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REVIEW

Food caramels: a review

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Abstract Caramel, defined as coloring agent and as an antioxidant, is being used in several kinds of food products. It has been classified into 4 classes to satisfy the requirement of several food and beverage systems. The variation in its consistency owing to its basic content of milk solids, sugars, and fat has been studied. Several methods have been found to estimate the amount of color provided by caramel in food products. Various formulations have been cited for the production of caramel by eradicating the frequent areas of problems during its processing. Caramel has been used as a synthetic colorant replacer in the baking and beverage industries. Researchers have aimed to ascertain the contribution to the antioxidant activity of some caramel-containing soft drinks. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established an acceptable daily intake (ADI) of Class I caramel color as "not specified"; that of Class II as 0–160 mg/kg body weight; that of Class III as 0–200 mg/kg body weight; and that of Class IV as 0–200 mg/kg body weight. This paper is an overview of the classification, physicochemical nature, formulations, coloring properties, antioxidant properties, and toxicity of caramel in different food systems.

Keywords Color · Caramel · Antioxidants · Maillard reaction · Toxicity

Introduction

Caramel is a complex blend of fat globules in varying size groupings surrounded by a high-concentration sugar solution in which milk solids-not-fat are dispersed or dissolved. It is generally manufactured by heating a mixture of glucose syrup, milk, and vegetable fat at a temperature ranging between 118 °C and 130 °C (Minifie 1989). Heating enhances the browning reaction and regulates the finished product moisture content (de Man 1990). The greatest single factor affecting the texture and chew is the amount of moisture left in the caramel. Color stability, and flavor are also considered important characteristics in applications. Besides these, recently caramel has also been highlighted as beneficial in non-enzymatic browning inhibition. A strong type of caramelization with yet another flavor is obtained by alkaline treatment; for example, by the reaction of sodium bicarbonate with boiling syrup at about 148.8 °C. The action of ammonia on certain reducing sugars also produces a caramel color. The polymeric material from plain caramel is generated from the condensation reactions of the aldehydes and ketones formed by heating the sugar with bases or acids. Ammonia caramel is formed in a Maillard-type reaction where carbonyl compounds react with amino groups or ammonia. Sulfite caramel is also a Maillard-type polymer and the information on that caramel composition is of practical importance.

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Classification of caramel

Caramel colors have been used for a long time and in a wide variety of food products so that consumers tend to think of them as a single substance, when in reality they are a family of similar materials with slightly different properties. Each type of caramel color has specific functional properties that ensure compatibility with a product and eliminate undesirable effects, such as haze, flocculation, and separation. Caramel colors are dark brown to black liquids or solids having an odor of burnt sugar and a pleasant, somewhat

bitter taste. They are totally miscible with water and contain colloidal aggregates that account for most of their coloring properties and characteristic behavior toward acids, electrolytes, and tannins.

Guelfi (1988) noted that American caramel is a distinctive class of confectionery, differentiated from European caramel by lighter color, opacity, and, generally, a predominance of dairy flavor over darker caramelized flavor. Hardt and Baltes (1989) classified the unknown caramel colors by Curie-point pyrolysis-high-resolution gas chromatography/mass spectrometry. Myers and Howell (1992) overviewed the chemical characterization and specifications of the 4 classes of caramel color, the historical development and the methods of manufacture.

There are 4 distinct types of caramel color to satisfy the requirements of different food and beverage systems (JECFA 1992; Codex 1996):

Caramel Color I (also known as plain or spirit caramel)
Caramel Color II (caustic sulfite caramel)
Caramel Color III (ammonia or beer caramel, baker's and confectioner's caramel)
Caramel Color IV (known as sulfite-ammonia, soft drink caramel, or acid-proof caramel).

Both the European Technical Caramel Association (EUTECA) and the International Technical Caramel Association (ITCA) have standardized the properties of 4 classes and 10 types of caramel.

Depending on their isoelectric points (pIs) caramels may be roughly divided into positive (pI 5.0–7.0), negative (pI 4.0–6.0), and spirit (pI<3.0) types. The pI determines the possibility of application of caramels. The average molecular weight of compounds in caramels is between 5 kDa (electropositive caramels) and 10 kDa (electronegative caramels). Other important properties are pH, aqueous solubility (should be completely soluble), specific gravity (usually 1.315–1.345), color intensity or tinctorial strength, hue index (called 'redness'), as well as flavor (taste and aroma). Its flavor has 2 organoleptic properties consisting of 2 components: taste arising from the acidity (modifiable) and a taste contribution attributed to the nature of the caramel (no-modifiable) (Tomasik 1993). GC/MS has been used for the rapid classification of caramels. The differentiation was done based on the nature of the compounds generated by pyrolysis. Using GC/MS analysis of the pyrolysates, the nature of the caramel can be obtained from the peak intensities (Moldoveanu 1998).

According to international standards, color intensity is defined as the absorbance of a 0.1% (w/v) solution of caramel color solids in water in a 1-cm cell at 610 nm. To express a parameter on a color equivalent basis, the parameter is determined for the caramel color and is expressed in terms of a product having a color intensity of 0.10 absorbance units. Table 1 lists the analytical requirements for classification of caramel colors. The complete analytical methodology for each of the above specifications can be found in the Compendium for Caramel Color (JECFA 1992) and the Food Chemicals Codex (Codex 1996). The Eighth Amendment to the Colors Directive of the European Union makes it clear that these 4 classes of caramel color are intended for coloring and are to be distinguished from and do not correspond to the sugary aromatic products obtained from heating sugar and used for flavoring foods and drinks (known as burnt or caramelized sugars). In the United States, the specifications for caramel color are found in the Code of Federal Regulations (CFR), Title 21, Section 73.85, and are very similar to those discussed above. The limits for all 4 types of caramel color are not more than 3 ppm of arsenic, not more than 10 ppm of lead, and not more than 0.1 ppm of mercury. Caramel may be used for coloring ingested food or for topically applied drugs.

High pressure liquid chromatoraphy analysis revealed unique profiles within each class and distinct differences between classes. The data indicated that each of the 4 classes can be considered as separate and that, throughout each class, the samples characterized constitute a homologous series of mixtures in terms of chemical composition (Licht et al. 1992b). Monosaccharide (D-fructose, D-glucose, anhydrosugars), disaccharide (glucobioses), and pseudodisaccharide (di-D-fructose dianhydrides) contents of D-fructose, D-glucose, and sucrose caramels were determined by gas–liquid chromatography–mass spectrometry (GLC–MS) as their trimethylsilyl (TMS) or TMS–oxime derivatives (Ratsimba et al. 1999).

Analytical determination of physicochemical properties of caramel system

Caramel is polymeric in its character. Among the numerous products of carbohydrate caramelization, most thoroughly characterized are the volatile substances and the group of nonvolatile carbohydrate oligocondensation products (Table 2).

Table 1 Codex classification of caramel

Parameters	Class I—E150 a	Class II—E150 b	Class III—E150 c	Class IV—E150 d
Color intensity	0.01–0.12	0.06–0.10	0.08–0.36	0.10–0.60
Total nitrogen (%)	<0.1	<0.2	1.3–6.8	0.5–7.5
Total sulphur (%)	<0.3	1.0–3.5	<0.3	1.4–10.0

(Codex 1996).

Table 2 Coloring and flavoring compounds of caramel

S. No	Coloring compounds of caramel (Non volatile compounds)
1	$C_{24}H_{36}O_{18}$ (caramelan)
2	$C_{36}H_{50}O_{25}$ (caramelen)
3	$C_{96}H_{102}O_{51}$ (caramelin)
	Flavoring compounds of caramel (Volatile compounds)
1	Acetylfuran
2	furfurol,
3	5-hydroxymethylfurfural
4	3-hydroxy- 2-acetylfuran
5	3-hydroxy-2(5H)-furanones
6	4-hydroxy-3(2H)-furanones
7	4-pyrone derivatives

In 1858, the French chemist M. A. Gelis authored the first known published technical study of caramel color (Gelis 1858). Gelis'work indicated that caramelized sucrose contains 3 main products: a dehydration product, caramelan $C_{12}H_{18}O_9$; and two polymers, caramelen $C_{36}H_{50}O_{25}$ and caramelin $C_{96}H_{102}O_{51}$. The presence of aldehydes of the furan series in caramel accounts for its antioxidant properties (Zenkevich et al. 2001). Ammonia caramels additionally contain melanoidin which is much darker in color than the 3 other components mentioned above. Hence, ammonia caramels are the most colored (Tomasik 1993). Vtllalon et al. (1999) using high-performance liquid chromatography determined the furanic aldehydes furfural and 5-hydroxymethyl furfural, in aged brandies and hydroalcoholic solutions of commercial caramels. Zenkevich et al. (2001) examined the possibility for quantitative determination of 5-(hydroxymethyl) furfural by chromatographic analysis.

Carbohydrate and fat contents in caramel largely dictate the structural and rheological properties of products; hence their constitution is essential to be analyzed. Maurice (2003) noted that caramel covers a wide range of textures and physical characteristics, from a soft, free-flowing liquid, suitable for an ice cream or dessert sauce, to a more viscous center for molded chocolate units, or from a firm, chewy texture, suitable for cut-and-wrap units, to hard English toffee. Caramel as fat droplets within a matrix of sugars was imaged by the use of pulsed-force atomic force microscopy (AFM) and scanning thermal microscopy (Morton et al. 2003).

Caramel is physically a glass consisting of viscous syrup with milk solids dissolved or dispersed in it and with fat emulsified into it. The viscosity of the syrup binding the whole structure together depends on many variable factors, particularly water content and anions present in the reaction, such as phosphate, carbonate, acetate, sulfite and so on which influence the reaction path.

It has been found that the physical and physicochemical properties of food polymers in the glassy state are much different from those in the rubbery state. Many researchers have demonstrated that the glass transition has a strong impact on food texture (Rogers et al. 1990), freeze drying (Roos and Karel 1991), frozen storage stability (Caldwell et al. 1990; Levine and Slade 1990), caking/clumping (Peleg 1995), and crystallization (Hartel and Shastry 1991; Jouppila and Ross 1994).

Unexplained variations in the rheological properties of caramel are an ongoing problem in the confectionery industry. A number of factors responsible for these variations have been suggested, such as protein concentration seasonal variations in the raw milk (Weckel and Steinke 1973), forewarming of milk, and salt balance. Milk proteins play a major role in modifying the textural properties of caramel. The resulting varying textural properties of caramel are presumed to be the consequences of structural/functional properties of milk proteins (Atapattu and Kakuda 1998). Cooking temperature of the caramel mix, and processing conditions used for sweetened condensed whole milk (SCWM), including time and temperature of the forewarming treatment, were the factors having the greatest effect on the textural properties of caramel (Tonucci and von Elbe 1988). Their study also revealed that caramel texture was not affected when calcium levels in milk used in the formulation were varied in the range of 20% above to 20% below the average value.

Molecular and mechanical relaxation properties of caramel systems as a function of temperature and composition using nuclear magnetic resonance (NMR) and dynamic mechanical analysis (DMA) were studied by Chung et al. (1999). Corn syrup, the major carbohydrate in the original recipe, was replaced by polydextrose to different extents. The glass transition temperature (Tg) of the caramel systems was estimated using NMR and DMA techniques; it increased with increasing polydextrose content. Cold flow, one of the most important rheological characteristics was retarded in the higher- polydextrose caramel system, coincident with the decrease in proton mobility of the caramel system.

Small-amplitude dynamic viscoelastic properties of 3 different commercial caramel formulations in the range of temperature and frequencies of 20–80 °C and 0.1–10 Hz, respectively, using a controlled-rate rheometer, were studied by Ahmed et al. (2006). Dynamic shear results revealed viscous behavior for caramel samples. Differential scanning calorimetry (DSC) was employed to examine the thermal transition of caramels. A shift in glass transition temperature (Tg) was noticed during thermal scanning (cooling and warming) of the caramel samples. Melting and crystallization temperatures were varied among the caramels. Testing temperature and variation in compositions resulted in differences in rheological parameters and melting and crystallization temperatures of the caramels.

Steiner et al. (2003) evaluated 6 caramel formulations by descriptive analysis. Mean texture values reflected that a slight increase in sweetened condensed skim milk and vegetable fat contents (1% w/w at a 2:1 ratio) significantly decreased stickiness ($P \leq 0.05$). Decreasing corn syrup dextrose equivalent (DE) decreased stickiness and increased hardness ($P \leq 0.05$). Pearson correlation coefficients revealed that stickiness to teeth while chewing, tooth packing, and tooth adhesiveness were highly correlated with one another ($P \leq 0.05$). Sensory hardness, cohesiveness, and number of chews were correlated with the rheological properties of storage modulus and viscosity, while stickiness was correlated with probe tack force ($P \leq 0.05$). The molecular mechanisms for sensory texture and stickiness of caramel can be used following such correlations with the help of equations of correlation coefficient.

Pons et al. (1991) presented a new device to avoid the preliminary extraction included in the identification of saccharides (sucrose, fructose, and glucose) and many of the degradation products which contribute to aroma and flavor of caramel. The process includes continuous trapping on adsorbent when heating, followed by a thermal desorption and overall analysis. Fifty-seven compounds were detected by this technique and also by solvent extraction and vapor analysis during cooking. Some unexpected intermediate volatile molecules were noted which can play an important role in the formation of the flavoring compounds.

Formulations and composition of caramel

Controlled heat treatment of carbohydrates produces caramel. The carbohydrate raw materials used are the monomers- glucose and fructose or polymers thereof (such as glucose syrups, sucrose or invert sugars, and dextrose). For promoting the process of caramelization, food-grade acids, alkalis, and salts may be used in amounts consistent with Good Manufacturing Practices (GMP) and subjected to the following stipulations.

For Class I caramel colors, ammonium and sulfite compounds cannot be used as reactants, for Class II caramel colors sulfite compounds must be used and ammonium compounds cannot be used as reactants, ammonium compounds must be used and sulfite compounds cannot be used as reactants for Class III caramel colors whereas both ammonium and sulfite compounds must be used as reactants for Class IV caramel colors. The ammonium compounds used are hydroxides, carbonates, bicarbonates, phosphates, sulfates, sulfites, and bisulfites, and the sulfite compounds are sulfurous acid and sulfites and bisulfites of potassium, sodium, and ammonium. Sulfuric acid and citric acid, and sodium, potassium, and calcium hydroxides are compounds that can be used for all 4 types of caramel color (Kamuf et al. 2003).

There are endless numbers of caramel formulations. Dairy products such as milk, milk protein, lactose, or butterfat formulates the final quality of caramel (Warnecke 1996). Since there is a lack of standards for the identity of caramel, the composition of caramel is dependent upon the confectionery technologist's imagination and the desired product.

Next to chocolate, caramel is the confection which is used in more types of candy than any other. Its formulations and process conditions must be modified to suit quite a wide range of textures. By far the most important ingredient in caramel is milk. Typically, finished caramel will contain from 1 to 3% milk protein. At the lower end of this range, the caramel must be boiled to a fairly low moisture content, to compensate for the lack of' 'body" imparted by the protein. At the higher end, the caramel can be soft, but still have a good "stand-up" property.

It was noted by Chirafisi and Milashouris (1981) that milk protein is the major contributer to the texture, body, and flavor of caramel. A milk processor generally considers milk as composed of water, milk solids nonfat, and milk fat. These components vary by breed of cattle, feed, and climate. The amount of water in milk makes the product very perishable due to the high water activity. The lactose in milk is a naturally occurring disaccharide sugar. When subjected to heat it contributes to the distinctive caramel color via the Maillard reaction by interaction with amino acids found in the protein. It is often used as a bulking agent as it does not provide much sweetness and can positively carry and enhance flavors.

Several studies have demonstrated that structural and physicochemical properties of proteins can have significant effects on the textural properties of food products (Marshall and Harper 1988; De Wit 1990; Patel and Kilara 1990). Chirafisi and Milashouris (1981) patented a caramel formulation by the use of 13.75 to 17.25% whey protein from a whey protein concentrate, 35 to 45% dry whey solids, and 8 to 12% sodium caseinate.

Umerie and Enebeli (1996) ascertained the feasibility of obtaining caramel from malted tubers of *Cyperus esculentus*. Mudadi et al. (1999) extracted the mucilage from *Azanza garckeana* fruit with water and the extract was heated at 130 °C in the presence of ammonium salts. When the mucilage, composed of galactose, glucose, arabinose, and rhamnose units, was heated, a brown color was formed. The presence of ammonium salts and pH had only a small effect on the development of color.

Rittenberg (2003) focused on the problems occurring while manufacturing caramel. Frequent problem areas encountered involve the proper melting of ingredients, those that need to be melted, such as fats and emulsifiers (10 °F

above the melting point). It is also desirable to determine the water and fat contents of caramel simultaneously in one single analytical step. In the classic time domain NMR methods, the differentiation of water and fat was not sufficiently good as the differences in relaxation times of oils and fats are relatively small. Therefore, Rudi et al. (2008) revealed a new TD-NMR method using a combined relaxation analysis where the magnetization at a certain time is determined by both T_1 and T_2. This combination leads to an increase of contrast and, therefore, opens up the possibility of quantification of water and fat by time domain NMR simultaneously.

Dai et al. (2003) compared extrusion production of solid caramel with the traditional process. The advantages were: production short reaction time, simple equipment, low cost, non-polluting, and others. Bainbridge (1997) put forth various methods for producing both caramel nutmeat clusters and typical chocolate clusters usually consisting of almonds, cashews, peanuts, coconut, or raisins, and also made comments pertaining to both manual and mechanical production of these items.

Application of caramel

Using caramel to replace synthetic colorants solves the common problem in digestion that occurs when the body absorbs red colors, leaving the blue and yellow to show as a "green effect" in pet stools. Bakers have been using caramel color to enhance the color and appeal of baked goods for decades. Caramel's high dispersibility in water and dough systems makes it well suited for such applications. Class III or IV caramel color is most often used in bakery applications. Caramel color can also be used to help reduce batch-to-batch color variations. (Kamuf et al. 2003).

Caramel is much darker than alternatives such as malt syrup (extract) and food-grade molasses and is often used for this reason. The wide selection of available caramel colors makes it a versatile tool for use in designing visually appealing baked products, ranging from tannish yellow to reddish brown to nearly black. Bakers can choose either liquid or powdered caramel colors depending on their process layout and equipment. Some select powder for its handling ease, longer shelf life, and performance in dry mixes.

Powdered caramel allows mix manufacturers to standardize the color of baking mixes. Bread, cake, and muffin mixes frequently contain caramel color to enhance the visual appeal of the final product (Table 3). Before the advent of powdered caramel colors, dry mixes for brown cakes, puddings, and other desserts contained several synthetic colorants used to replace cocoa.

Today, bakers concerned with labeling often formulate using powdered caramel in dry mixes to "clean" the ingredient label by reducing or eliminating certified colorants. Caramel color can also be used in pet foods to replace a combination of 3 certified colorants, FD&C Red #40, FD&C Yellow #5 (or #6), and FD&C Blue #1, which when blended together make brown. The result is a product with a cleaner label and a meaty appearance at a cost equivalent to that for synthetic colorants.

Caramel color is 2–6 times darker than most cocoa powders in baking systems. If the purpose of adding extra cocoa powder is to darken a product, as opposed to adding flavor to the baking system, then using caramel color is a cost-effective way to reduce the amount of cocoa required.

Compared with other natural colorants, caramel does not deteriorate under the high temperatures and pressures of extrusion processes. Typically a Class I, III, or IV caramel color is used in these types of applications. More than 50 different breakfast cereal products found in U.S. supermarket shelves list caramel color on their ingredient labels. Snack and confectionery processors use powdered caramel color to standardize the color of spice mixes and other seasoning blends. Processors also apply liquid or powder forms of caramel in water-soluble, extruded products to boost adhesion in rice cakes, granola, and energy bars (Kamuf et al. 2003).

Table 3 Suggested usage levels (%) in typical applications for different classes of caramel color

Applications	Class I (Liquid) (CI 35)	Class III (Liquid) (CI 110)	Class III (Powder) (CI 190)
Bread, Multi grain		1.0	0.5
Breakfast cereal			2.0
Cookies, biscuits			0.5–5.0
Ice cream cones		1.0	
Muffin Mix			1.0
Muffin, chocolate			1.0–3.0
Nutrition bar	1.0–2.0		
Rice cake	0.5–1.0		

(Kamuf et al. 2003)

Improved carbonated soft drinks and other beverages sweetened with aspartame and colored by caramel were patented by Zablocki and Vevang (1997). Their work focused the production of beverages in which positively charged caramel is used as a replacement for the portion of the negatively charged caramel conventionally found in beverages.

Synthetic red dye stuffs such as FD &C #2 and FD &C #40 have been found to be unsuitable for use in conjunction with foodstuffs like ice cream and maraschino cherries. Van Praag et al. (1978) used caramel for intensifying the color of natural red dye stuffs having the same intensity and quality of red color as the known synthetic dyes and with color fastness and brightness comparable to previously known synthetic dyestuffs.

Caramel as a colorant

There are more than 6,000 additives available to the food industry. More than half are flavors, both natural and synthetic, while the remainder includes colorants, preservatives, antioxidants, emulsifiers, thickeners, acids, bases, anticaking agents, flavor enhancers, glazing agents, improvers, bleaching agents, sweeteners, solvents, and a miscellaneous category (Millstone 1985).

Food products are colored to make them more appealing to consumers, to allow consumers to identify what taste to expect from a product, and to protect sensitive flavors from light. Colorants added to foods must also be proven safe, stable, legally permitted, and effective in a particular application. Color has always played a vital role in food selection and acceptance, and colorants are added to foods to make up for color that may be lost during processing. Caramel color, from the palest yellow to the deepest brown, accounts for more than 80% (by weight) of all colorants added to the foods we eat and drink.

Caramel colors used in the manufacture of a wide variety of foods and beverages have been in commerce for more than 100 years. The regulatory history of these additives in the US, the UK, the JECFA, and the EC has been reviewed and an introduction to the safety studies of caramel colors has been provided by Chappel and Howell (1992).

There are 2 types of browning reactions in food products: enzymatic browning, which is seen when damaged or cut fruit darkens at the exposed surface, and nonenzymatic browning, which occurs when food products, such as coffee beans, meats, breads, or sugars, are heated (Richardson and Hyslop 1985; Mathewson 1999).

Desirable brown color formation is generally associated with nonenzymatic browning which occurs in several ways. Two of the most important are the Maillard reaction in which sugars, aldehydes, and ketones react with naturally occurring nitrogen-containing compounds, such as amines and proteins, to form brown pigments known as melanins; and caramelization reactions in which sugars are heated in the absence of nitrogen-containing compounds. During a caramelization reaction the sugars initially undergo dehydration and then condensation or polymerization into complex molecules of varying molecular weights. Lightly colored, pleasant-tasting caramel flavors are produced during the initial stages, but as the reaction continues higher-molecular-weight color bodies are produced, and the flavor characteristics become more bitter. Caramel color first gained commercial importance as an additive in brewery products (such as porter, stout, dark beers, and ales) and as a colorant for brandy. Greenshields (1973) indicated that it is common for both Maillard and caramelization reactions to yield aldehydes and dicarbonyl compounds, but the former reaction incorporates nitrogen-containing components.

Yang et al. (2002) studied the relationship between the color ratio of the caramel pigment and its red index theoretically, and then caramel pigment could be produced with fresh color, homogeneous texture, and better salt-tolerant stability, which can be tailor-made for different requests by adjusting temperature, time, pH in the reaction, and adding a specific catalyzator. Licht et al. (1992a) concluded that caramel color IV exhibits compositional uniformity within the range of color intensity required by the food industry worldwide. Chen and Li (2002) studied the relationships of coloring property, hue, and color intensity of caramel pigments. After a dying experiment with bean curd the results showed that the coloring property and hue stability of caramel had no direct relation with hue and color intensity. But the processing technology had some influence on it.

Determination of caramel color

A common method of caramel color determination in Europe involves the use of a colorimeter or comparator to match a solution of caramel color to a series of standardized colored glasses and the use of the appropriate multiplier to determine color strength in European brewery convention (EBC) units (EBC Method 1950). The color of the glass standards used has a hue that is the same as beer, and solutions of Class III, which is the caramel color type used in beer, exactly match the hue of the glass, making it easy to determine their EBC value. The other 3 classes of caramel color have varying hues and determining a match between solution and glass can be very difficult. During the 1970s, JECFA and the industry came up with a tentative correlation that relates a certain amount of absorbance of a 0.1% (w/v) caramel color solution at 610 nm for each class of caramel to 20,000 EBC units. For Class I, for example, 0.053 absorbance units correlates to 20,000 EBC; the correlation for

Class III is 0.076 absorbance units; and the correlation for Class IV is 0.085 absorbance units. Experience has shown that the correlation value for a double-strength Class IV (color intensity 0.200–0.270) caramel color should be 0.104. This system works well; however, the analyst has to know which type of caramel colorant is being checked. Linner (1970) developed an equation based on spectrophotometric readings at 510 and 610 nm to determine the hue index or the "redness" of a particular caramel color. The range of the hue index for caramel color is approximately 3.5–7.5; generally, the higher the value the redder or more yellow the color. Grover (1968) studied the color of commercial caramel by measuring the extinction of monochromatic light (λ 0.4 to 0.7 μ) of solutions in water over a concentration range of 0.02–2.5 g/100 ml. The Lambert-Beer 'law'was proved to be applied within the accuracy of measurement, and a straight line relationship between log E and λ was established.

Wilks et al. (1973) confirmed the presence of small quantities of 4-methylimidazole (4-MeI) in caramel color by retention time data and infrared and mass spectroscopy. This study describes a method for the quantitative extraction of 4-MeI from caramel color, as well as 2 chromatographic procedures, thin-layer and GLC. Coffey and Castle (1994) developed ion-pair high performance liquid chromatography and capillary electrophoresis methods to distinguish Class III caramels from those of Classes I and IV. Coffey et al. (1997) developed and validated an ion-pair HPLC method for the estimation of Class III caramel added to foods. A variety of beers, biscuits, gravy powders, savory spreads, confectionery products, and baked foods were tested using the method. It was concluded that the HPLC method provides a simple, robust, and semi-quantitative measure of the presence of Class III caramel in foods. Fernandes and Ferreira (1997) developed a procedure for the gas chromatographic-mass spectrometric quantification of 4-(5-) methylimidazole in ammonia caramel colors by using isobutylchloroformate as dervatizing reagent. Wilks et al. (1977) presented an improved method for isolation and quantification of 4-methylimidazole (4-MeI) in caramel color. The method consisted of a methyl chloride extraction of a semidry mixture of the sample and celite 545, followed by concentration and GLC analysis of the elute. Quantification was done using 2-methylimidazole (2-MeI) as an internal standard. Afterwards Thomsen and Willumsen (1981) revealed a procedure for quantitative ion-pair extraction of 4(5)-methylimidazole from caramel color using bis(2-ethylhexyl)phosphoric acid as ion-pairing agent. Furthermore, a reversed-phase ion-pair liquid chromatographic separation method has been established to analyze the content of 4(5)-methylimidazole in the extracts. 2-Acetyl-4-(1,2,3,4-tetrahydroxybutyl) imidazole (noted as THI) in Class III caramel was detected by Moretton et al. (2008) using two-dimensional liquid chromatography/UV. Leggett (2008) summarized the user choices involved in measuring the color of each of the optical categories of food products and provided the best options for color measurements of each.

Analytical determination of antioxidants in caramel

Caramelization, is a pyrolysis reaction of certain sugars and is a good source of food color and antioxidant capacity, depends on pH and sugars. Tsai et al. (2009) heated 4 sugars (including monosaccharide and disaccharide) with different concentrations (1–40%) at pH 3, 7, and 10 at 90 °C for various durations (0–42 h). Results from 240 samples indicated that caramels from monosaccharide with higher concentration exhibited better antioxidant capacity at more alkaline condition. Further statistical analysis through principal component analysis and structural equation model revealed that browning pigment, the interactive result of sugar concentration and pH, instead of colorless intermediate, was the major contributor to classifying the caramels or exhibiting antioxidant capacity. Antioxidant properties of foods and beverages have been widely studied; however, few data have been reported on the antioxidant capacity of soft drinks. Payet et al (2005) investigated seven cane brown sugars for their polyphenol content and volatile composition in relation to their free radical scavenging capacity determined by 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assays. The brown sugar extracts showed interesting free radical scavenging properties despite the low concentration of phenolic and volatile compounds.

Brenna et al. (2009) aimed to ascertain the contribution to the antioxidant activity of some caramel-containing soft drinks, such as cola drinks, and chinotto, an original Italian soft drink. Some commercial caramel colors were analyzed for main parameters, namely, 5-(hydroxymethyl)-2-furfural (HMF), residual glucose and fructose content, total reducing compounds by the Folin–Ciocalteau reagent, and the antioxidant activity by the ferric reducing-antioxidant power and DPPH methods. Similar analyses were performed on various soft drinks colored with E150 d. The results showed that even if soft drinks have a lower antioxidant activity than other beverages such as tea, coffee or chocolate, they may contribute to the antioxidant pool assumed with the diet.

The Cu (II)-catalyzed oxidation of sulphite by molecular oxygen in a caramel-containing model system is characterized by the induction time for browning to begin and the rate of loss of oxygen. Whilst caramel (ammonia-sulfite) and citric acid are both good antioxidants, the induction time is increased with caramel concentration up to 0.05 wt%, but falls to zero at twice this concentration. Wedzicha and

Clayton (1994) concluded that sucrose acts as an antioxidant but saccharin and salt have no effect, while benzoic acid has some antioxidant behavior. Schwarz et al (2009) reported an increase in antioxidant activity of brandy de jerez on addition of caramel color. Caramellization of sucrose added in mulberry extract proved to increase the anti oxidant capacity of extract Tsai et al (2005).

The interactions between carbohydrates (carbonyl groups) and amino acids or proteins (free amino groups) in a non-enzymatic condensation process is called Maillard reaction. Furfural and hydroxymethylfurfural are characteristic flavor compounds of the Maillard reaction. Furfural is the result of a reaction with a pentose sugar (such as ribose); HMF is the result of a reaction with a hexose sugar (glucose, saccharose). The antioxidant capacity of Maillard reaction products (MRPs) was first investigated by Franzke and Iwainsky, (1954).

Chawala et al (2009) reported that MRPs, especially melanoidins, have antioxidant activity through scavenging oxygen radicals or chelating metals. MRPs from histidine had the highest antioxidant activity determined by conjugated diene formation. Andrade and Morales (2005) described a new concept of the overall antioxidant properties of food melanoidins, where chelating ability toward low molecular weight antioxidant compounds is connected to the stabilization of these compounds involved in the shelf life of the product. Andrade et al, (2005) analyzed the antioxidant activity of different coffees. The higher contribution of melanoidins to the total antioxidant activity of coffees was shown to be caused by the low molecular weight (LMW) fraction noncovalently linked to the melanoidin skeleton. The antioxidant activity of MRPs is often associated with increased stability and shelf life of food systems vulnerable to oxidation reactions. Nondialyzed, high-molecular weight (HMW = >3500 Da) MRPs were recovered from three model sugar-lysine (glucose-lysine, Glc-Lys; fructose-lysine, Fru-Lys; and ribose-lysine, Rib-Lys) reactions, heated at 121 °C for 1 h. Samples were characterized by UV and fluorescence spectra and assessed for antioxidant activity using both standard chemical methods. Antioxidant activity of Glc-, Fru-, and Rib-Lys HMW-MRPs (50 μg/mL) produced protection ($P<0.05$) against $H_2O_2^-$ (Kitts and Chun 2005).

McGookin and Augustin (1991) studied the antioxidant activity of casein and Maillard reaction products obtained by reaction of casein with glucose or lactose. Casein was antioxidative and heating casein in the presence of glucose or lactose resulted in enhancement of antioxidant activity. The development of antioxidant activity in reacted casein–sugar mixtures was determined as a function of initial casein and sugar concentration. The observed antioxidant activity of reacted casein–sugar mixtures was due to casein itself and Maillard reaction products resulting from reacting casein with sugar. Butter cookies were also analyzed by Bressa et al, (1996) for a chain breaking antioxidant activity in extract of butter cookies and result supports the concept that functionally relevant antioxidants are generated by Maillard reaction. Yoshimura et al (1997) used a glucose−glycine system as a Maillard reaction model, and investigated the rate of inhibition toward active oxygen by MRPs by electron spin resonance. MRPs obtained through heating a glucose−glycine mixture for 1 h inhibited more than 90% of active oxygen species existing in the form of hydroxyl radicals (•OH). Morales and Perez, (2001) estimated the free radical scavenging activity of MRPs produced by heating glucose or lactose with lysine, alanine or glycine directly by means of a DPPH method. This study showed that fluorescence measurement of heated sugar/amino acid systems is more effective than browning to follow the formation of MRPs with free radical scavenging activities.

Caramel toxicity

The JECFA has established an acceptable daily intake of 200 mg/kg/day for caramel Color III. The safety of **c**aramel Color III has been questioned during recent years following feeding studies in the rat that were associated with reduced white cell and lymphocyte counts. These effects have been attributed to the presence of 2-acetyl-4(5)-tetrahydroxybutylimidazole in this class of caramel color (MacKenzie et al. 1992a).

Food additives can be regarded as the safest constituents of our daily food. Nevertheless, complicated issues with respect to their safety evaluation do also occur. Houben and Penninks (1994) illustrated some of these issues by the description and evaluation of the research on the immunotoxicity of the food additive caramel Color III which is commonly used as a color additive in many products for human consumption. Toxicity studies conducted in the 1970s demonstrated that administration of caramel Color III can cause a reduction in total white blood cell counts in rats due to reduced lymphocyte counts. The imidazole derivative 2-acetyl-4(5)-(1,2,3,4-tetrahydroxybutyl)-imidazole was found to be responsible for the immunotoxicity.

Salmonella typhimurium plate incorporation assay (Ames test) was used by Allen et al. (1992) to examine a total of 15 caramel colors for genotoxic activity. Representatives of all 4 classes of caramel color were tested for genotoxic potential in the Ames test. None of the 15 caramel colors tested exhibited genotoxic potential.

Caramel Color III is used as a color additive in beers and a variety of other foods. Beer is the most important single source of Caramel Color III in the human diet, although consumption of dark beers has been decreasing in recent years. MacKenzie et al. (1992a) conducted short-term oral

toxicity studies which were conducted on low-THI and high-THI samples of Caramel Color III (13 week) and on a sample of THI (28 days). All treated groups given Caramel Color III had lower food and fluid consumption than the controls. There were no toxicologically important pathological findings. Caramel Color III containing 23 (commercial sample) or 143 (research sample) ppm THI and administered at a level of acceptable daily intake of 200 mg/kg body weight/day for 7 days did not affect any of the factors investigated (Houben et al. 1992)

MacKenzie et al. (1992b) evaluated caramel color IV, a type of caramel color used in the manufacture of cola soft drinks for subchronic and chronic toxicity in rats, and carcinogenicity in Fischer-344 (F344) rats and B6C3F_1 mice. A number of changes were not considered to be toxicologically significant. There were no toxicologically important pathological findings. The high molecular weight color fractions were filtered by Selim et al. (1992) from tissues like mesenteric lymph nodes, liver, kidney, and tissues of the gastro-intestinal tract, in which radioactivity was found. No major differences were observed in the absorption, distribution or excretion patterns between the single and multiple oral dose regimens.

Evans et al. (1977) added caramel produced by a straight ammonia-catalyzed 'half open-half closed pan' process at levels of 0 (control), 1, 3, or 6% to the diet of groups of 48 male and 48 female rats for 2 years. In both sexes there was a reduced rate of body-weight gain at all dose levels. In the males, this was accompanied by a reduction in the cumulative food intake. There was evidence that dilution of the diet by the caramel also contributed to the reduction in body-weight gain and there was no evidence of a carcinogenic effect. Brusick et al. (1992) used results from a battery of short-term tests *in vitro* and *in vivo* to assess the genotoxicity of colors in relation to reports from the literature. No evidence of genotoxicity was found in the Salmonella plate incorporation test using 5 standard strains or in the *Saccharomyces cerevisiae* gene conversion assay using strain D4, either with or without S-9 for activation. The results support the conclusion that the colors do not pose a genotoxic hazard to humans.

Ammonia caramel (AC) color was supposed to reduce blod lymphocytes counts specifically in rats fed a diet low in vitamin B_6. This effect is associated with 2-acetyl-4(5)-(1,2,3,4-tetrahydroxybutyl)imidazole. To characterize and compare the effects of AC and THI and to study the influence of dietary pyridoxine, studies in rats were conducted by Houben et al. (1988). Spector and Huntoon (1982) investigated the ability of color (ammonia process) (AC) to inhibit rabbit brain pyridoxal kinase (EC 2.7.1.35) and pyridoxine uptake and release by rabbit brain slices. AC contains a heat stable, competitive inhibitor of pyridoxal kinase that can be removed by dialysis or activated charcoal. A concentration of 0.6% AC in the pyridoxal kinase assay reduced activity by 50. The inhibition of pyridoxine uptake and acceleration of vitamin B_6 release from brain slices by AC are probably related to the inhibition of pyridoxal kinase since accumulation and retention of vitamin B_6 in brain slices depend on pyridoxal kinase activity.

Caramelization of a 1% sucrose solution at 180 °C accompanied characteristic changes in pH, M_r, UV-absorbance, and fluorescence values, as well as increased reducing power activity after 40–60 min. Similar changes occurred to sucrose heated at 150 °C, after 150–240 min. Kitts et al (2006) tested bioactivity of caramelized sucrose samples for mutagenic activity, using *Salmonella typhimurium* strains TA-98 and TA-100, respectively, as well as the *Saccharomyces* D7 yeast strain for mitotic recombination and Chinese hamster ovary cells (CHO) to assess clastogenicity. Caramelized sucrose expressed no mutagenicity in the TA-98 strain, similarly, mitotic recombination in the *Saccharomyces* D7 yeast strain and clastogenic activity in CHO cells were induced when exposed to caramelized sucrose.

Conclusions

Researchers have classified caramel in 4 classes according to its use in different systems of food and beverages. Many formulations for caramel have been published focusing on the changes in physicochemical properties of caramel owing to change in quantity and quality of its basic ingredients like fat, sugar, and milk solids. Researchers have identified the key role of milk solids in the preparation of caramel. There are established methods cited for determining the color of caramel but a rapid method for determining its color and antioxidant properties is yet to be established. Permissible limit of all the classes of caramel colors in food products and have also studied the toxic levels of caramel in human food and their consequences on human health. Genotoxicity analysis results reflected that caramel colors do not pose a genotoxic hazard to humans. The wide use of caramel as a food additive requires standardization of both the substance as such and the methods employed for the quantitative determination of this substance in various products.

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