino acid (Ashor & Zent, 1984; O'Brien & Massey, 1989) due to the fact that it has two available amino groups. However, the reactivity of lysine would appear to be dependent on the conditions of the reaction being studied.

The reactivity of peptides has hardly been studied and, unfortunately, many of the studies comparing peptide reactivity with that of amino acids measured the rate of browning to quantify the Maillard reaction. In general, the amount of browning has been shown to increase with chain length of the peptide. However, the amount of browning is not, per se, proportional to the conversion of amino acid or peptides, as the degree of browning depends on the type of melanoidin formed during the Maillard

reaction (de Kok & Rosing, 1994). Motai (1973) concluded that melanoidins from peptides exhibited a darker degree of color than those from amino acids. When the degree of sugar decomposition was measured it appeared that small peptides (up to 3 amino acid groups) are more reactive than the corresponding amino acids (de Kok & Rosing).

Reactant Ratio

The extent of browning seems to vary according to the sugar:amine ratio. At a molar ratio of 1:1 the development of color in a glycine-glucose reaction system was higher than at a ratio of 10 sugar to one amino group (Wolfrom *et al.*, 1974). However, a study by Warmbier *et al.* (1976a) on a glucose-lysine model system showed that the rate of browning increased to a maximum at a ratio of one glucose to 3 lysine. One reason for this is that the initial step of formation of the Schiff base is dependent on the concentration of both sugar and amino acid. Table 4 & 5 (Baisier and Labuza, 1992) show that the ratio of amino acid to reducing sugar can effect the rate of browning. The

Table 4

Effect of increasing the molar glucose ration for the browning of a glucose - glycine (0.1 M) solution at pH 7 and 37° **from** Baisier and Labuza (1992)

glucose M	browning rate OD/h x 10 ⁴	glucose ratio	browning ratio
0.5	625	5	11
0.3	326	3	6
0.2	196	2	3.2
0.1	55	1	1.0
0.06	24	0.6	0.4
0.04	11	0.4	0.2

effect of increasing the amino concentration show a greater increase in browning than that of increasing the sugar content on a molar basis and the increase for both is greater than the relative concentration increase (2 times greater for sugar and 2 to 3 fold times greater for amine). Thus one can use this information to change color and flavor. It also suggests that as one reduces sugar content to get a lower calorie candy,

Table 5
Effect of increasing the molar glycine ration for the browning of a
glucose (0.1 M) - glycine solution at pH 7 and 37° (Baisier and Labuza1992)

glycine M	browning rate OD/h x 10 ⁴	glycine ratio	browning ratio
0.4	68	10	23
0.3	62	7.7	21
0.2	49	5	16
0.1	26	9	15
0.04	3	1	1

the amount of flavor and color changes dramatically. As an example, Table 6 shows the typical composition of a caramel, with Maillard reactants marked. The potential

Table 6
Typical caramel composition

- **sucrose ~30-35 %**
- **glucose ~25 - 35%**
- **milk solids ~10-15%**
- **hard fat ~10-15%**
- **butter fat ~10 - 12%**
- **salt ~ 0.2%**

reactivity of a typical caramel will change as some ingredients are replaced for others. For example, at present there is a large consumer concern over fat content and high calorie food. Many of the fat replacers to lower the lower calorie content of foods are protein based. This will increase the color development and change flavor formation, but the extent that this will occur is unknown.

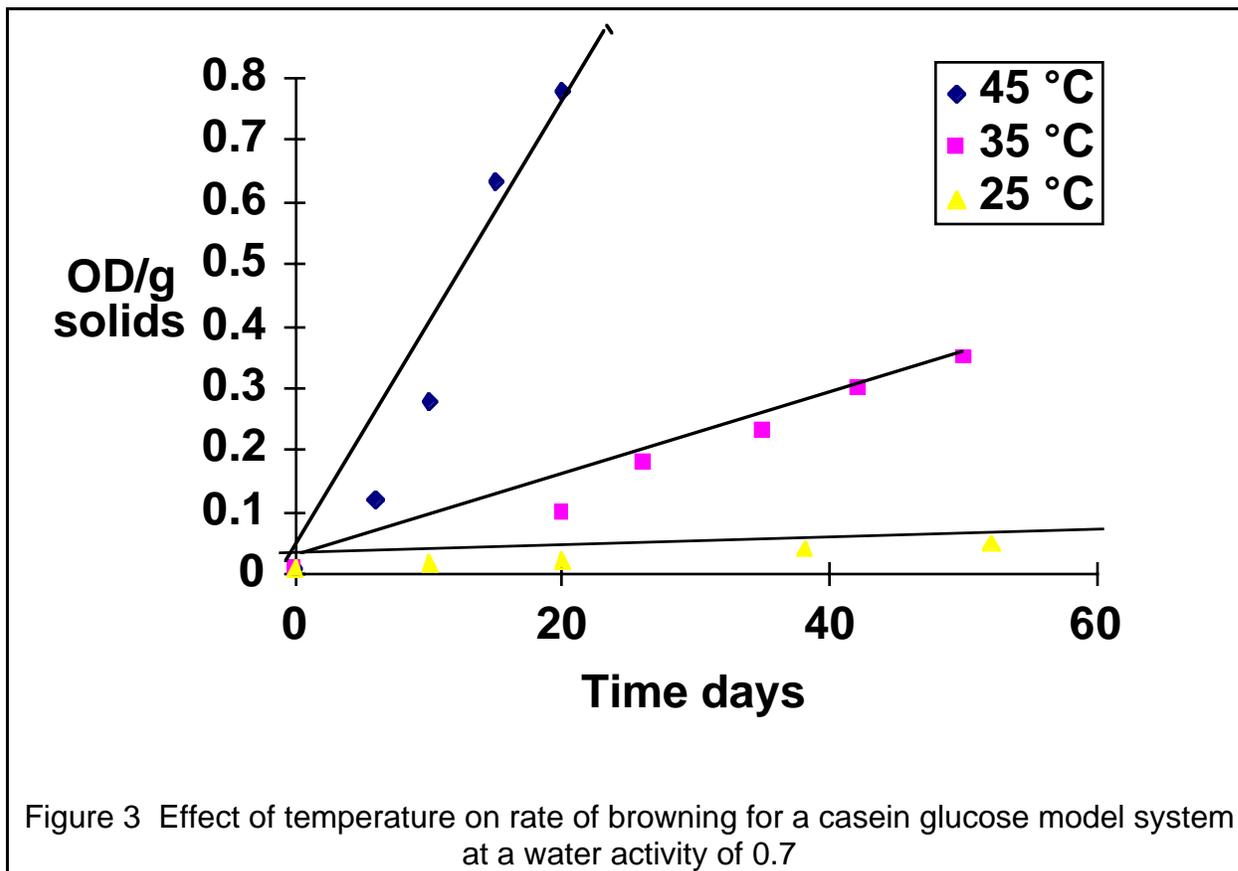
Temperature and time

To make candies it is necessarily to heat the ingredients through a boiling phase, reduce the moisture content and then cool. Different candies are differentiated by their textures which is determined by the cooking and cooling procedure (Table 7).

Table 7
✿ Typical cooking ranges for confectionery

- fondant
 - ✿ 235 - 250° F
 - ✿ 10 - 12.5% moisture
 - ✿ rubber
- caramel
 - ✿ 240 - 270 °F
 - ✿ 5% (hard toffee) - 25% moisture (soft caramel)
 - ✿ glass to rubber
- nougat
 - ✿ 250 - 270 °F
 - ✿ 8 - 11% moisture
 - rubber

The effect of temperature and duration of heating on browning was actually studied by Maillard, who reported that the rate of browning increases with temperature. This has been confirmed many times as shown in Figure 3. The Strecker degradation is favored



by high temperatures and, as mentioned previously, different flavors are formed at different temperatures. The length of heating is also important as the formation of melanoidins usually occurs at a rate which increases in proportion to the square of the reaction time at any give temperature (Mauron, 1981) and different flavors are formed depending on the extent of reaction (Rizzi, 1991).

The effect of temperature is best defined by the temperature dependence of the rate constant as shown by the Arrhenius equation where:

$$k = k_0 (\exp [-E_a/RT])$$

k_0 = reference rate constant

E_a = activation energy or temperature sensitivity of the reaction

R = gas constant

T = temperature in °K

This relationship can be determined by drawing a semi-log plot of rate versus inverse absolute temperature as is shown in Figure 4a. More conveniently, for food processing,

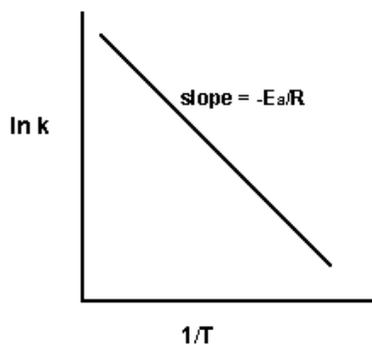


Figure 4a. Arrhenius plot of \log_e of rate constant vs. inverse absolute temperature

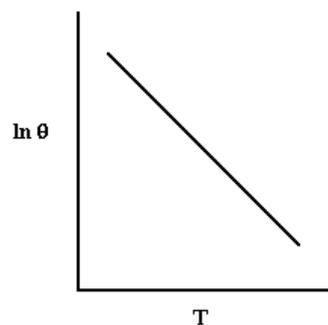


Figure 4 b. Shelf life plot of \log_{10} shelf life θ_s vs. temperature.

is to determine the Q_{10} value where the Q_{10} is related to the activation energy for the reaction. This is the amount that the rate increases or decreases for every 10 °C changes in temperature. Q_{10} can also be calculated by plotting the time it takes to reach a predetermined endpoint against temperature (Fig 4b) on a semi-log plot. The Q_{10} value varies depending on which stage of the reaction is being measured and the temperature range looked at. For example, the initial step has a Q_{10} of 2 and the formation of flavor intermediates has a value of 4 - 6, depending on which intermediate

and the Q_{10} for brown pigment formation has been determined to be between 3 and 8 (Labuza 1994). Thus, the Maillard reaction has a relatively high temperature coefficient of 2-8 (Labuza & Saltmarch, 1984). Most temperature studies have only considered color development and, thus, there is only a little information on the effect of temperature on the effect on flavor formed during browning. For example, if a glucose-glycine model system is heated at 90 °C for an hour or at 70 °C for 20 h, the same amount of glycine is lost from each system but the former has twice the UV absorbance (Kaananne & Labuza, 1989) indicating different intermediate compounds. The aroma profile of a product thus varies with the temperature and time profile during cooking or extrusion heating (Ames, 1990). An example of the effect of Q_{10} is given in Figure 5.

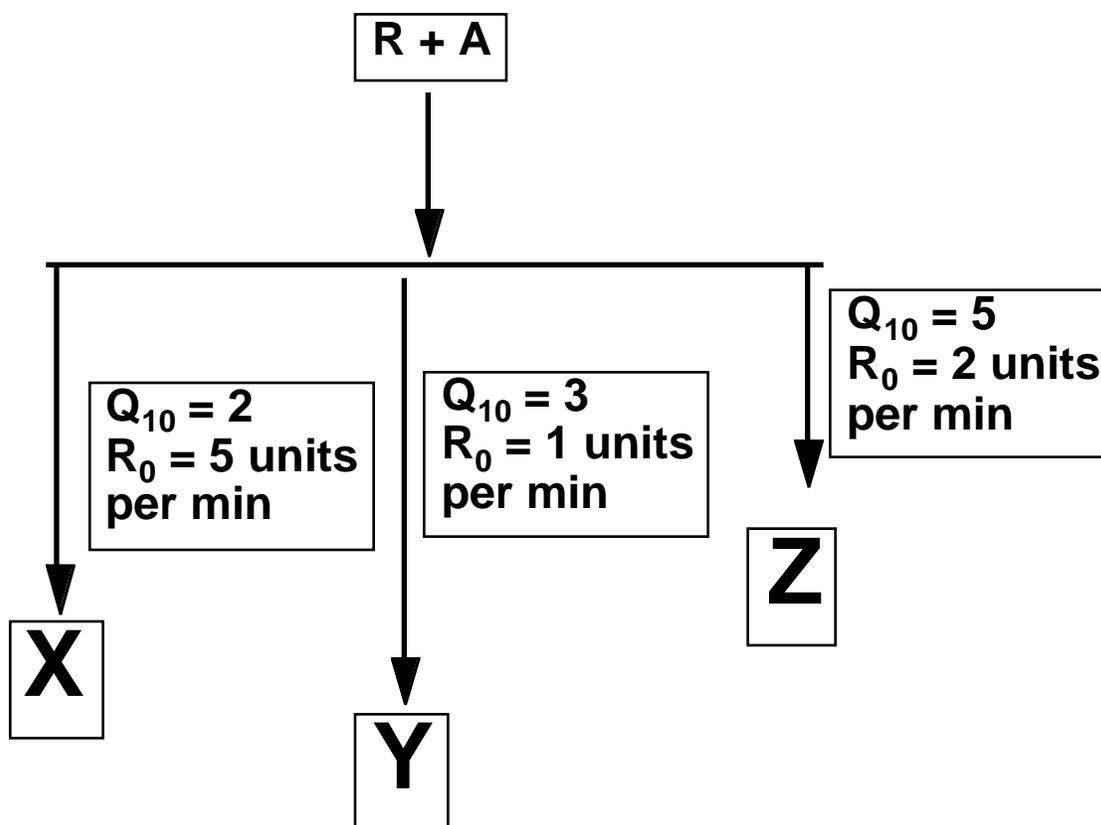


Figure 5

Example of the formation of three different intermediate Maillard flavor compounds with different reaction rates and temperature sensitivities.

Each pathway Figure 5 represents the formation of a different intermediate flavor compound (X, Y, Z). Each of these have a different Q_{10} , temperature dependence, and

a difference rate at the reference temperature T_0 . Now consider two different processes as follows:

Process 1 - cooked at $T = T_0 + 20^\circ\text{C}$

Process 2 - cooked at $T = T_0 + 50^\circ\text{C}$

For Process 1, if we assume the rate is constant over time (apparent zero order) the:

$$\text{Amount formed} = [\text{Rate at } T_0] \times (Q_{10})^{\Delta T/10} \times \text{time}$$

Thus the amount formed for each flavor is:

$$\text{Amount X} = 5 \times (2)^{20/10} \times 10 = 200 \text{ units}$$

$$\text{Amount Y} = 1 \times (3)^{20/10} \times 10 = 90 \text{ units}$$

$$\text{Amount Z} = 2 \times (5)^{20/10} \times 10 = 500 \text{ units}$$

The ratio of X:Y:Z is 2:1:5.

For process 2 we have:

$$\text{Amount X} = 5 \times (2)^{50/10} \times 1 = 160 \text{ units}$$

$$\text{Amount Y} = 1 \times (3)^{50/10} \times 1 = 243 \text{ units}$$

$$\text{Amount Z} = 2 \times (5)^{50/10} \times 1 = 6250 \text{ units}$$

The ratio of X:Y:Z is 0.6:1:26

Thus for the higher temperature shorter time process, the amount of X decreased by 20% while B increased three fold and Z increased 12 fold. Since overall flavor perception is a harmonization of individual compounds, the perceived flavor for the HTST process will be very different than the lower temperature longer time process. There is only a limited amount of information on the effect of temperature and time on the development of each flavor, very little data exists on rates or activation energies. Thus, at any given temperature-time combination, a unique aroma profile is produced. Unfortunately carrying out a method at a higher temperature for a shorter time to increase the rate of production of a candy would probably result in the formation of a completely different flavor profile as is shown in the example (Ames, 1990).

Water content

For the first stage of the Maillard reaction to occur, water is essential. Thus, the rate of this reaction is dependent on the amount of free water available as related to water activity. The rate of brown pigment formation is also dependent on the water content of the system (Reynolds, 1963). Overall the Maillard reaction shows a classic max/min response to change in water activity which is shown in Figure 6. As it is likely that the

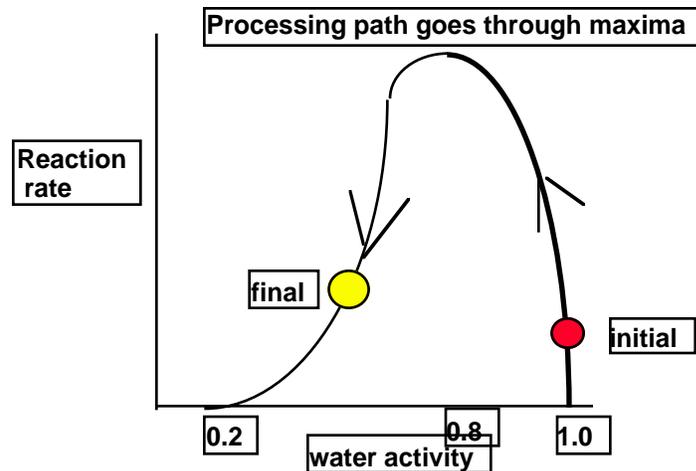


Figure 6. Influence of water activity on the rate of the Maillard reaction

initial formulation before processing will have a high water content, during processing the product is likely to dry and thus move from right to the left on the line shown in Figure 6. Maximum browning occurs at 30% moisture (Wolfrom et al., 1953) which corresponds to a water activity of 0.6 - 0.8. Thus, candies with a higher water content (i.e. chewy and soft candies) may be more susceptible to the Maillard reaction than candies with a lower water content, especially during storage.

The use of certain compounds such as glycerol or sorbitol in confectionery products may change the rate-water activity profile. As shown in Figure 7, glycerol shifts the rate maximum to a lower water activity as compared to a control while sorbitol seems to act as an inhibitor at all aw values, probably due to its high viscosity. Thus even at low moisture there can be browning in the glycol phase. It should be noted

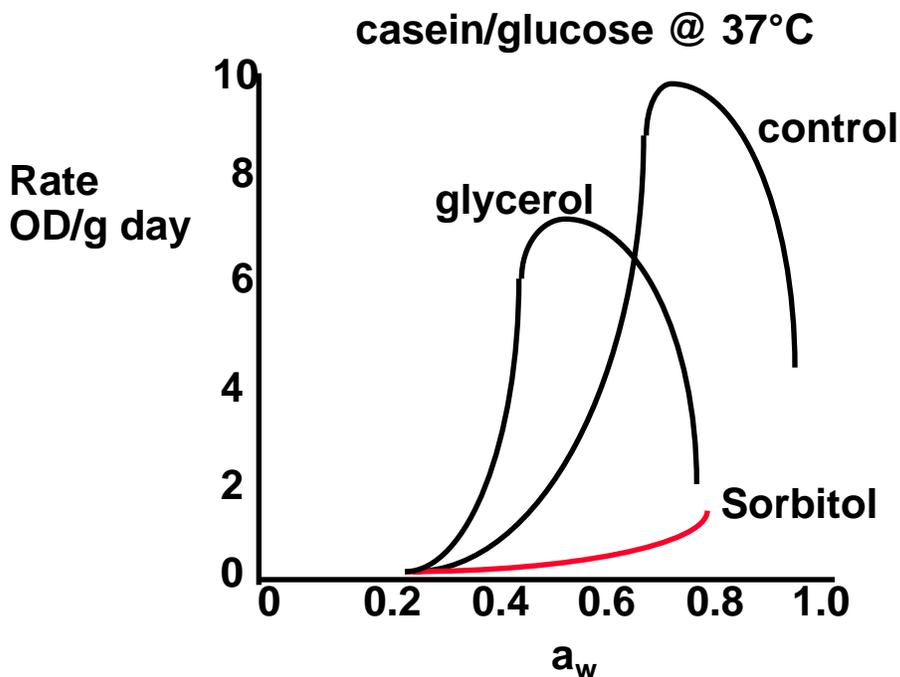


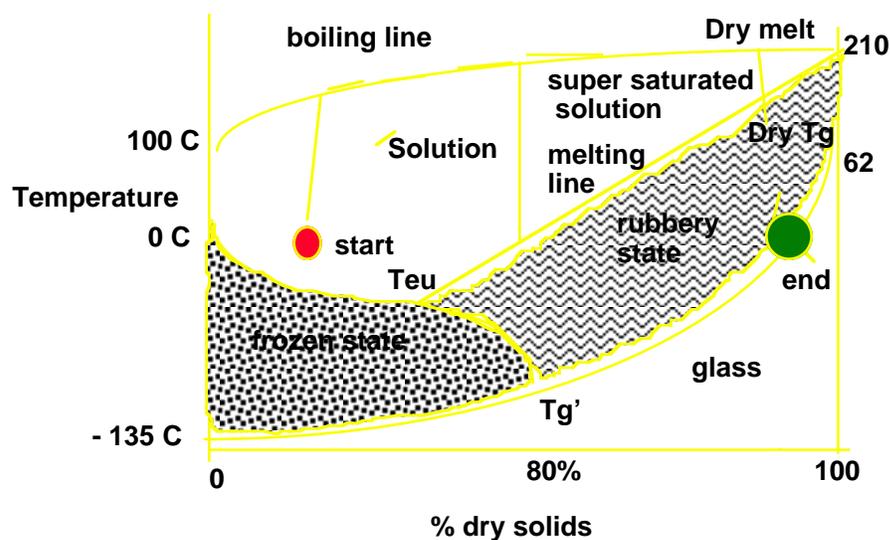
Figure 7. Effect of glycerol and sorbitol on the Maillard reaction rate in a model system

that there is little available on the water activity - temperature profile for candy products. Table 8 shows such data for a pure sucrose - water system, assuming no hydrolysis which would lower the a_w further.

Table 8
Sucrose composition as a function of boiling point

sucrose molality	%	water activity	~boiling point °F
0.77	16	0.99	212.5
1.00	28	0.98	213.1
1.35	37	0.97	213.6
1.92	43	0.96	214.1
2.56	51	0.95	214.8
3.30	59	0.93	215.9
4.03	67	0.90	217.5
5.51	73	0.88	219.3
6.79	81	0.81	223.9
amorp	90	~ 0.7	253.4
amorp	95	~ 0.5	284
amorp	98.2	~ 0.2	320
amorp	99.6	< 0.1	340

The influence of the moisture and temperature can be further examined by looking at the state diagram which superimposes the freezing - melting - boiling equilibrium lines on the glass- rubber amorphous temperature moisture line known as the T_g curve. (Roos and Karel, 1992) as shown in Figure 8. This T_g curve is characterized by a T_g



dry which is the melting point of a non-crystalline solid phase, T_g/C_g , which is that point at the maximum concentration of solids in the liquid phase and the T_g of pure water at about -135°C. The dashed line represents the state changes as a liquid formulation is

heated to boiling, loses water and increases solids content and then is cooled back to room temperature. This temperature-moisture profile will control the rate of flavor and color development. If the end point at room temperature is in the glass state (e.g. a hard candy) then the product is stable during storage, if it is in the rubbery state then further Maillard reactions can occur generally producing undesirable off flavors.

It also should be noted that if the product is in the rubbery state, crystallization and graininess of the sugars can occur, generally the temperature needs to be about 20 to 30 °C above the T_g for the given moisture. As the crystallization occurs, water is given up to other regions which can then increase the browning reaction during storage (Kim et al 1981). Thus crystallization inhibitors like starches and gums are required. The position of the T_g line is thus important and can be manipulated by changing the amounts of various sugars. Table 9 lists the T_g dry and the T_g' for some common sugars as compared to starch. As seen fructose and glucose have lower lines and thus would lead to a more rubbery product with more sensitivity to the Maillard reaction.

Table 9

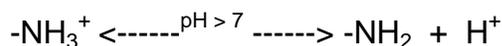
Glass transition data for carbohydrates

sugar	Tg'	Cg' (%solids)	Tg (anhydrous)°C
starch	-6	73	243
lactose	-40	82.5	101
maltose	-41	81.5	87
sucrose	-46	79.5	62
glucose	-57	79,2	31
fructose	-58	78.6	5
sorbitol	-43.5	88	-9
trehalose	?	?	100
pullulan		74	
maltotriose		81	

pH

The pH has a significant effect on the Maillard reaction. In general, the rate and extent of browning increases with increasing pH (Wolfrom & Rooney, 1953). The reaction generally has a minimum is at pH 3 (Lea & Hannan, 1949). At a pH < 3 and > 9, other nonenzymic interactions (i.e. sugar-sugar and protein-protein) compete with the Maillard reaction. Thus, the Maillard reaction has an optimum above pH 7 which is also above the typical pH for most candy products. The rate of formation of ketosamines increases with pH and is acid-base catalyzed. Lee & coworkers (1988) showed that the formation of Amadori products, monofructosyllysine and difructosyllysine in a glucose-lysine mixture increased as the pH was increased from pH 4-8. A change in pH also leads to a change in the mechanism of the reaction and, hence, to the formation of different volatile and colored products (Kroh & Westphal, 1988).

The pH dependence of the initial step of the reaction can be related to the amount of unprotonated amine present, which is controlled by the following equilibrium:



At the pK_a of the amine group, by definition, half the amine is present as the protonated NH₃⁺ state preventing electron transfer. Thus, the rate of the Maillard reaction is lower at a pH lower than the pK_a of the reactive amino group. Theoretically for a pure system

a unit change in pH causes a change of 10 fold in rate but the change in complex systems like candies would be much less, perhaps 2 to 3 fold. Table 10 shows the effect of pH on the amount of unprotonated and thus reactive amine for glycine and lysine. From this it can be seen that lysine is about 10 times more reactive than glycine. Schnickles et al (198) showed that the rate of browning for proteins during storage was directly correlated with the lysine content. If ingredients are changed, such as in changes made to lower calories, it is expected that the pH and buffering capacity will also change, thus there can be significant impact on the types of flavors developed as well as the final color.

Table 10
Influence of pH on the amount of unprotonated amine

<u>pH</u>	<u>glycine</u>	<u>Lysine</u>
1	2.5×10^{-7}	2.45×10^{-6}
3	2.5×10^{-5}	2.45×10^{-4}
5	2.5×10^{-3}	2.45×10^{-2}
7	0.25	2.45
8.9	17	50
9	20	80
9.6	50	84
11	96	~100

One should also note that as temperature increases the pH decreases. The pH of pure distilled water at 25°C is 7 but it drops to 6 at 100; C because of the temperature dependence of the equilibrium constant. Thus although the increase of T during processing increases the reaction rate but this is partially ameliorated by the reduction of pH.

The rate of the reaction is also dependent on the concentration of acyclic sugar present as noted earlier. As was seen in Table 2, the amount of acyclic sugar increases with increasing pH. Hence, as pH increases so does the rate of the Maillard reaction.

Role of buffer

The Maillard reaction forms H^+ ions, so decreasing the pH of the system. As mentioned above, as the pH falls the rate of the Maillard reaction decreases. Consequently, buffers

are necessary when studying the Maillard reaction in a model system. It is not, however, clear as to whether or not confectionery products contain natural buffers (for example, protein and ascorbate have been shown to act as a buffer). Buffers have been shown to increase the rate of browning. It is possible that the buffer mops up the H^+ ions keeping the pH and the reaction rate constant or may interact with the reactants in some way enhancing the Maillard reaction (de Kok & Rosing, 1994). More research is needed in this area.

Oxygen

Recent studies on the Maillard reaction *in vivo* have shown that glucose autoxidation plays a significant role in the glycation of proteins (Ahmed *et al.*, 1988; Hunt *et al.*, 1988; Wells-Knecht *et al.*, 1995). However, it is not clear as to whether oxygen is important for the Maillard reaction of candy systems. It is likely that oxygen will be able to dissolve through the matrix of certain candies especially those in a rubbery state such as gums. Comparatively few studies have been carried out on the effect of metal ions on the Maillard reaction and browning; and as with many other studies the results are conflicting. A recent study on the effect of the addition of Cu^{2+} ions showed that their influence was concentration dependent and dependent on the pH of the system (Rendleman & Inglett, 1990). At pH 3, the addition of Cu^{2+} increased the absorbance of a glucose-glycine reaction mixture. It also seemed copper became bound to the melanoidins at the highest concentration of 100 ppm. At pH 6.18 - 6.86, the addition of Cu^{2+} only had a small effect on the absorbance of a glucose-glycine model system heated at 50 °C. However, these systems were unbuffered so there was a tendency for the pH to decrease most rapidly in the systems with the greatest copper content.

When lipids are present in a food system it is possible that the presence of tocopherol (Vitamin E) reduces the rate of the Maillard reaction (Hermosin, 1992). If, however, the lipids become oxidized, the products may well react with amino acids, forming compounds which are similar to the Maillard intermediates. Also free radicals and peroxide products formed during lipid peroxidation may react with the Maillard intermediates, increasing the rate of the reaction.

Storage

The significance of the Maillard reaction during storage of candies has not been studied to any extent. It is obvious that the water content/water activity is important as is the position in the state diagram (rubber vs glass). Stability of confectionery after manufacture is controlled through use of suitable packaging material and by carefully controlling and monitoring of the conditions of temperature, humidity and light of the warehouse or storage facility. If products are kept over a long period of time, especially under adverse conditions the Maillard reaction may occur causing discoloration and the development of off-flavors. The rate will probably be lower during storage than during processing as there is a lower temperature (25 vs. 120 °C), and lower moisture (10% v. 40-50%). However, at lower temperatures the role of lipid oxidation will become more significant resulting in different reaction products. One factor not considered is that a high processing temperature will result in the formation of more intermediates, causing more reaction products to be formed during storage. This is indicated in Figure 9 for the formation of isovaleraldehyde, a Maillard off flavor formed during storage of dehydrated carrots (Eichner & Wolf, 1983).

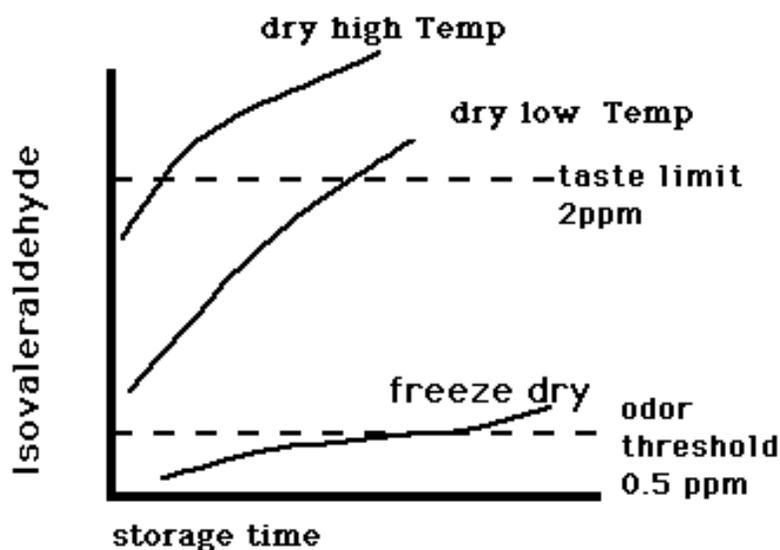


Figure 9

Effect of processing conditions on evolution of Maillard reaction flavors during storage.

Considerations for the Maillard reaction in confectionery products

This review shows that the Maillard reaction is responsible, at least in part, for the final color and flavor of confectionery. Thus, the rate and extent of formation of the Maillard reaction products are dependent on the content of acyclic reducing sugar, the percent of amine that is protonated, reactant ratio and concentration, temperature, pH, water content and matrix state. However, most research on the Maillard reaction has been done on simple model systems generally containing a sugar and a single amino acid, heated at a known temperature for a fixed length of time. Thus, it is difficult to transfer these results to a food processing situation. In a food, the situation is further complicated by composition and structure.

Despite the extensive amount of work done on the Maillard reaction over the past 85 years, the situation in the processing plant is still a case of “cook and look”. The reason for this is that during processing the temperature or moisture content change with time, resulting in a concentration change with different diffusion rates for the reactants. During processing, the pH is not controlled, buffers are rarely added to food and it is quite often assumed that natural buffers are present. If pH is not controlled, the Maillard reaction releases hydrogen ions, causing a fall in pH; this decreases the rate of the Maillard reaction and thus impacts on flavor. As mentioned early, there are over 2000 types of candy, each with over a hundred different formulas and processing methods. Thus, for a particular candy, the actual amount of reactive reducing compound and reactive amine is unknown as this will vary with the formula used.

Confectionery, like all food, is a heterogeneous mixture of ingredients and structures. In such a system, many reactions may be occurring at different rates and in different domains. These reactions may or may not influence the progress of the Maillard reaction. For example, during chocolate manufacture, lipid peroxidation may occur and the products may react with amino acids to undergo stage 3 Maillard-type reactions forming melanoidins and flavors. Also in candy production, the high processing temperatures and the lower fat content could lead to the occurrence of sugar-sugar interactions. Many of the products from this reaction are similar to Maillard reaction products and, thus, change the flavor and color of the final product.

The composition of confectionery products may be altered by additives, e.g. flavor and color compounds added to confectionery or proteins and carbohydrate gums added to

candies to give structural stability or to change caloric value in making of low fat or low sugar products. These additives may, however, react with food constituents, including Maillard reaction intermediates and products. This might reduce the potency of the additive by decreasing its concentration. Also the interactions of the additives with food constituents may alter the progress of the Maillard reaction during candy production and cause the formation of unexpected flavor products.

Food structure and texture has only been considered in a few studies. Of the few reports on non-liquid model systems, most considered the Maillard reaction in dried-powder systems, either from fruit juices or milk (e.g. some flavor studies considered the Maillard reaction in a dry mixture of sugar and amino acid). These did not take into account the complex structure of a "solid" food such as the confectionery products considered here. Even though a few studies have considered the migration of reactants through a cellulose matrix, these studies did not consider the effect of reactants trapped in the matrix of a food such as a candy or in dual texture products, e.g., those with a higher moisture soft center.

Finally, the importance of carefully monitoring the processing conditions to obtain a consistent product must be emphasized. Especially, kinetics studies have shown that a small change in temperature of processing (and/or other parameters) can have a large effect on the final food product in terms of Maillard reaction products. For example, a change in heating (time or temperature) may cause the concentration of volatile flavors to change by releasing more at higher temperatures and longer times.

Conclusion

Kinetic studies on model systems of the browning reaction are beginning to allow the prediction of browning of simple model systems containing one sugar and amino acid. This work needs to be extended into the area of flavors and into the progress of Maillard browning in mixtures of sugars, amino acids, proteins etc. Also kinetic studies directly on food systems or on the interactions which may occur between the products of the different reactions in food must be undertaken.

However, there are still many unanswered questions concerning the Maillard reaction. In particular, the interaction of the Maillard reaction with other nonenzymic reactions such as lipid peroxidation, caramelization, protein denaturation, etc. Also a study on the

progress of the Maillard reaction in a heterogeneous substrate such as food must be investigated to give the whole picture of reactions in confectionery. Knowledge of the Maillard and other non-enzymatic browning reactions which occur during food is important for the control of the sensory properties of confectionery products.

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