Core course 13: Unit 3 (Part 1): Food Chemistry II

Browning reactions in food

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Dear viewers, in today’s session under Food technology we will be learning about Browning reactions in food.

Browning of foods is defined as the process in which there is a change in the color of the food due to a chemical process. Browning reaction is broadly classified into two types i.e., Enzymatic browning and non-enzymatic browning. Non-enzymatic browning of foods includes browning reactions without the involvement of enzymes. Although most non-enzymatic browning in food materials is undesirable when it indicates deterioration in flavor and appearance of the product involved, the development of brown colors in some products is entirely acceptable. It is desirable in caramelization of sugars, browning of tea which imparts flavor, browning of the crust in bread and roasting of meat. To understand more about non-enzymatic browning, the topic is divided into seven major topics:

1. Types of browning reactions
2. Maillard reaction
3. Caramelization
4. Ascorbic acid browning
5. Lipid and protein browning
6. Factors influencing non-enzymatic browning reactions
7. Implications of non-enzymatic browning reactions

1. **Types of Browning reactions:** Browning reactions in food are broadly classified as enzymatic and non-enzymatic browning reactions. Enzymatic browning occurs usually in fruits and vegetables. It is defined as a process wherein enzymes such as phenolase oxidize phenols to orthoquinones which rapidly polymerize to form undesirable brown or black pigments such as melanins. This process takes place in the presence of oxygen. Enzymatic browning becomes evident when fruits and vegetables are subjected to processing or to mechanical injury. These enzymes are present in fruits such as apples, banana, pears, gooseberry and kiwi and; in vegetables such as lettuce, brinjal, mushrooms, asparagus, broccoli, carrot and potato. Non-enzymatic reactions on the other hand include chemical reactions involving reducing sugars which individually undergo caramelization or sugars along with proteins which undergo Maillard reaction.

2. Maillard reaction: The Maillard reaction is the action of amino acids and proteins on sugars. The reaction was first reported by Maillard in 1912. The sugar moiety is a reducing sugar with a free carbonyl group. This reaction occurs in many foods causing browning. The end-product is the melanoidins, which are the brown pigments. The brown color results from the following three stages of development.

I. Initial stage with no formation of color
A. Sugar-amine condensation
B. Amadori rearrangement
II. Intermediate stage with formation of yellow pigments
    C. Sugar dehydration
    D. Sugar fragmentation
    E. Amino acid degradation
III. Final stage with the formation of highly colored dark pigments.
    F. Aldol condensation
    G. Aldehyde-amine polymerization and formation of heterocyclic nitrogenous compounds.

Maillard reaction is called the carbonyl-amine reaction, between free amino groups from amino acids, peptides, or proteins and the carbonyl group of a reducing sugar such as ribose, glucose, fructose and galactose. Among the most reactive carbonyl compounds are α,β-unsaturated aldehydes such as furaldehyde, and α-dicarbonyl compounds such as diacetal and pyruvaldehyde. The reaction which is reversible starts between an aldose or ketose sugar and a primary or secondary amine, the product of which is a glycosylamine. The yield of glycosylamine is higher when the amount of water present is low. The formation of glycosylamine is important in the browning of concentrated and dried foods. The initial product of the reaction between glucose and ammonia is glucosylamine. This rearranges to form 1-amino-1-deoxy-D-fructose in the presence of an acid catalyst which is called Amadori rearrangement. The dehydration of sugar leads to the formation of Amadori Rearrangement Products or ARPs such as furfural, reductones and fission products such as acetol, pyruvaldehyde, diacetyl groups. The reaction takes place in two ways, in the presence of neutral or acid solutions furfurals are formed and in the dry state or in non-aqueous solvents when amines are present, reductones are formed. The Heyns rearrangement shows the reaction starting with fructose instead of glucose. Both of these rearrangements bring about the same transformation where α-D-fructopyranosylamine is converted to 2-amino-2-deoxy-α-D-glucopyranose. The order of formation of compounds in Heyns rearrangement is ketoseamine followed by diketoseamine and diamino sugar in the presence of aldose.

The intermediate stage leads to breakdown of Amadori compounds and the formation of degradation products, reactive intermediates such as 3-deoxyglucosone or formation of osones by Strecker's reaction and volatile compounds that lead to the formation of flavor. The 3-deoxyglucosone participates in cross-linking of proteins at much faster rates than glucose itself, and further degradation leads to two known advanced products: 5-hydroxymethyl- 2-furaldehyde (HMF) and pyraline.

The main pathway for the formation of brown color in foods appears to be degradation and condensation by way of the 1,2-enol forms of the aldose or ketose amines. The aldol condensation mechanism for the α,β-dicarbonyl compounds formed seems to be involved. The formation of these brown pigments via the carbonyl-amine reactions has similarities to the formation of caramels. Another step in the formation of brown pigments is the Strecker degradation of the amino acid moiety. This degradation of the alpha amino acids results in aldehydes containing one less carbon atom than the amino acid. The loss of the carbon atom is accounted for by the release of carbon dioxide. In addition to CO_2, it produces carbonyl compounds and amines. It is also necessary that the amino group of the amino acid be at the alpha position to the carbonyl group and the CO_2 comes from the carboxyl group of the amino acid moiety and not from the sugar. These dicarbonyl compounds are osones and are active agents for Strecker degradation. Pyrazine compounds with different amounts of substitution are formed in carbonyl-amine reactions and can cause Strecker degradation of the amino acid to form 2,5-Dimethylpyrazine from a glucose and glycine reaction.

The final stage is characterized by the production of nitrogen-containing brown polymers and copolymers known as melanoidins. They have been described as low-molecular weight colored substances that are able to cross-link proteins via *ε*-amino groups of lysine or arginine to produce high molecular weight colored melanoidins. Also, it has been postulated that they are polymers consisting of repeating units of furans and/or pyrroles, formed during the advanced stages of the Maillard reaction and linked by polycondensation reactions. Chemical structure of melanoidins can be mainly formed by a carbohydrate skeleton with few unsaturated rings and small nitrogen components; in other cases, they can have a protein structure linked to small chromophores.

3. Caramelization: When sugars are treated under anhydrous conditions with heat, or at high concentration with dilute acid, caramelization occurs, with the formation of anhydro sugars. Glucose forms glucosan or l,2-anhydro-α-D-glucose and levoglucosan or 1,6-anhydro-β-D-glucose with a specific rotation of +69° and -67° respectively. With similar treatment, fructose gives rise to levulosan or 2,3-anhydro-β-D-fructofuranose. Simultaneous hydrolysis and dehydration take place when sucrose is heated at about 200°C, and followed by a rapid dimerization. These compounds are characterized by isosacchrosan, which is a sucrose molecule one molecule of water lesser. It is not sweet, but mildly bitter. When dilute solutions of reducing sugars are used, the beginning stages of caramelization involve enolization, isomerization, dehydration, and fragmentation. Following this, polymerization reactions take place, which in the end form pigments similar to those formed in more concentrated solutions, or at higher temperatures. Caramels for commercial use are made from glucose syrups, but usually caramelization is the result of reactions that take place when sucrose is heated. The reaction takes place at 200°C. There are three stages during this process, during which water is lost and isosacchrosan formed first followed by formation of other anhydrides. The first stage starts with melting of the sucrose, followed by foaming which continues for 35 min. During this period one molecule of water is lost from a molecule of sucrose. The foaming then stops. Shortly after this, a second stage of foaming starts which lasts 55 min. During this stage about 9% of the water is lost, and the compound formed is caramelan, a pigment with the molecular formula of $C_{24}H_{36}0_{18}$.

$$2\ C_{12}H_{22}0_{11} - 4\ H_{2}0 = C_{24}H_{36}0_{18}$$

Two molecules of sucrose lose four molecules of water to form caramelan.

Caramelan melts at 138°C, is soluble in water and ethanol, and is bitter in taste. The pigment caramelen with the molecular formula $C_{36}H_{50}0_{25}$ is formed during the third stage of foaming which starts after about 55 min.

$$3\ C_{12}H_{22}0_{11} - 8H_{2}0 = C_{36}H_{50}0_{25}$$

Three molecules of sucrose lose eight molecules of water to form caramelen.

Caramelen melts at 154°C and is soluble in water. When the heating is continued, the result is the formation of humin, which is an infusible, dark mass with a high molecular weight, and is called caramelin. The molecular formula of caramelin is $C_{125}H_{188}0_{80}$. The reaction rate of caramelization is ten times greater at pH 8 than at lower pH.

4. Ascorbic Acid Browning

Browning of ascorbic acid can be briefly defined as the thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions, by oxidative or non-oxidative mechanisms, either in the presence or absence of amino compounds. Non-enzymatic browning is one of the main reasons for the loss of commercial value in citrus products. These damages, degradation of ascorbic acid followed by browning is also a concern in non-citrus foods such as asparagus, broccoli, cauliflower, peas, potatoes, spinach, apples, green beans, apricots, melons, strawberries, corn, and dehydrated fruits. In citrus juices, non-enzymatic browning is from reactions of sugars, amino acids, and ascorbic acid. In freshly produced commercial juice packed in Tetrapak, is mainly due to non-enzymatic browning involving carbonyl compounds formed from L-ascorbic acid degradation. The presence of amino acids and possibly other amino compounds enhance browning.

When oxygen is present in the system, ascorbic acid is degraded primarily to dehydro ascorbic acid (DHAA). DHAA is not stable and spontaneously converts to 2,3-diketo-l-gulonic acid

Under anaerobic conditions, ascorbic acid undergoes the generation of diketogulonic acid via its keto tautomer, followed by β elimination at C-4 from this compound and decarboxylation to give rise to 3-deoxypentosone, which is further degraded to furfural. Under aerobic conditions, xylosone is produced by simple decarboxylation of diketogulonic acid and that is later converted to reductones. In the presence of amino acids, ascorbic acid, DHAA, and their oxidation products furfural, reductones, and 3-deoxypentosone may contribute to the browning of foods by means of a Maillard-type reaction.

5. Lipid and protein browning: Lipid oxidation occurs in oils and lard, and also in foods with low amounts of lipids, such as products of vegetable origin. The reaction is both desirable and undesirable. It is desirable in the production of cheese or fried food aromas and undesirable when odor, appearance and flavor are affected. Moreover, toxic compound formation and loss of nutritional quality can also be observed. Although the lipids can be oxidized by both enzymatic and non-enzymatic reactions, the latter is the main involved reaction. This reaction arises from free radical or reactive oxygen species generated during food processing and storage, hydroperoxides being the initial products. These compounds are quite unstable further leading to many reactions and pathways. The enzymatic oxidation of lipids occurs sequentially. Lipolytic enzymes can act on lipids to produce polyunsaturated fatty acids that are then oxidized by either lipoxygenase or cyclooxygenase to form hydroperoxides or endoperoxides, respectively. Later, these compounds suffer a series of reactions to produce, among other compounds, longchain fatty acids responsible for important characteristics and functions. Via polymerization, brown-colored oxypolymers are produced subsequently from the lipid oxidation derivatives. Radical transfer occurs early in lipid oxidation, and this process underlies the antioxidant effect for lipids. In addition, protein radicals can also transfer radicals to other proteins, lipids, carbohydrates, vitamins, and other molecules, especially in the presence of metal ions such as iron and copper The interaction between oxidized fatty acids and amino groups has been related to the browning detected during the progressive accumulation of lipofuscin an age-related yellow-brown pigments in lysosomes of men and animals. According to the mechanism proposed for the protein browning caused by acetaldehyde, the carbonyl compounds derived from unsaturated lipids readily react with protein-free amino groups to produce, by repeated aldol condensations, the formation of brown pigments. More recently, another mechanism based on the polymerization of the intermediate products 2-(1-hydroxyalkyl) pyrroles has been proposed. 2-

(1-Hydroxyalkyl) pyrroles (I) have been found to be originated from the reaction of 4,5-epoxy-2-alkenals formed during lipid peroxidation with the amino group of amino acids and/or proteins, and their formation is always accompanied by the production of N-substituted pyrroles (II). Compounds derived from reaction of 4,5-epoxy-2-alkenals and phenylalanine have been found to be flavor compounds analogous to those of Maillard reaction. Therefore, flavors traditionally connected to Maillard reaction may also be produced as a result of lipid oxidation. However, the N-substituted 2-(1-hydroxyalkyl) pyrroles are unstable and polymerize rapidly and spontaneously to produce brown macromolecules with fluorescent melanoidin-like characteristics.

6. Factors influencing non-enzymatic browning reactions:

A number of factors can affect the formation of these pigments. Among these are pH, temperature, moisture content, time, concentration, and nature of the reactants. Also, one of these factors may affect another.

Temperature: The rate of browning increases with rising temperature. In model systems the development increases 2 to 3 times for each 10°C rise in temperature. In natural systems, particularly those high in sugar content, the increase may be faster. Two methods have been used to measure these changes: (1) measure of color development and (2) measure the evolution of CO_2.

pH: Although browning reactions usually slow down as the pH decreases until the optimum stability pH for reducing sugars is passed, this is not so important for food products. Maillard reaction and ascorbic acid degradation is faster at an alkaline pH or a nearly dry state.

Moisture: The moisture content seems to have an important effect on the rate of browning. It is quite likely that for moisture contents above 30% a decrease in reaction is caused by dilution.

Acids: The development of brown color in dried fruits is largely caused by the reaction between amino acids and glucose, a reaction which is speeded by the presence of organic acids.

Fermentation: The formation of ketoseamines in dried whole egg or egg white is avoided by fermentation of the glucose before drying.

Oxygen: Removal of oxygen decreases the rate of browning reactions especially in ascorbic acid oxidation.

Technologies: Modified-atmosphere packages, microwave heating, ultrasound-assisted thermal processing, pulsed electric field processing and carbon dioxide-assisted high-pressure processing are some examples of technological processes that allow ascorbic acid retention and consequently prevent undesirable browning.

7. Implications of non-enzymatic browning reactions

Advantages: Processing such as baking, frying, and roasting are based on the Maillard reaction for flavor, aroma, and color formation. Maillard browning may be desirable during manufacture of meat, coffee, tea, chocolate, nuts, potato chips, crackers, and beer and in toasting and baking bread. In foods, antioxidant properties of Maillard reaction products have been found in honey and in tomato purees. The antimicrobial activity of coffee melanoidins against different pathogenic bacteria has been reported.

Disadvantages: In processes such as pasteurization, sterilization, drying, and storage, the Maillard reaction often causes detrimental nutritional and organoleptic changes such as lysine damage. Other types of undesirable effects produced in processed foods by Maillard reaction may include the formation of mutagenic and carcinogenic compounds. Frying or grilling of meat and fish may generate low (ppb) levels of mutagenic/carcinogenic heterocyclic amines via Maillard reaction. The formation of these compounds depends on cooking temperature and time, cooking technique and equipment, heat mass transport, and/or chemical parameters. The formation of carcinogen acrylamide is observed in a range of cooked foods. Moderate levels of acrylamide (5–50 μg/kg) is found in heated protein-rich foods, and higher levels (150–4000 μg/kg) in carbohydrate rich food, such as potato, beet root, certain heated commercial potato products, and crisp bread. It is absent in raw or boiled foods, but it is present at significant levels in fried, grilled, baked, and toasted foods. On the basis of the large number of existing studies, the International Agency for Research on Cancer has classified acrylamide as “probably carcinogenic” to humans. The Maillard reaction is one of the main reactions causing deterioration of proteins during processing and storage of foods. This reaction can promote nutritional changes such as loss of nutritional quality, attributed to the destruction of essential amino acids or reduction of protein digestibility and amino acid availability.

Conclusion:

To sum up, the Maillard reaction results in the formation of brown nitrogenous polymers and copolymers. The reaction involves the sugar-amine condensation, the Amadori rearrangement resulting in the keto form, and the Strecker degradation which results in action on the alpha amino acids with the loss of one molecule of CO_2 and the formation of an aldehyde followed by formation of colored pigments. When sugars are treated under anhydrous conditions with heat, or at high concentration with dilute acid, caramelization occurs, with the formation of anhydro sugars. Ascorbic acid browning occurs due to thermal decomposition of ascorbic acid under both aerobic and anaerobic conditions, by oxidative or non-oxidative mechanisms, either in the presence or absence of amino compounds. These reactions can be controlled with factors such as pH, temperature, moisture content, time, concentration, and nature of the reactants. Browning reactions in food can therefore be advantageous and disadvantageous depending on its use in food system.