J Food Sci Technol (October 2014) 51(10):2271–2288
DOI 10.1007/s13197-012-0718-8

REVIEW

Non-enzymatic browning in citrus juice: chemical markers, their detection and ways to improve product quality

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Revised: 9 November 2011 / Accepted: 26 April 2012 / Published online: 10 May 2012

Abstract Citrus juices are widely consumed due to their nutritional benefits and variety of pharmacological properties. Non-enzymatic browning (NEB) is one of the most important chemical reactions responsible for quality and color changes during the heating or prolonged storage of citrus products. The present review covers various aspects of NEB in citrus juice *viz*. chemistry of NEB, identifiable markers of NEB, analytical methods to identify NEB markers and ways to improve the quality of citrus juice. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) is one of the promising marker formed during browning process with number of analytical methods reported for its analysis; therefore it can be used as an indicator for NEB process. Amongst analytical methods reported, RP-HPLC is more sensitive and accurate method, which can be used as analytical tool. NEB can be prevented by removal of amino acids/ proteins (*via* ion exchange treatment) or by targeting NEB reactions (e.g. blockage of furfural/ HMF by sulphiting agent).

Keywords Browning · Non-enzymatic browning · Citrus juice · Maillard reaction · Chemical markers

Abbreviations

CDA	Color dilution analysis
DHA	Dehydroascrobic acid
DMHF	2,5-dimethyl-4-hydroxy-3(2H)-furanone
GLC	Gas–liquid chromatography
HMF	5-(hydroxyl methyl)furfural
HPLC	High performance liquid chromatography
NEB	Non-enzymatic browning
PDA	Photo-diode array
PPO	Polyphenol oxidase
RP-HPLC	Reverse-phase high performance liquid chromatography
TBA	Thiobarbituric acid
TLC	Thin layer chromatography
UPLC	Ultra performance liquid chromatography
UV	Ultra-violet

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Introduction

Citrus fruits have long been valued as part of a nutritious and tasty diet. The flavors provided by citrus are among the most preferred in the world, and it is increasingly evident that citrus not only tastes good, but is also good for people. It is well established that citrus and citrus products are a rich source of vitamins, minerals and dietary fibre (non-starch polysaccharides) that are essential for normal growth and development and overall nutritional well-being. However, it is now beginning to be appreciated that these and other biologically active, non-nutrient compounds found in citrus and other plants (phytochemicals) can also help to reduce the risk of many chronic diseases. Citrus do not contain fat and being a plant food, no cholesterol. The average energy value of fresh citrus is also low, which can be very important for consumers concerned about putting on excess body weight. For example a medium orange contains 60 to

80 kcal, a grapefruit 90 kcal and a tablespoon (15 ml) of lemon juice only 4 kcal (Whitney and Rolfes 1999).

Citrus juice is known for their variety of pharmacological and healing effects on human health. For example, lemon fruits possess anti-bacterial properties which make these fruits very effective for treating various throat infections, including tonsillitis, sore throat, colds and others. Lemon juice is also very helpful for effective dental care. Lemons and lemon juice contain calcium, which is vital for healthy bones and teeth. Lemon juice can be used to calm down toothache, banish bad breath and stop gum bleeding. Skin care is another very common application of lemon juice and lemon products. As a great antiseptic, lemon juice solution can be applied to burns and sun burns, assist in would healing and getting rid of such problems as eczema and acne. As lemon juice contains lots of antioxidants, it can act as a natural remedy that prevents aging and wrinkle formation. Regular consumption of lemon juice can help those people who suffer from high blood pressure and other related problems. A great natural source of potassium, lemon provides relaxation from such symptoms as nausea, dizziness, mental stresses, headaches, anxiety and depressions. Lemon juice health benefits also include natural abilities to stop any type of internal bleeding. Those who suffer from frequent nasal bleeding can use a piece of cotton wool with several drops of lemon juice to stop the bleeding. Lemon is considered to be an excellent natural diuretic and using lemons is recommended to those who have high uric acid problems, urinary tract infections and other related ailments. Health benefits of lemon juice also include relieving the symptoms of respiratory diseases, such as asthma, etc (Attaway 1994; Deyhim et al. 2006; Duyn and Pivonka 2000).

Citrus juices are very easy to prepare. A simple and more commonly used method on small scale to prepare fresh unpasteurized juice involves cutting the fruit into half and squeezing each half to collect the juice. The self-life for unpasteurized juice is <3 days and must be stored at >10 °C. Further the citrus juice concentrate can be prepared by separating collected juice into particulate solids portion (such as pulp or pectin) and a serum portion. The serum portion which comprises 7–20 % and from 80 to 93 % water is concentrated by removing essentially pure water. The steps involved in preparation of canned citrus juice include: deaeration, deoiling, pasteurization and canning. Juice deaeration reduces the levels of dissolved oxygen, reduces flavor deterioration, prevents degradation of ascorbic acid and reduces frothing during filling step. Juice deoiling controls the peel oil level of freshly extracted juices prior to packaging. Juice pasteurization is required to inactivate enzymes and destroy microbial contaminants. Finally juice canning include filling juice into cans and after filling, live steam is injected into the headspace followed by closing of the can. Glass and plastic containers should be avoided. In case of glass containers, deterioration of color, flavor and loss of ascorbic acid occurs whereas in plastic containers, orange flavor compounds migrate into the plastic container (Linton et al. 1999; Nienaber and Shellhammer 2001; Strobel 1984).

Browning in food products is the well known phenomena that take place during processing and storage leading to brown coloration of juice due to chemical reactions such as caramelization, ascorbic acid degradation and the Maillard reaction. It is the most common quality problem of many concentrated fruit juices and causes loss of nutrients and the formation of intermediate undesirable compounds like furfural and 5-hydroxymethylfurfural (5-HMF) (Clegg 1964). Due to tremendous health benefits and their wide use as health drinks, quality of the juice should be monitored and maintained with great care. Non-enzymatic browning (NEB) is one of the most detrimental chemical reactions responsible for quality problems of citrus juice. Extensive amount of literature on markers of NEB and analytical methods to monitor NEB in citrus juice have been reported; but no comprehensive review published on these aspects. Recently Perez-Cacho and Rouseff published a review on the processing and storage effects on orange juice aroma (Perez-Cacho and Rouseff 2008). Herein, we have reviewed the chemistry of NEB, markers of NEB, analytical methods to monitor NEB or detect markers of browning and ways to improve the quality of juice.

Chemistry of non-enzymatic browning

NEB is one of the most important chemical reactions responsible for quality and color changes during the heating or prolonged storage of citrus products (Buera et al. 1987; Rodriguez et al. 1991). It is referred as a Maillard reaction when it is initiated by the condensation of the carbonyl group of reducing sugars with free amino groups of amino acids and/or proteins. It is a chemical process that produces a brown color in foods without the activity of enzymes. Melanins and other chemicals are responsible for the brown color. It is generally understood that the degradation products of L-ascorbic acid and/ or sugars, e.g. furfural, 5-(hydroxymethyl) furfural (HMF), or other carbonyl compounds, participates in juice browning and polymerize with each other or react with amino acids to yield browning materials (Clegg 1964; Kacem et al. 1987; Kennedy et al. 1990; Lee and Nagy 1988a, b; Robertson and Samaniego 1986; Sawamura et al. 1991). A mixture of dehydroascorbic acid (DHA) and an amino acid in non-aqueous solution produced a red or yellow pigment as an intermediate in the browning pathway (Hayashi et al. 1983a, b; Kurata et al. 1973). It is believed that a similar browning reaction occurs in citrus juice as well as in the DHA aqueous model systems (Sawamura et al. 1991).

In citrus juices, NEB is due to the reactions of sugars, amino acids and ascorbic acid. However, the decomposition of ascorbic acid is reported to be the major deteriorative reaction occurring during the storage of orange juice (Solomon et al. 1995). Further there exists a high correlation between the percentage loss of ascorbic acid and an increase in browning in grapefruit juices (Lee and Nagy 1988b). On the other hand, sugar-amino acid reactions of the classical Maillard type are of minor importance in citrus juice browning because of the high acidity (pH 2.0–4.0) involved (Clegg 1964). However, the presence of amino acids in ascorbate systems is also considered a major contributor to the development of browning (Robertson and Samaniego 1986). This is illustrated by the fact that the main degradation product of juices with pH values below 4.0 is furfural (Huelin et al. 1971). Furfural is known to undergo polymerization and, as an active aldehyde, may combine with amino acids and contribute to the browning of the juice (Solomon et al. 1995). Likewise, HMF concentration has a high correlation with the level of browning in lemon juice and therefore plays an important role in the formation of brown pigments (Robertson and Samaniego 1986). The process of browning is also dependent on storage material used. Browning takes place faster in bottled juices than in canned juices (Nagy et al. 1990).

Two main forms of NEB are caramelization and the Maillard reaction. Both vary in reaction rate as a function of water activity. The Maillard reaction is a chemical reaction between an amino acid and a reducing sugar, usually requiring the addition of heat. The sugar interacts with the amino acid producing a variety of odors and flavors. The Maillard reaction is the basis of the flavoring industry, since the type of amino acid involved determines the resulting flavor (Handwerk and Coleman 1988). This reaction involves series of complex consecutive and interconnected processes, involving formation of glucosamines, ketosamines *via* Amadori rearrangements, diketosamines, and degradations and polymerizations of these compounds. Because heating may accelerate the Maillard reaction, juices that undergo processing steps which involve heat may be particularly susceptible to NEB (Daniher and Furrer 2003). The Maillard reaction and Amadori rearrangement is depicted in Fig. 1. The lone pair of nitrogen of amine attacks on the carbonyl carbon of glucose (**1**) to form structure **3**. This species (structure **3**) is unstable and loses water to produce the open chain form of the glycosylamine (**4** or **5**) with a C=N bond. Rearrangement of compound **4** or **5** yields the Amadori compound **7** or **8**. This sequence of reactions is known as the Amadori rearrangement. Attack by O-6 on the carbonyl group closes the ring producing a 1-deoxy-1-amino-D-fructopyranose compound (the Amadori product, **8**). The 1,2-eneaminol (**6**) on dehydration yields 2,3-enol compound **9** which further on reaction with water results in formation of dicarbonyl compound **10**. Dicarbonyl compound **10** on removal of two water molecules finally leads to formation of furan compound, HMF (**12**) as depicted in Fig. 1 (Hodge and Rist 1953; Yaylayan and Huyghues-Despointes 1994).

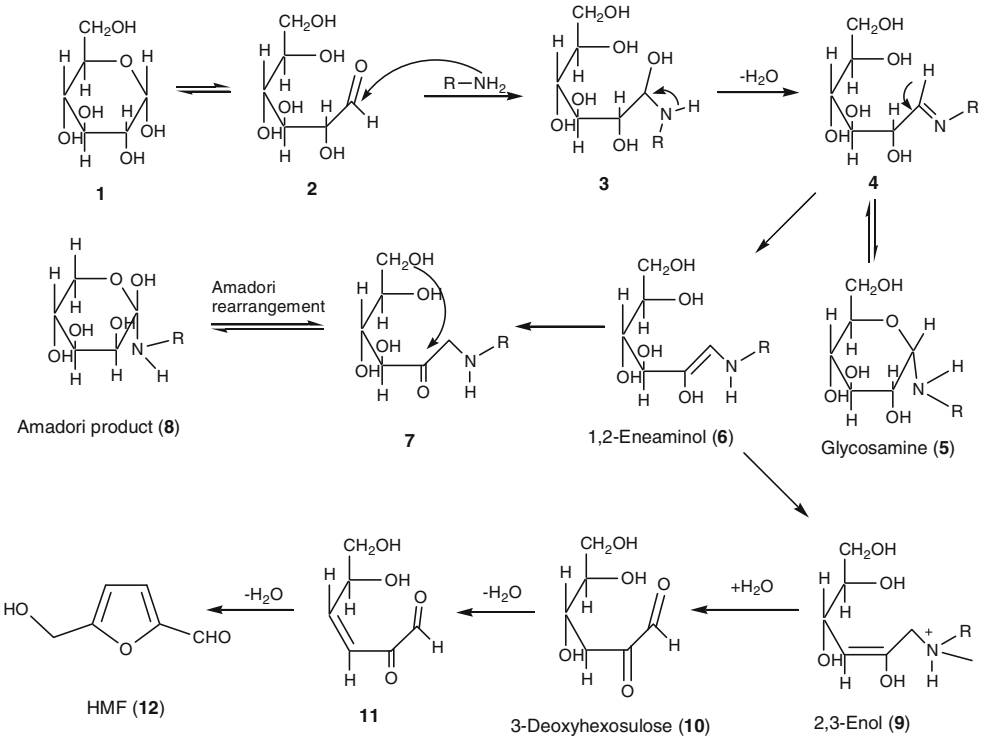


Fig. 1 Maillard reaction, Amadori rearrangement and formation of HMF

Caramelization is the oxidation of sugar. It is used extensively in cooking for the resulting nutty flavor and brown color. As the process occurs, volatile chemicals are released producing the characteristic caramel flavor. Like the Maillard reaction, caramelization is a type of NEB. However, unlike the Maillard reaction, caramelization is pyrolysis, as opposed to reaction with amino acids. When caramelization involves the disaccharide sucrose, it is broken down into the monosaccharides fructose and glucose. Caramelization generally occurs at high temperatures (~150 °C), low water content or high sugar content. It leads to nice flavors and colors in many foods and however it also leads to undesirable flavors and colors e.g. 'burnt sugar smell' (Hodge 1953).

Amino acids, ascorbic acid and sugars play key role in NEB of citrus juice. Huffman (1974) and Varsel (1980) made direct claims that naturally occurring amino acids cause or accelerate flavor damage in orange juice. A great number of studies with products other than citrus and models have demonstrated that amine-assisted sugar breakdown is the source of most cooked, baked, or otherwise heated food aromas. It should be noted, however, that there is a great similarity between acid-catalyzed and amine catalyzed degradation of sugars. The major difference is that milder conditions of heat and acidity are needed in the later case. It has been reported that there is a loss of three mole of water during interaction of glycine, D-glucose, D-fructose and HMF leading to formation of a polymeric structure (Handwerk and Coleman 1988). Of the specific components of citrus juices that react in Maillard browning, the amino acids have the most prominent and diverse set of roles. The role of amino acids in NEB of citrus products was confirmed by their removal by ion-exchange resins, which increased stability against deteriorative change. Several researchers investigated ion-exchange treatment for stabilizing citrus products (Bruemmer and Bowers 1977; Clegg 1964; 1966; Huffman 1974; Joslyn 1957; Onayemi and Bruemmer 1984). Numerous model studies, simulating citrus juice composition confirm that amino acids cause an acceleration of browning (Clegg 1964; Joslyn 1957).

Ascorbic acid degradation has been associated with browning of orange juice since long time (Joslyn and Marsh 1935). It has been demonstrated that amino acids accelerate ascorbic acid breakdown (Clegg 1964; Joslyn 1957), and in the presence of amine it is dehydroascorbic acid that is the reactive intermediate (Clegg 1964) in the pathway to furfural and brown pigment production. When ascorbic acid breaks down by acid catalysis, furfural is also the main end product (Kurata and Sakurai 1967a; Tatum et al. 1969). Acid catalysis is functional even under oxidative conditions and that ascorbic acid is partially responsible for orange juice discoloration. Since it was observed that ascorbic acid breakdown in orange juice results in furfural production and furfural buildup closely parallels quality loss in citrus products, furfural indexing has been proposed as a basis for quality control (Dinsmore and Nagy 1972; Nagy and Dinsmore 1974; Nagy and Randall 1973). In addition to ascorbic acid, pentoses and uronic acids are also known to produce furfural on breakdown. Ascorbic acid is used for the prevention of browning and other oxidative reactions in food products (Bauernfeind and Pinkert 1970) as well as it acts as an oxygen scavenger for the removal of molecular oxygen in polyphenol oxidase reactions. Ascorbic acid is however irreversibly oxidized to dehydroascorbic acid during the reduction process, thus allowing browning to occur upon its depletion (Fig. 2) (Bauernfeind and Pinkert 1970).

Two degradation products *viz*. 3,4-dihydroxy-5-methyl-2(5H)-furanone (MW 130; brown color) and 2-furancarboxylic acid (MW 112; colorless) were formed in the aqueous L-dehydroascorbic acid (DHA) solution. The DHA solution produced brown color under non-oxidative conditions than under oxidative conditions. The former degradant increased under non-oxidative conditions during storage. However, the later product consists of explicit white crystals, and its aqueous solution never becomes colored during storage (Sawamura et al. 1994).

Ascorbic acid degradation occurs slowly in clear orange juice concentrate at 4 °C with a rate constants ranging from 4.79×10^{-4} to 3.13×10^{-2} per week (Lee and Chen 1998). Suppression of the conversion of ascorbic acid into DHA in the absence of O_2 would result in little browning of the juice under anaerobic conditions. Browning proceeds under an anaerobic conditions more intensely than in the aerobic case, if DHA has already been formed in juices. The primary effect of oxygen is to oxidize ascorbic acid producing dehydroascorbic acid, which contains α-dicarbonyl group and can take part in the production of Strecker aldehydes from the corresponding amino acids (Fig. 3). The production of methional from methionine is probably due to this process. Common Strecker aldehydes include ethanal (fruity, sweet aroma), methylpropanal (malty) and 2-phenylethanal (flowery/honey like aroma). Condensation of two aminoketones

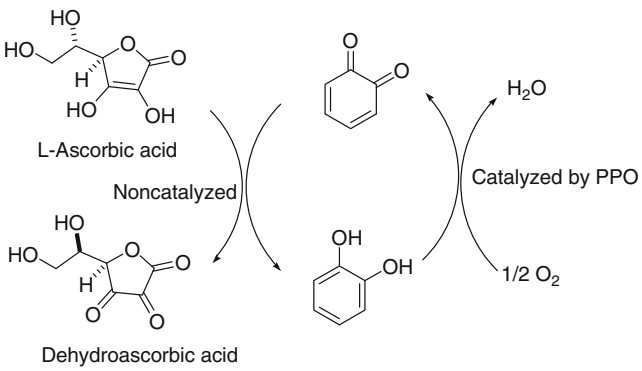


Fig. 2 Formation of dehydroascorbic acid (DHA) under enzymic and nonenzymic conditions

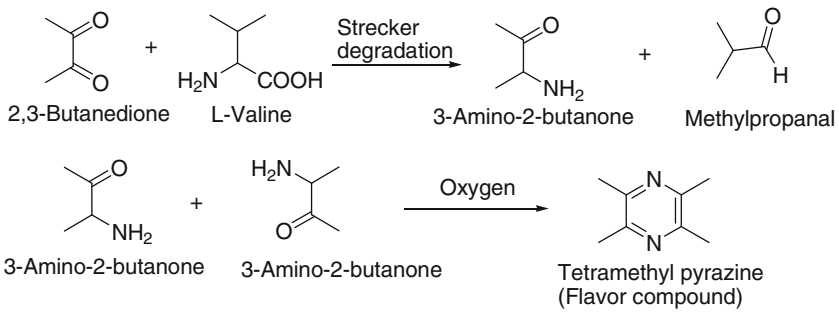


Fig. 3 Strecker degradation of α-dicarbonyl compounds

may yield pyrazine derivatives that are also powerful aroma compounds (Fig. 3) (Li et al. 1989).

Ascorbic acid is readily converted to dehydroascorbic acid by mild oxidation, but the loss of vitamin activity only arises after hydrolysis of the lactone to form 2,3-diketogulonic acid (DKG) (Tannebaum et al. 1985). Under anaerobic conditions, ascorbic acid reacts *via* its keto tautomer which is in equilibrium with its anion and undergoes delactonisation to form DKG. Decarboxylation of DKG results in the formation of xylosone and 3-deoxypentaone, which is formed by β-elimination at the C4 of DKG, followed by decarboxylation. It is at this point that the pathways begin to assume the features of NEB reactions. Xylosone is further degraded to form reductones and ethylglyoxal, whereas 3-deoxypentaone degrades to form furfural and 2-furancarboxylic acid. These compounds may combine with amino acids to form brown pigments (Fig. 4) (Kurata and Sakurai 1967b; Tannebaum et al. 1985). Further, several researchers suggested that NEB follows ascorbic acid loss (Clegg and Morton 1965; Clegg 1966; Solomon et al. 1995; Trammell et al. 1986; Zerdina et al. 2003).

Like ascorbic acid, sugars also play vital role in NEB process. Citrus juices contain mainly sucrose, fructose and glucose, however number of other sugars are also present in minor amounts. The main difference in the way that fructose and glucose react lie in the position of the reactive carbonyl. Glucose reacts with amino acids and then rearranges to produce 1-amino-1-deoxyfructose (ADF) while fructose yields 2-amino-2-deoxyglucose (ADG). ADG undergoes only the 1,2-enolization to produce 1,2-dicarbonyl-3-deoxy osulose. Other sugars are present in orange juice at concentrations high enough to be precursors for the trace amounts of flavor compounds necessary to render a product objectionable. Rhamnose reacts with alanine at pH 3.5 to produce the highly flavorful 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) (Fig. 5) (Shaw and Berry 1977). Under same conditions fructose did not yield a furanone; instead, two furans and two pyrroles gets formed (Shaw and Berry 1977). The reason for this may be that, in the case of hexoses, the products arising from 2,3-enolization, maltol and isomaltol are not stable in aqueous medium and, if formed, disappeared rapidly. Rhamnose is a minor constituent of citrus pectin while galacturonic acid is the main constituent. Rhamnose and galacturonic acid become constituents of citrus juices when enzymic degradation of pectin occurs in juice processing (Handwerk and Coleman 1988).

A pyrone (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone) has also been isolated from dehydrated orange juice (Shaw et al. 1967) from acid-catalyzed fructose degradation (Shaw et al. 1967) and from a D-glucose, methylamine and acetic acid degradation reaction (Fig. 6) (Jurch and Tatum 1970). This pyrone undergoes acid-catalyzed rearrangement to give isomaltol, a furan derivative, while dehydration affords maltol (Shaw et al. 1971). It was also demonstrated (Mills 1978) that this compound underwent ring contractions to produce the important pineapple-like furanone (Rodin et al.

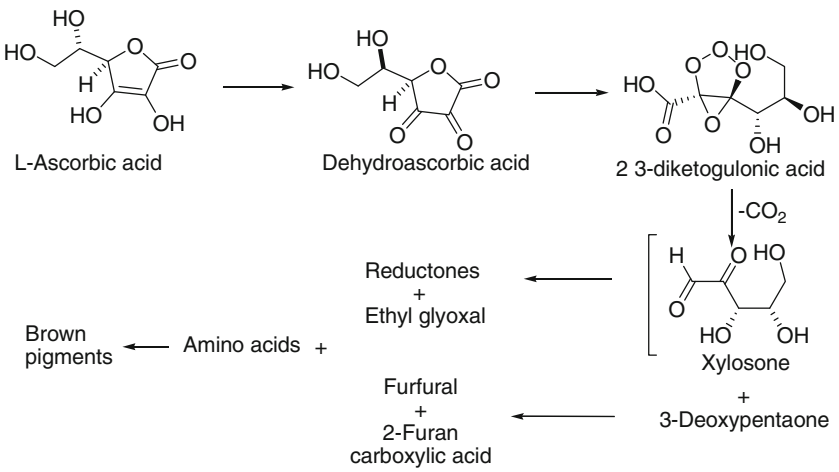


Fig.4 Ascorbic acid degradation pathway

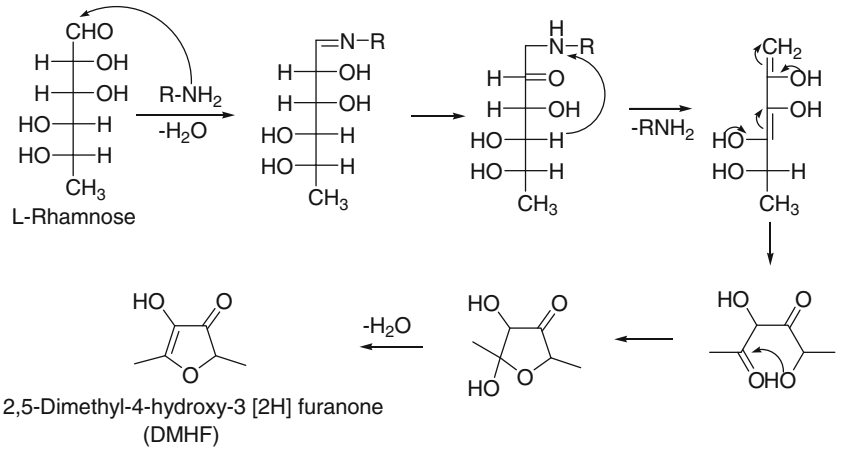


Fig. 5 Formation of DMHF (Furaneol) from rhamnose and amino acid

1965) and a nearly odorless hydroxymethyl derivative, 2-methyl-4- hydroxy-5- (hydroxymethy1)-3(2H)-furanone (Fig. 6). In case of orange juice, the pyranone is found in the dehydrated product, and the furanone occurs in canned juice. The model study (Shaw and Berry 1977) showed that rhamnose may be the carbohydrate source. The furanone has a taste threshold of 0.05 ppm, and the pyranone has a taste threshold greater than 200 ppm. This 4,000-fold difference accounts for the fact that the furanone imparts an undesirable taste. Unfortunately, studies of the reaction kinetics have emphasized the more easily measured changes in color and other empirical indices and have developed very little data on those reactions producing compounds of taste significance (Handwerk and Coleman 1988).

Markers of citrus juice browning

Several chemical markers responsible for NEB have been identified which mainly include furans and furanones, Amadori compounds, hydroxycinnamic acids, sugars, alpha-terpienol and PVG. In stored canned orange juice, *p*-vinyl-guaiacol (PVG), α-terpineol, and 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone (furaneol, DMHF) have been proposed to be the principal detrimental, off-flavor compounds (Tatum et al. 1975) which reach their taste-threshold levels under typical processing and storage conditions (Handwerk and Coleman 1988). Of these three compounds, PVG is considered to be the most detrimental.

Furans and furanones

Thermal degradation of sugars, amino acids and ascorbic acid can produce off-flavors and NEB products (Lee and Nagy 1988a). These heat-induced degradations produce acids (acetic or butyric acid), furans (e.g., furfural and 5-methyl furfural), furanones (e.g., 2,5-dimethyl-4-hydroxy-3 (2H)-furanone and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone), ketones, cyclopentanones, pyranones, and pyrroles (Tatum et al. 1975). Some of them impart sweet, caramel-like, or burnt sugar-like sensory impressions. However, other reaction products such as furfural and 5-methylfurfural, whose concentration increases due to thermal processing (Tonder et al. 1998) are rarely aroma active as they have high odor thresholds. Because furfural is formed by oxidative degradation of ascorbic acid, it has been used as an indicator of thermal abuse. Furanones are Maillard reaction products which have also been identified in heated orange juices

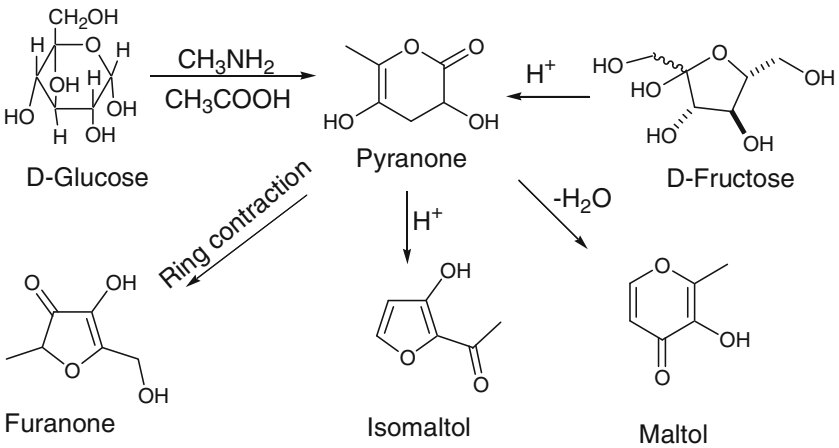


Fig. 6 Formation of pyranone/furanone from D-glucose/fructose

(Tatum et al. 1975). Furaneol and homofuraneol have been identified as odor-active compounds in canned orange juices inducing caramel odor qualities (Ruiz Perez-Cacho et al. 2007).

Two main compounds which have been isolated in degraded samples of L-ascorbic acid were furaldehyde and 5-(hydroxymethyl)furaldehyde (5-HMF) (Huelin 1952, 1953; Huelin et al. 1971; Kanner et al. 1981; Robertson and Samaniego 1986). Their presence has been proposed as index of browning (Berry and Tatum 1965; Kanner et al. 1981; Meydav and Berk 1978; Robertson and Samaniego 1986). The rate of formation of 5-HMF is dependent on the loss of L-ascorbic acid and is likewise directly related to storage temperature (Kennedy et al. 1990). High temperature storage (76 and 105 °C) remarkably accelerated the formation of 5-HMF similar to the accelerated decomposition of L-ascorbic acid. The formation of HMF is depicted in Fig. 1. There is extensive evidence on the formation of 5-HMF and furaldehyde caused by the acid-catalyzed dehydration of sugars (Roig et al. 1994). Subsequent browning from these solutions was observed and its rate was intensified by the presence of amino compounds. Huelin et al. reported that sugars do not contribute to 5-HMF or furaldehyde formation in juice (Koca et al. 2003). Formation at high temperature showed that whilst the intensity of browning was increasing, 5-HMF was continuously accumulating although L-ascorbic acid concentration was already at a minimum. This could be explained by the relatively low reactivity of 5-HMF towards browning (Flink 1983). Other more reactive carbonyls such as the αβ-unsaturated carbonyls which have formed as a result of the oxidation of L-ascorbic acid appear to be the more potent browning agents (Clegg and Morton 1965). Therefore, the possible reaction taking place during the initial stage of browning is between amino compounds and some other degradation products of L-ascorbic acid followed by condensation of the products with each other or with nitrogen-free intermediates (Roig et al. 1999).

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF, furaneol) is believed to be a key flavor constituent in many fruits and baked foods (Zabetakis et al. 1999). DMHF (furaneol) is formed in stored citrus juice and has been reported to reduce juice quality. The source and pathways by which DMHF is formed in citrus products have not been delineated. In model orange juice solutions, DMHF was formed only when rhamnose (6-deoxyhexose) was present. Arginine was the major amino acid to react with rhamnose *via* the Maillard reaction. In buffer solutions, the formation of DMHF was affected by pH, temperature and time of storage. Under acidic conditions, DMHF is formed only from arginine and rhamnose, whereas at pH 6–8, DMHF was also formed from glucose and fructose, albeit in smaller amounts. The formation of DMHF from hexoses required a reducing step, which was apparently contributed by Maillard-producing reductones. The formation of DMHF from L-rhamnose is depicted in Fig. 5. The accumulation of DMHF to taste-threshold levels, which occurs under the acidic conditions of citrus juices, is related, at least in part, to the reportedly small amounts of rhamnose interacting with arginine *via* the Maillard reaction (Haleva-Toledo et al. 1997). Analytical techniques such as HPLC with either UV (Lee and Nagy 1987) or Photo Diode Array (PDA) detection, GLC-FID (Williams and Mottram 1981) and GLC-MS (Withopf et al. 1997) have been used for the analysis of DMHF and derivatives.

Amadori compounds

Determination of the level of Amadori compounds present in foods allows one to detect the onset of the reaction before detrimental changes occur (del Castillo et al. 1998) as well as to retrospectively assess the heat treatment or storage conditions to which a product has been submitted. This is the first stable compound of the Maillard reaction and are very sensitive indicators to detect changes in food quality (Sanz et al. 2003). These compounds can be measured after their transformation into 2-furoylmethyl amino acids (2-FMAA) by acid hydrolysis. 2-Furoylmethyl derivatives of alanine (2-FM-Ala), asparagine (2-FM-Asn), proline (2-FM-Pro), γ-aminobutyric acid (2-FM-GABA), glutamic acid (2-FM-Glu), and arginine (2-FM-Arg) have also been reported as indicators of the Maillard reaction development in orange juice (del Castillo et al. 1999, 2000).

Hydroxycinnamic acids

Hydroxycinnamic acids have also been associated with accelerated browning. Early studies employed TLC to separate and quantify hydroxycinnamic acids (Schmidtlein and Herrmann 1975). Spots were scraped off from plates and hydroxycinnamic acids quantified using spectrophotometric techniques. Later, GLC procedures were developed (Schulz and Herrmann 1980) but required the acids to be derivatized into more volatile forms. HPLC is the current procedure of choice because it requires minimal sample preparation, is relatively rapid, and allows easy quantitation. It also has the ability to quantify esterified (conjugated) forms of hydroxycinnamic acids (Risch and Hermann 1988).

L-rhamnose

Rhamnose, which is a sugar precursor for DMHF production under acidic conditions (Shaw and Berry 1977) is present in small amounts in citrus juice (Lanza et al. 1991; Stepak and Lifshitz 1971), but nevertheless was found to be

a building block for glycosides and polysaccharides in orange juice (Kauschus and Thier 1985). Quantification of rhamnose in orange and grapefruit juices is being reported. Presumably the galacturonic acid and rhamnose found in citrus juice result from the enzymic degradation of pectin which occurs during processing and storage. Apparently, the enzymic release (e.g. via naringinase) of rhamnose from the flavonoids naringin (grapefruit) or hesperidin (orange) may contribute additional free rhamnose (Romero et al. 1985). Although overall rhamnose content is low, it is higher in commercial juices than in fresh juice, probably due to processing and pasteurization of the former. After storage, rhamnose content does not change significantly (Haleva-Toledo et al. 1997).

α-terpineol

α-Terpineol was reported to produce a stale, musty, or piney off-flavor when added to orange juice (Tatum et al. 1975); however, GC-olfactometry (GCO) studies (Bazemore et al. 1999) have demonstrated that this compound is rarely aroma active in commercial orange juices, suggesting that the added α-terpineol may have contained aroma active impurities. The odor threshold for α-terpineol in orange juice is very high, 16.6 mg/L (Plotto et al. 2004) and rarely exceeded in commercial orange juices. α-Terpineol exists at low levels in pasteurized juice. A linear increase in α-terpineol with increasing storage time due to limonene degradation in a non-oxidative pathway is reported (Durr et al. 1981). In another storage study comparing concentration differences due to storage temperature, commercial juices stored for 12 weeks at −18 °C contained approximately 1 ppm of α-terpineol, whereas the same canned juices stored at 35 °C for the same time period reached concentrations ranging from 3.4 to 5.5 μg/mL (Tatum et al. 1975). Since α-terpineol is formed more rapidly from linalool than limonene, the linalool/α-terpineol ratio has been suggested as a means of evaluating orange juice storage time/conditions (Askar et al. 1973).

p-vinylguaiacol (PVG)

PVG has been proposed to be formed in citrus products from free ferulic acid due to nonenzymic decarboxylation following the ferulic acid's release from bound forms (Peleg et al. 1992). Although ferulic acid occurs mainly in bound form, the amount of free ferulic acid present in citrus fruit before processing exceeds the amount needed to form an above-taste-threshold level of PVG during processing and storage (Peleg et al. 1991). PVG formation was found to increase under practical storage conditions for orange juice (Naim et al. 1988) and this formation was accelerated when the juice was fortified with free ferulic acid, resulting in inferior aroma quality (Naim et al. 1988).

D-galacturonic acid

For pectinase clarified citrus juices, D-galacturonic acid can be detected. D-galacturonic is a reactant in the Maillard reaction and due to its relative stability; it is also a marker for browning. It has also been reported that presence of D-galacturonic acid increases the browning of juices (Ibarz et al. 2008; Jayaraman and Buren 1972). Chemical structures of browning markers are shown in Fig. 9.

Analytical methods for determination of markers as an index of browning

Monitoring of browning process/ identification of browning markers is very important quality control process for fruit juices. Different analytical methods reported for identification of chemical markers of browning are discussed.

UV spectroscopy

Browning in citrus juices has been studied by a number of investigators (Handwerk and Coleman 1988; Nagy et al. 1992). The most common method for characterizing browning is the measurement of absorbance at 420 nm (A420) (Valdramidis et al. 2010; del Castillo et al. 1998, 2000; Meydav et al. 1977; Solomon et al. 1995). Briefly, the method is as follows: A 10 mL orange juice sample was centrifuged (10 min) to remove coarse particles from the sample. Five milliliters of ethyl alcohol was added to 5 mL of juice supernatant and centrifugation was repeated. The absorbance of the supernatant was obtained at 420 nm using a UV–Vis spectrophotometer (Meydav et al. 1977). Another simple three-step solvent extraction procedure was used to evaluate the extent of NEB; which involves centrifugation of the sample, addition of alcohol, chilling in an ice bath, recentrifugation and reading the absorbance at 420 nm. It has been used to record NEB reaction rates in both orange and grapefruit juices (Klim and Nagy 1988).

Browning development in citrus juice concentrates can be determined using two color measurement methods: determination of soluble brown pigments as absorbance at 420 nm (A420) and measurement of CIE L* (lightness), and b* (yellowness) values. Significant correlations were obtained between A420 and CIE-Lab color parameters (L*, b*), suggesting that all three values would be suitable as indices of browning (Koca et al. 2003).

The content of furfural in juice can also be determined using UV spectroscopy method. The method consisted of distilling 200 ml of single-strength orange juice from which 2 mL was taken and to this was added 2 ml of 95 % ethyl alcohol and 1 ml of aniline reagent (5 ml of freshly distilled aniline made up to 50 ml with glacial acetic acid). The color

which developed after 15 min was measured at 515 nm (Dinsmore and Nagy 1972; Nagy and Randall 1973). Examination of canned juice showed that for every 5 °C temperature rise in storage there was an approximate doubling of the furfural content.

Further HMF content can be determined by derivative spectroscopic method using a color reaction between HMF and 2-thiobarbituric acid (TBA) (Meydav and Berk 1978). A color complex formed was measured by reading the absorbance at 550 nm by UV–vis spectrophotometer (del Castillo et al. 2000). TBA based derivative spectrophotometry method can also be used for simultaneous determination of HMF and furfuraldehyde. The yellow reaction products show high absorption in the visible region. Their simultaneous determination was accomplished by taking the first-derivative signal at 436 nm for furfuraldehyde determination and at 414 nm for HMF determination, respectively. The method has been applied to a commercial orange juice and oral rehydration salt formulations (Tu et al. 1992).

UV spectrophotometric determination of hexoses, pentoses, and uronic acids has also been reported. The method is based upon the formation of UV-absorbing furan aldehydes in strong H_2SO_4. The molar absorptivity of the 5-hydroxymethyl-2-furaldehyde formed from hexoses increases appreciably with increasing H_2SO_4 strength from 70 to 93 %, thereby increasing the sensitivity of hexose measurements in the higher acid concentrations. Uronic acids react with 89 % H_2SO_4 to give 5-formyl-2-furoic acid as a major product. Ribose and 2-deoxyribose can be determined in 79 % H_2SO_4, with some advantage in sensitivity to ribose as compared to current procedures which use 95 % H_2SO_4 (Scott et al. 1967).

Most identification experiments have been focused on quantitatively predominating Maillard reaction products, rather than using their color activity as the selection criterion for their identification. To bridge the gap between color activity and structural chemistry, the Color Dilution Analysis (CDA) has been developed by Hofmann and Frank (2002) offering the possibility of screening and characterizing the key chromophores formed in NEB reactions. To gain a better understanding of the puzzling browning mechanisms, in addition, quantitative precursor studies and ^{13}C-labeling experiments have been performed to monitor how the carbohydrate skeleton is shifted into these chromophores. An aliquot of the browned solution was separated by RP-HPLC into several fractions, and the effluents of peaks were separately collected. To rank them in order of color intensity, these fractions were made up with water to the same volume, and were then diluted stepwise (1:1) until the visual detection threshold was reached. The dilution, at which a color difference between the diluted fraction and two blanks (water) could just be visually detected, was defined as the Color Dilution (CD)-factor. As this CD factor is a measure of color activity, it allows the ranking of fractions according to their color intensities. Further separation of selected fractions, which were evaluated with high CD factors, followed by HPLC purification and structure determination of the most intense colorants by means of ^{1}H, ^{13}C- and ^{15}N-NMR as well as LC/MS (Hofmann and Frank 2002).

HPLC

A reversed-phase HPLC method for rapid analysis of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) with an UV detection at 290 nm has been reported. DMHF content ranged from 1.6 to 27.3 ppm in ten fresh pineapple juices. Using this method, the production of DMHF was monitored in canned grapefruit juices during storage at varying temperatures (Lee and Nagy 1987). HPLC-UV method for simultaneous estimation of furans DMHF, DMHF-glucoside and mesifuran in strawberries has been reported (Sanz et al. 1994). An ion-pair RP-HPLC method for determination of Amadori compounds is also reported (del Castillo et al. 1999; Resmini et al. 1990). A ternary solvent system consisting of water, acetonitrile, and tetrahydrofuran was used to separate browning pigments formed in grapefruit juice stored in cans and bottles. Pigments were spectrophotometrically characterized using a PDA detector. The use of 390 nm to monitor NEB of grapefruit juice was found to be more appropriate than the previously used 420 nm (Nagy et al. 1992).

The simultaneous separation and identification of furanic compounds as the possible degradation products of sugars and ascorbic acid on heating was reported using ion-exchange HPLC method. 5-(Hydroxymethyl) furfural (5-HMF), furfural, 2-furoic acid, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), 2-acetylfuran and six unknown compounds were separated and detected in fruit juice concentrates, wine, beer and beverage samples. Peaks were detected at wavelengths of 254 and 280 nm, respectively to facilitate the detection of these compounds (Yuan and Chen 1998).

HPLC determination of hydroxycinnamic acids in orange juice using solvents containing THF has been reported. Mobile-phase solvents containing THF substantially improved chromatographic resolution between the four hydroxycinnamic acids of interest compared to solvents employing methanol or acetonitrile. The mobile phase consisting of 21 % THF/79 % water (with 2 % acetic acid) produced the best separation and allowed the most accurate quantitation of the four hydroxycinnamic acids from the components in orange juice (Rouseff et al. 1992). An isocratic ion-pair HPLC method using UV detection at 280 nm was reported for determination of a 2-furoylmethyl derivative of lysine, furosine in baby cereals (Guerra-Hernandez and Corzo 1996).

To investigate the generation of limonene and isolimonene/C_{17}-epilimonene during storage of orange juice, freshly squeezed orange juice (pH 3.5) was stored for up to 4 weeks at 4 and 20 °C and then limonene and isolimonene/C_{17}-epilimonene were quantitatively determined by means of HPLC-MS/MS using the multiple reaction monitoring mode. The concentration of limonene in orange juice increased slightly during storage from 70.0 μg/100 g in the freshly squeezed juice to 80 and 85 μg/100 g when maintained for 2 weeks at 4 and 20 °C respectively. Storage of the orange juice induced also an increase in the concentration of C_{17}-epilimonene, e.g. in the samples maintained for 4 weeks at 4 and 20 °C respectively, a 1.5- or 2-fold increase of the amount of C_{17}-epilimonene was observed. Comparing the storage-induced increase of the amount of limonene with that of isolimonene clearly demonstrated that the formation of limonene was more favored upon storage of the orange juice than that of its C_{17}-epimer, thus during storage, limonoate A-ring lactone present in the juice is slowly converted to limonene upon acid catalysis. For quantitative analysis, aliquots of the fresh and treated orange juice were centrifuged and the supernatant was passed through water-conditioned C18-E SPE-cartridge, which was eluted with methanol. Aliquot from the methanolic effluent was analyzed for limonene and isolimonene/C_{17}-epilimonene by means of LC-MS/MS. Using negative electrospray ionization, limonene and isolimonene/C_{17}-epilimonene were analyzed in the multiple reaction-monitoring (MRM) mode using the mass transition m/z 469→229. Quantitative analysis was performed by comparing the peak areas obtained for the mass trace with those of defined standard solutions of limonene and C_{17}-epilimonene in methanol (Havekotte et al. 2009).

GCMS / GC-FTIR

The use of GC-MS to identify DMHF and mesifuran (compounds responsible for a strawberry-like flavour note) together with nasal appraisal by sniffing the GC eluate led to the identification of these furanones in berries and wines of inter-specifc grapewine breeding (Rapp et al. 1980). The presence of DMHF in stored orange and grapefruit juice was verified by GCMS analysis which indicated the expected fragments: 128 [M^+], 85 [M-CH_3OH] and 57 [M-CH_3OH-CO] at 0.94: 0.33: 1 ratio respectively. In another study of pineapple flavor using capillary GC coupled to Fourier transform IR spectroscopy (Fehl and Marcott 1989) identified DMHF and phenylacetaldehyde, an unwanted off-flavor compound.

GLC

D-Glucosylamines and their Amadori rearrangement products, 1-deoxy-1-(N-substituted)-amino-D-fructoses formed in the Maillard browning reaction of D-glucose with various amines are shown to be readily detectable together with the reactants, by gas–liquid chromatography of the trimethylsilylated reaction mixture. The procedure affords a convenient method for monitoring the course and extent of the browning reaction. A mixture of *N,*0-bis(trimethylsilyl)acetamide, *N*-(trimethylsilyl)imidazole, and chlorotrimethylsilane was used for trimethylsilylation. Reaction of D-glucose with amino acids involves heating the solution of D-glucose, D-glucitol and an α-amino acid in N,N-dimethylformamide. Aliquots were subjected to direct trimethylsilylation and samples were analysed using GLC with a flame ionization detector. GLC with trimethylsilyl derivatization has also been used for analysis of hydroxybenzoic acid/ hydroxyl cinnamic acid derivatives in plants (Schulz and Herrmann 1980).

TLC

Detection of Amadori compounds in reaction mixtures (carbohydrates and amino acids) and stored dehydrated orange juice was carried out by TLC on cellulose aluminum plates. The solvent system was 1-butanol/pyridine/water, 2:3:1 (v/v). Plates were sprayed with 0.5 % 2,3,5-triphenyl-2*H*-tetrazolium chloride in 0.5 mol/L sodium hydroxide. The identification of Amadori compounds in stored dehydrated orange juice and model systems was achieved by comparison with the Rf values of synthesized standard. TLC analysis of model systems allowed the detection of Amadori compounds formed during storage. The Rf values of the spots ranged from 0.06 (model system containing arginine) to 0.23 (model system containing proline) and the Rf of the isolated fructosyl-γ-aminobutyric acid was 0.13. In the case of model systems containing serine or aspartic or glutamic acids, only traces of Amadori compounds were detected, in agreement with the negligible development of color observed. TLC of stored dehydrated orange juices showed the presence of spots with *Rf* values similar to those observed in stored model systems. These spots were absent in fresh dehydrated orange juice (del Castillo et al. 1998).

UPLC–MS/MS

A rapid method for qualitative and quantitative analysis of 17 phenolic acids (comprising ferulic acid) in different beverages as well as grapefruit juice using UPLC–MS/MS linked simultaneously to both a PDA 2996 photo diode array detector and a Micromass Quattro microTM API benchtop triple quadrupole mass spectrometer has been reported. The ferulic acid appeared at retention time of 7.04 min (Gruz et al. 2008).

Reflectance measurements

This method was used to compare the relative effectiveness of a series of compounds in inhibiting browning in freshly prepared and commercial fruit juices including apple, grape, grapefruit, orange, and pineapple juices. The potential inhibitors tested include ascorbic acid, a commercial formulation called Sporix, sodium sulfite, N-acetyl-L-cysteine, L-cysteine, and reduced glutathione (Molnar-Perl and Friedman 1990).

The extent of browning was measured by recording the reflectance or *L* values with a Minolta Chroma Meter, Type CR 100. The values of the tristimulus coordinates L, *a,* and *b* were recorded and used to calculate the extent of browning in the absence and presence of inhibitors. A Radiometer pH M26 meter and a Beckman 39030 thin-probe combination electrode were used to record pH values. The following equation was used to calculate the percent inhibition of browning under a variety of conditions

$$\%\ Inhibition\ of\ browning = \frac{\Delta L\ control\ sample - \Delta L\ control\ sample}{\Delta L\ control\ sample} \times 100$$

where Δ *L* is the difference between the measured *L* value at time *t* and the corresponding value at zero time. Zero time was defined as (a) about 1–2 min after the pressing of the fresh juices, held at room temperature, (b) after purchase of commercial juices in a local store, and (c) after preparation of the protein foods at room temperature (Molnar-Perl and Friedman 1990).

Micellar electrokinetic chromatography

This method was used for determination of 5-Hydroxymethylfurfural (HMF) content in various food products including orange juice. The MEC procedure utilizes capillary electrophoresis instrument equipped with a PDA detector and the separation was carried out using uncoated fused silica capillaries with capillary voltage of +25 kV (Teixidó et al. 2011).

Ways to improve citrus juice quality

A large consumption of citrus juice and its associated nutritional and pharmacological benefits to the human health creates the impetus on juice manufacturers to maintain the quality of juice. Quality of the juice can be improved by avoiding off-flavor components to be incorporated in the juice product during manufacturing or by inhibiting the process of browning.

Precautions to be taken during juice preparation

Controlling the extraction process to avoid mixing of undesired constituents like Naringin, isolimonene, D-limonene is very important. The extractors most commonly used for citrus juices are designed to operate on unpeeled fruit. In most designs the fruit is halved by being forced against a sharp knife. Halves are caught in semispherical cups and are gently pressed against a semispherical or conical member or burr, conforming to the shape of the halved fruit, to express the juice. In soma types of equipment the burr rotates and has a serrated surface. In one type of citrus extractor widely used in Florida, the fruit is quartered and the quarters are forced between two rolls, one convex and the other concave. In expressing citrus juices from unpeeled fruit, great care must be taken in the construction and adjustment of the extractor to avoid introduction of undesirable flavoring principles into the juice. For example, in the grapefruit there resides, primarily in the albedo and locular wall tissue, a bitter water-soluble glucoside, naringin. Too severe pressing or abrasion of the naringin-bearing tissues will permit some of the glucoside to be leached out by the juice after liberation from the juice vesicles. The possibilities of contamination with naringin are greatest in immature fruit. Another principle in California navel oranges, isolimonene; when extracted with the fruit juice, it gets converted to an intensely bitter lactone form. Isolimonene apparently occurs in the albedo, the center fibrovascular bundle, and in the locular wall (Havekotte et al. 2009).

Another important factor in the expression of citrus juice is the control of peel oil extraction. Directly underlying the thin cuticle on the outside of the peel is an epidermal layer of cells containing numerous oil vesicles. Citrus oils contain about 90 % *D*-limonene. On storage, juices containing excessive quantities of this oil develop an objectionable turpentine flavor. The oil content of citrus juices should never exceed 0.030 %, and with proper equipment and precaution this value can be maintained below 0.01 %. To avoid unpalatable flavors in citrus juices, extractors must be adjusted to prevent incorporation in juice of *D*-limonene from peel oil and the glucoside, naringin, from albedo and locular wall tissue. Thus, control of the relative concentrations of D-limonene, isolimonene together with control of the sugar concentration, in juice controls the sensation of bitterness in the juice (Havekotte et al. 2009). Chemical structures of these undesired bitter compounds are depicted in Fig. 7.

Sterilization is another important parameter during juice preparation. The pulp and juice of sound citrus fruit are sterile. However, after the fruit is cut and the juice extracted, it can become burdened with microorganisms of all types. In normal canned grapefruit or orange juice there is a slight settling of insoluble solids and the body of the product remains clouded or opaque. This opacity is apparently due

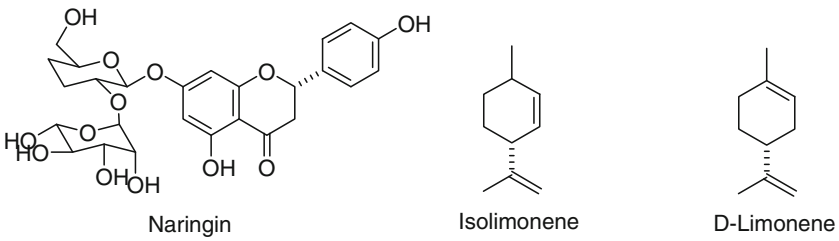


Fig. 7 Structure of naringin, isolimonene and D-limonene

to a colloidal suspension of solids stabilized by naturally occurring pectins. The juice needs to be rapidly heated to 90 °C in the flash pasteurizer and held for about 30 s in order to ensure inactivation of the pectin splitting enzymes and destroy microbial contaminants (Lueck and Pilcher 1941).

Inhibition of browning process

The best way to improve the citrus juice quality is to inhibit/slow down process of browning. Researchers have achieved success in inhibiting this process *via* number of approaches, which are discussed below.

Ion-exchange treatment

Amino acids play very important role in NEB of citrus juice and it has been documented that their presence in the juice leads to acceleration of the process of browning (Clegg 1964; Handwerk and Coleman 1988; Joslyn 1957; Wolfrom et al. 1974). It has been reported that the removal of amino acids from citrus products by ion-exchange resins increases the stability against deteriorative change. Stabilization of colors of grapefruit syrup (Onayemi and Bruemmer 1984), orange syrup (Bruemmer and Bowers 1977; Huffman 1974) and lemon juice (Clegg 1964, 1966) *via* treatment with cation-exchange resin has been reported by several researchers. It has also been known that removal of both anions and cations by ion exchange are beneficial in delaying development of brown color (Joslyn 1957). Recent findings further support the beneficial role of ion-exchange treatment in controlling browning process. The control of visual color production in Maillard reactions was obtained by adding ionic species to model systems consisting of various monosaccharides and amino acids in a neutral aqueous buffer at 100 °C. Addition of Na, K, Mg or Ca ions or choline, chloride led to increased browning measured by absorbance at 420 nm. Bidentate anions like phosphates, carboxylates and tetraborate also led to enhanced browning as a consequence of their bifunctional structures. A reduction in browning was observed by addition of an ammonium salt to the sugar/amino acid reactions (Rizzi 2008, 2011).

Further, the ion-exchange treatment was found to be beneficial in reducing the contents of principle bitter-flavor components (naringin and limonene) of natural grapefruit juice. A weak anion exchange resin having a styrene polymer matrix carrying functional groups derived from a mono or polyamine is required for this beneficial effect. The ion exchange treatment also reduces the acid content of the grapefruit juice and most importantly this treatment does not impair the nutrient content or the desirable flavors in the treated juice (Mitchell et al. 1985; Principe and Lozano 1991).

Addition of sulphiting agents

In addition to proper control of variables such as temperature, oxygen and light to prolong the shelf-life of products containing L-ascorbic acid, some chemical substances have been added to arrest flavour and quality deterioration. Of the food additives commonly used, sulphiting agents have the longest history of use. Sulphiting agents are used to control enzymatic and NEB, to arrest microbial spoilage and to act as an antioxidant, as a reducing agent and as a bleaching agent (Taylor et al. 1986). In synthetic systems containing L-ascorbic acid and tryptophan, polyphosphates have been shown to reduce browning (Yu et al. 1974). An assessment of the degree of browning in presence of additives showed that the addition of sodium metabisulphite tends towards reducing browning, particularly at the higher storage temperatures of 76 and 105 °C where a greater accumulation of 5-HMF was observed. Nonetheless, even at the lower storage temperatures where the same level of 5-HMF was formed in samples stored at the same temperature; the degree of browning was still much lower in samples preserved with sodium metabisulphite. These results could be explained by the two-fold effect of sulphite species on the oxidative browning resulting from L-ascorbic acid decomposition. Firstly, the additive inhibits auto-oxidation of L-ascorbic acid. Secondly, it prevents subsequent browning due to L-dehydroascorbic acid and other carbonylic degradation products. The degradation of L-ascorbic acid causes the formation of carbonyl compounds, which can undergo a series of reactions leading to the formation of browning. The attack of sulphites on the carbonyl groups forms hydroxysulphonate compounds which are less reactive

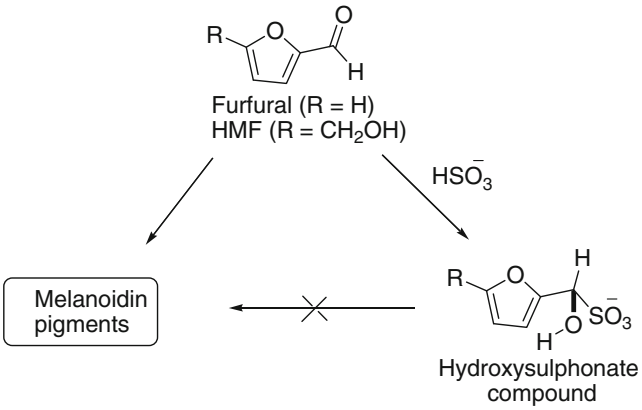


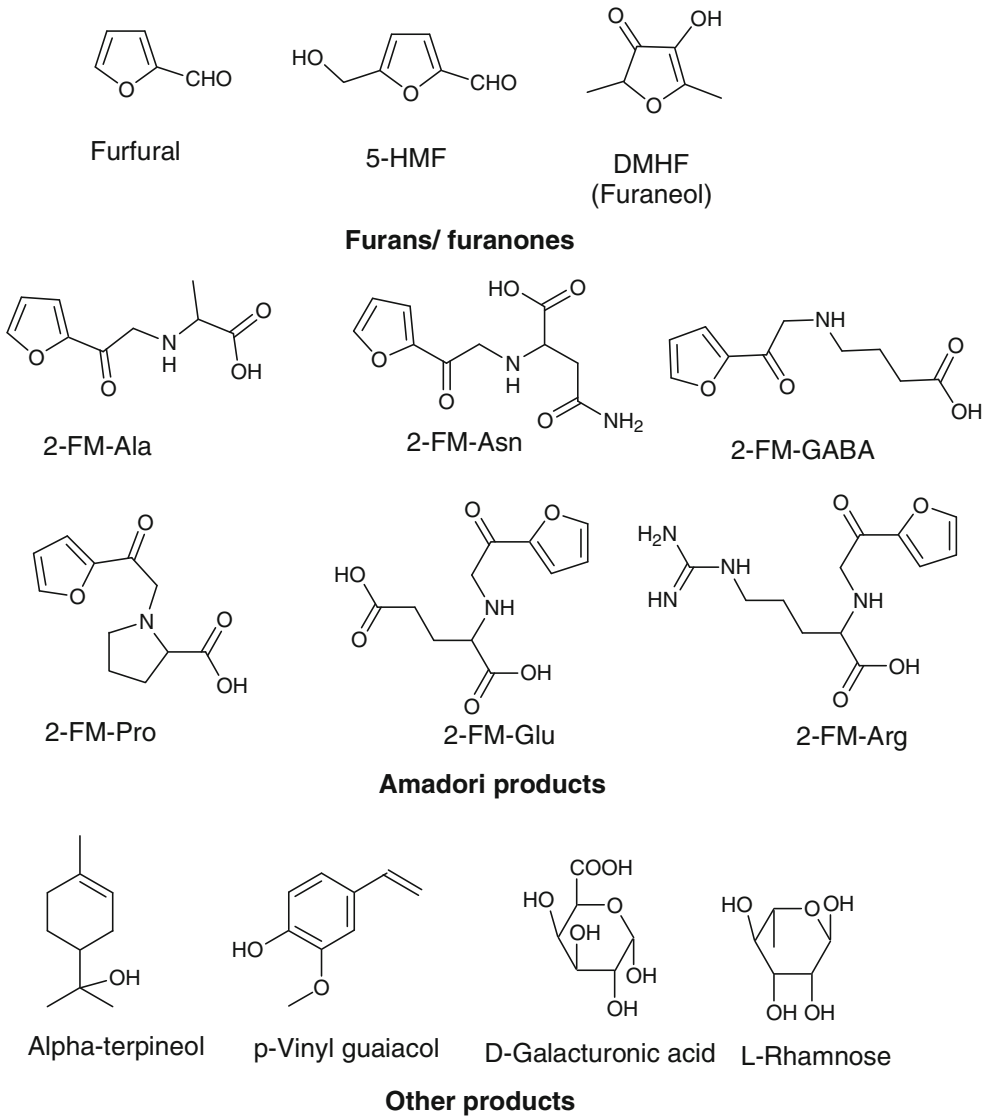
Fig. 8 Blockage of furfural/ HMF by sulphiting agent

towards browning than their precursors (Figs. 8 and 9) (Roig et al. 1999).

Addition of L-ascorbic acid or its more stable derivatives

L-Ascorbic acid in its reduced form is of utmost importance in nutrition and in food processing; however, once it gets degraded, it results in the production of reactive carbonyl compounds which act as intermediates in NEB in foods (Roig et al. 1999). L-Ascorbic acid and its various neutral salts and other derivatives have been the leading GRAS antioxidants for use on fruits and vegetables and in fruit juices for the prevention of browning and other oxidative reactions (Bauernfeind and Pinkert 1970). In citrus products, addition of L-ascorbic acid is not effective in extending flavor and color qualities. Ascorbic acid has also been reported to inhibit formation of pyrazine type of compounds, which gets formed by Strecker degradation of α-dicarbonyl compounds. Addition of 1 % ascorbic acid was able to show 52 % inhibition of 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine in open glucose/ glycine system

Fig. 9 Chemical structures of browning markers in citrus juice



(Porter et al. 2006). More stable forms of ascorbic acid derivatives such as erythrobic acid, 2- and 3-phosphate derivatives of ascorbic acid, phosphinate esters of ascorbic acid and ascorbyl-6-fatty acid esters of ascorbic acid have however been developed to overcome problems associated with ascorbic acid (Sapers et al. 1989; Sapers and Ziolkowski 1987; Seib and Liao 1987).

Addition of SH-containing compounds

Thiol compounds have been proposed to inhibit NEB in fruit juices (Molnar-Perl and Friedman 1990) and off-flavor formation in citrus juice (Naim et al. 1993b). Addition of L-cysteine to the peel juice prior to evaporation and / or pasteurization during processing removes bitterants and thereby retard the aggregate of polyphenolic components. Thus by retarding the accumulation of polyphenolics, there is a inhibition of browning of peel juice (Chu et al. 2006; Chung et al. 2007). Use of L-cysteine to inhibit NEB (Arnold 1969; Montgomery 1983) did not attract further research as cysteine is a flavor source in some foods which can be objectionable in fruit juice (Molnar-Perl and Friedman 1990; Roig et al. 1999). Nevertheless, some thiol compounds are natural components of human diets and play significant physiological roles in vivo as nucleophiles and scavengers of free radicals. Accelerated storage of citrus juice under laboratory conditions (up to 14 days at 45 °C) has shown that fortification with L-cysteine, and to a lesser extent with *N*-acetyl-L-cysteine (at concentrations below 5.0 mM), reduces DMHF and PVG content, as well as ascorbic acid degradation in orange juice without significant production of sulfur-containing off-flavors (Naim et al. 1997, 1993a, b).

Addition of low concentrations of L-cysteine has been reported to reduce formation of DMHF (Lee and Nagy 1987; Naim et al. 1993a) in commercial orange juice stored under accelerated conditions (Naim et al. 1993a). The role of SH-containing amino acids in minimizing browning varies depending on juice varieties, maturity of the fruits used to make the juices, storage times, the content of polyphenol oxidases, free amino acids and reducing sugars. Simple thiol compounds such as 1,3-propane dithiol, propane dithiol resin, ethyl 3-mercapto propanoate, cysteine, N-acetyl-L-cysteine, cysteine hydrochloride and ethyl acrylate showed inhibition of browning by covalently attaching them to a solid support structure permitting them to interact with browning precursors/ intermediates. These compounds can be easily separated for the foodstuff due to presence of solid support (Daniher and Furrer 2003).

Flavonoid extract and hespidin were able to inhibit browning only upto 10 % while thiol compound cysteine showed more than 90 % inhibition. A non-thiol compound ethyl acrylate also showed more than 85 % inhibition. Results of the Molnar-Perl and Friedman's work revealed that under certain conditions, SH-containing N-acetyl-L-cysteine and the tripeptide-reduced glutathione may be as effective as sodium sulfite in preventing both enzymatic and non-enzymatic browning. Molnar-Perl and Friedman have also discussed the unique electronic and nucleophilic properties of sulfhydryl compounds that enable them to act as inhibitors of both enzymatic and non-enzymatic browning (Molnar-Perl and Friedman 1990).

Redox potential modification

The proper adjustment of redox potential of the juice is necessary in order to ensure complete microbial destruction. Alwazeer et al. studied effect of both redox potential and pasteurization of orange juice on color and ascorbic acid stability and growth recovery of microorganisms during storage at 15 °C for 7 weeks. Three conditions of redox potential, +360 mV (ungassed), +240 mV (gassed with N_2), and −180 mV (gassed with N_2–H_2) were applied to orange juice. Both thermal destruction and recovery of sub-lethally heat-injured cells of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* were investigated. While oxidizing conditions were the most effective for thermal destruction of *L. plantarum* and *S. cerevisiae*, reducing conditions decreased recovery of heated cells of *S. cerevisiae*. In addition, gassing the juice with N_2 or N_2–H_2 increased color retention and ascorbic acid stability. The redox potential of juice must be adjusted just after the heat treatment in order, firstly, to maximize microbial destruction during pasteurization, and secondly, to prevent the development of microorganisms and stabilize color and ascorbic acid during storage (Alwazeer et al. 2003).

Miscellaneous

Other than above-mentioned strategies for prevention of browning in citrus products, several other methods, which are reported for non-citrus products can also be attempted for citrus juices. Peng and Jiang studied the potential usage of salicylic acid as a powerful anti-browning agent in fresh-cut Chinese water chestnut. Salicylic acid treatment delayed discoloration, maintained eating quality with higher content of the quality attributers, and reduced activities of or delayed the increases in activities of PPO, POD and PAL in fresh-cut chestnut (Peng and Jiang 2006). Gui et al. investigated the inactivation of polyphenol oxidase (PPO) in cloudy apple juice exposed to supercritical carbon dioxide ($SCCO_2$) treatment. Higher pressure, higher temperature, and longer treatment time caused more inactivation of PPO. The maximum reduction of PPO activity reached more than 60 % at 30 MPa and 55 °C for 60 min (Gui et al. 2007). Further the addition of chitosan was able to control the enzymatic

browning in apple juice. Browning could be prevented in McIntosh apple juice by the addition of at least 200 ppm chitosan, irrespective of the chitosan product tested, followed by filtration with diatomaceous earth filter aid. Chitosan at 1,000 ppm was required to prevent browning in juice from ripe Bartlett and Bosc pears. Juice from very ripe Bartlett pears did not respond to chitosan treatment. Chitosan addition interfered with the prevention of browning in apple and pear juice by centrifugation (Sapers 1992). NEB can be reduced by maintaining the acidic pH (3–4) of the juice and storage at lower temperature. Under acidic condition, the amine group remains in protonated form and thus no free lone pair becomes available for reaction to take place with carbonyl compounds (Kvamme 1996).

Onion by-products showed remarkable antioxidant activity, high bioactive composition (total phenols and quercetin) and an excellent anti-browning effect (Roldan et al. 2008). Kim et al. reported prevention of enzymic browning of pear by onion extract (Kim et al. 2005). Orange juice adulteration with water extracts of pulp or peel can be determined from 'phlorin' content or its hydrolysis product content (phloroglucinol) (Scordino et al. 2004) using HPLC (Johnson et al. 1995; Louche et al. 1998) or capillary electrophoresis analysis (Braddock and Bryan 2001).

Discussion

Citrus consumption has tremendous health benefits. Citrus fruits are nutrient-dense foods that can be good sources of carbohydrates, including dietary fibre, and many vitamins and minerals. Citrus fruits are equally valuable among populations who need to overcome and prevent micronutrient deficiencies as well as those concerned with problems of over-nutrition, obesity and diet-related chronic diseases. For example, citrus is an ideal component of low-fat, sodium-restricted diets. Over the past two decades, rising incomes and the shift in consumer preferences towards healthier, more convenient products have contributed to a growth in demand for citrus. Income levels also influence the variety and form of citrus consumed; in developed countries more processed citrus is consumed than in developing countries where people consume more fresh citrus. The increased demand of processed citrus i.e. citrus juice provides impetus on citrus juice manufacturers to maintain the quality of juice to maintain original nutritional value of citrus fruits. NEB has been proved to be detrimental to the quality of citrus juice. Several chemical compounds, which get formed during this browning process, have been served as markers of browning. DMHF is a good marker to be used as a indicator of browning process; as well as several analytical methods are reported for determination of DMHF. Different analytical methods have also proven their potential to identify these chemical markers. UV spectroscopy is the simplest and widely adopted quality control method for browning of citrus juice. TLC is another very easy method for monitoring the formation of Amadori compounds (brown pigments) in the juice on storage, whereas, HPLC is a more accurate and precise way to identify browning markers in the juice and therefore should be implemented. Different analytical methods can be recommended for different stages of browning process in citrus juice. During initial stage, there is no presence of UV absorptive products (Schiffs bases, Amadori products), therefore RP-HPLC for determination of Amadori products should be used. At intermediate stage, there is presence of strong UV absorptive compounds (dicarbonyls, HMF etc.), thus UV spectroscopy based method should be handy and fast way to monitor browning process. There is appearance of dark brown color at final stage of browning which can be measured at 420 nm.

Amongst different methods covered in the present article for inhibition of browning, following strategies are promising: (a). ion exchange treatment (to remove amino acids/ proteins) and (b). addition of more stable derivatives of ascorbic acid to the citrus juice; (c). blockage of furfural/ HMF by addition of sulphiting agent, which reacts with furfural/ HMF carbonyl to form less reactive hydroxysulphonate compound. Later approach targets the end stage of NEB reactions thereby prevent the formation of brown pigments. Currently researchers are searching for the natural replacements for sulfating agents for prevention of NEB.

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