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Changes in the Soluble and Insoluble Compounds of Shelf-Stable Orange Juice in Relation to Non-Enzymatic Browning during Storage

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ABSTRACT: For the first time in literature, this study revealed the participation of polymeric components of orange juice cloud and pulp (such as proteins, arabinogalactan proteins, or protein-pectin complexes) during nonenzymatic browning. In a quest to better understand the nonenzymatic browning of shelf-stable orange juice during storage, the juice was fractionated into different fractions depending on the solubility in water/ethanol and the obtained fractions were characterized. The results showed that brown compounds that were formed during storage of orange juice were distributed over water insoluble (pulp), ethanol insoluble (cloud), and ethanol soluble (serum) fractions. In the ethanol insoluble fraction, the brown compounds are hypothesized to be associated with proteins, arabinogalactan proteins, and/or protein-pectin complexes of this fraction without significantly changing their molecular weight distributions, monosaccharide compositions, and protein contents. The changes in the ethanol soluble fraction including ascorbic acid degradation, acid-catalyzed hydrolysis of sucrose, and formation of furfural and 5-hydroxymethylfurfural were highly correlated to the browning development of the juice during storage.

KEYWORDS: *Nonenzymatic browning, orange juice, storage, fractionations, soluble compounds, insoluble compounds*

INTRODUCTION

Orange juice is one of the most popular and widely consumed juices in the world owing to its attractive color, refreshing taste, and high nutritional value. Among the quality attributes of orange juice appreciated by consumers, its bright orange color has been highlighted as largely influencing consumer acceptance.[1] Unfortunately, upon storage, an unacceptable brownish color appears which limits the product's shelf life and negatively influences consumers' purchasing behavior.[2] In shelf-stable orange juice, the formation of a brown color during storage is mainly attributed to nonenzymatic browning (NEB).[3−5] Besides orange juice, NEB is also known to be a main cause of color deterioration during storage of other shelf-stable fruit juices such as lemon juice, grapefruit juice, strawberry juice, mango juice, and apple juice.[6−10]

Nonenzymatic browning in orange juice has been proposed in literature to involve multiple pathways, namely, ascorbic acid (AA) degradation, acid-catalyzed sugar degradation, and Maillard-associated reactions.[3,11−14] Most studies have attempted to quantitatively investigate the changes in NEB precursors such as AA, glucose, and fructose or the formation of NEB intermediates like furfural and 5-hydroxymethylfurfural (HMF) and their relationship to browning during storage.[5,13,15,16] Nonetheless, the mechanisms of NEB are rather complex and have not been fully elucidated. Although the chemical structures and the reaction pathways leading to various chromophores in model systems of amino acids and pentoses or hexoses have been described,[17] this classical Maillard reaction type is reported to be of minor importance in citrus juice browning (including orange juice) during storage because of the high acidity involved.[15]

Orange juice is a complex system consisting of a serum phase (e.g., organic acids, sugars) and cloud and pulp (e.g., proteins, polysaccharides) which have different solubilities in water and/or ethanol thereby enabling the separation of orange juice into different fractions.[18,19] Although all these fractions are simultaneously present in the orange juice, only the soluble compounds present in the serum such as AA and sugars have received attention so far in studies related to NEB in citrus juices.[3,5,12] The contribution of insoluble compounds present in the cloud and pulp to the browning has not been investigated yet. Nevertheless, in other food matrices such as coffee brew, brown compounds (i.e., melanoidines) are commonly found to be associated with polymers including polysaccharides and proteins.[20] Additionally, it has also been proposed that the brown melanoidins might be formed by the cross-linking of low molecular weight chromophores and high molecular weight colorless proteins.[21] Therefore, we hypothesize that the brown compounds which are formed in pasteurized shelf-stable orange juice during storage might also be associated with naturally occurring polymers in the juice cloud and pulp. In addition, although the composition of orange juice cloud has been well documented in literature,[19,22,23] its changes during storage have not been studied

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yet. Hence, it was of interest to investigate the changes in the insoluble (cloud and pulp) and soluble (serum) compounds of orange juice in relation to NEB during storage.

The main objective of this study was to gain a better understanding of NEB of orange juice during storage through simultaneously (i) investigating the distribution of brown compounds over different fractions of orange juice obtained through (stepwise-)fractionation and (ii) characterizing the changes in insoluble and soluble compounds in relation to NEB. For these purposes, pasteurized shelf-stable orange juice was stored at an elevated temperature (42 °C) for varying times. The nonstored and stored orange juices were fractionated into water/ethanol insoluble and soluble fractions. The insoluble compounds were characterized for their molecular weight distribution, monosaccharide composition, and protein content, and changes in the soluble compounds such as AA, sugars, furfural, and HMF with storage time were quantified.

MATERIALS AND METHODS

Materials. A single batch of freshly produced single strength shelf-stable orange juice (Minute Maid) filled in 60 polyethylene terephthalate (PET) bottles of 330 mL was used in this study. The juice was reconstituted from concentrate. The pH and total soluble solid content (°Brix) of the orange juice were 3.9 and 11.3, respectively, which remained unchanged during storage.

Storage and Sampling of the Orange Juice. To generate samples at different storage moments in a short time frame, an elevated temperature of 42 °C was applied for storage in the present study. This temperature was selected since previous work[13] has confirmed that storage of shelf-stable single strength orange juice at 42 °C only accelerates the NEB process without including new reaction pathways. The orange juice was stored for 15 weeks at 42 °C in incubators protected from light. Sampling was done prior to the storage and at three different storage moments (5, 10, and 15 weeks). At each sampling moment, 15 orange juice bottles were randomly taken from the incubators; the juice was mixed and uniformly divided into smaller portions. These tubes were frozen in liquid nitrogen and stored at −40 °C until use. Prior to analysis, samples were thawed in a circulating water bath at 20 °C for a standardized time and vortexed.

Color Measurement. The color of the orange juice was measured using a Hunterlab ColorFlex EZ colorimeter (D65 illuminant, 45°/ 0° geometry, and 10° observer angle, operating in a reflection mode) at room temperature. Prior to the measurement, the instrument was calibrated with a standard black and white ceramic tile. To make the measurements, a glass container was filled with 5 mL of sample, closed with a white ceramic plate, and covered with a black cylinder. Color measurement was performed in triplicate with five readings for each sample. Color results were expressed as the CIE $L^*a^*b^*$ value. The L^* value represents lightness, ranging from black (0) to white (100). The a^* and b^* values indicate color direction green ($-a^*$)/red ($+a^*$) and blue ($-b^*$)/ yellow ($+b^*$), respectively. These values were used to calculate the total color difference (ΔE^*) (eq 1) which is a measure of the difference in color between the pasteurized stored sample and the pasteurized nonstored sample (i.e., a control sample, subscript 0 in eq 1).

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

Browning Index Measurement. Browning index (BI) of the orange juice was determined using a spectrophotometric method described by Klim and Nagy[24] with slight modifications. The juice was centrifuged at 1000xg, 4 °C for 15 min (J2-HS centrifuge, Beckman, Brea, CA, U.S.A), and the supernatant was collected. Subsequently, the supernatant was mixed with 95% ethanol in a ratio of 1:1, and the obtained solution was placed in an ice bath for 15 min for flocculation, followed by a second centrifugation step (1000xg, 4 °C, and 15 min). The supernatant was filtered (0.45 μm, hydrophilic), and the absorbance was measured at 420 nm using a spectrophotometer.

Fractionation of the Ethanol Soluble and Ethanol Insoluble Fraction of Orange Juice. Orange juice is comprised of serum, cloud, and pulp. While the orange juice pulp consists of the large particles (>2 μm) that tend to settle upon storage and are pelletable under a mild centrifugation,[18] the orange juice cloud can be separated from the serum based on its difference in ethanol solubility. Therefore, centrifugation and ethanol precipitation (at 70% ethanol) were used to separate these three fractions of orange juice.[25−27] Furthermore, the orange juice cloud (ethanol insoluble fraction) is rich in polymers such as pectin, proteins, celluloses, and hemicelluloses[18,19] which may be selectively precipitated at different ethanol concentrations.[26] Hence, a stepwise ethanol precipitation procedure with increasing ethanol concentrations was carried out to further fractionate the ethanol insoluble fraction into different subfractions.

Ethanol Precipitation. Centrifugation and ethanol precipitation were applied to separate the serum, cloud, and pulp fractions. A schematic overview of this step is shown in Figure 2A in the Results and Discussion section. Specifically, 200 mL of the juice was mildly centrifuged (1000xg, 4 °C, and 10 min) (J2-HS centrifuge, Beckman, Brea, CA, U.S.A.) to separate the large cell wall fragments (i.e., orange juice pulp) from the supernatant which contains orange juice serum and cloud. The pellet was washed with ultrapure water, centrifuged (10000xg, 4 °C, and 15 min), suspended in ultrapure water, and lyophilized. To the supernatant, absolute ethanol was added under continuous stirring until an ethanol concentration of 70% (v/v) was reached. The mixture was kept overnight in centrifuge bottles at 4 °C for precipitation. The precipitation conditions (70% ethanol and 4 °C) were chosen to maximize the amount of precipitated polymers. The mixture was centrifuged (10000xg, 4 °C, and 15 min), and the ethanol precipitated material was washed with 70% (v/v) ethanol solution, centrifuged (10000xg, 4 °C, and 15 min), dissolved in ultrapure water, and lyophilized resulting in a 70% ethanol precipitated fraction (EP). The supernatant was subjected to a solvent evaporation using a rotavapor at 40 °C to remove ethanol yielding an ethanol soluble fraction (ES). In addition, carotenoids were removed from all the fractions by extraction with a solvent mixture containing hexane, ethanol, and acetone (2:1:1 (v/v/v)). After that, they were lyophilized to yield three fractions, namely, the pellet, the EP, and the ES which were stored in a desiccator until further use or analysis.

Stepwise Ethanol Precipitation. Since different types of polymers (polysaccharides and proteins) may be precipitated at different ethanol concentrations, stepwise ethanol precipitation was used to further fractionate the 70% ethanol precipitated fraction (EP) following a procedure adapted from Bekedam et al.[28] Readers are referred to Figure 2B in the Results and Discussion section for a visualization of this step. The lyophilized EP was dissolved overnight in ultrapure water, and then absolute ethanol was slowly added until an ethanol concentration of 20% was reached. The solution was kept for precipitation and then centrifuged (10000xg, 4 °C, and 15 min) (J2-HS centrifuge, Beckman, Brea, CA, U.S.A.) to separate the supernatant and the residue. The residue was washed with 70% ethanol and centrifuged (10000xg, 4 °C, and 15 min) while the supernatant was subjected to further ethanol precipitation in which the ethanol concentration was increased stepwise to 40 and 60% following the same procedure. Ethanol was removed by evaporation, and the obtained fractions were lyophilized to yield EP20, EP40, and EP60 for the materials that precipitated at 20, 40 and 60% ethanol, respectively, and ES60 for the soluble material at 60% ethanol. All the lyophilized fractions were stored in a desiccator until further analysis.

Characterization of the Insoluble Compounds. *Molecular Weight Distribution.* The molecular weight distribution of the samples was analyzed based on the work of Shpigelman et al.[29] High performance size exclusion chromatography was performed on a series of three columns with exclusion limits of 8×10^4, 4×10^6, and 1×10^7 g mol^{-1}, respectively, in combination with a guard column. Elution took place at 35 °C with 0.1 M acetic acid in 0.1 M NaCl (pH

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4.4) at 0.5 mL min^{-1}. The eluent was monitored by refractive index detection in combination with light scattering detection, and the absorbance was measured at 280 nm by diode array detection in the Agilent 1200 system. Lyophilized samples were dissolved overnight in eluent buffer and filtered (0.45 μm, hydrophilic) prior to injection (100 μL). All the samples were analyzed in duplicate.

Monosaccharide Composition Analysis. Monosaccharides, including neutral sugars and galacturonic acid, were determined using high performance anion exchange chromatography combined with pulsed amperometric detection by following the method that was described previously[30] with some modifications. For hydrolysis of polysaccharides to monosaccharides, 1 mg of the lyophilized sample was dissolved overnight in 1 mL ultrapure water, and a 80 μL portion was dried in a test tube under a nitrogen evaporator at 45 °C. Next, 2 mL of 2 M methanolic HCl was added to the tube and gently mixed, and then the methanolysis was carried out at 80 °C for 16 h in an oil bath. After being cooled to ambient temperature, the samples were dried at 30 °C under a nitrogen evaporator. Subsequently, 2 mL of 2 M trifluoroacetic acid (TFA) was added to the samples and acid hydrolysis was performed with incubating the samples at 121 °C for 1 h. The samples were cooled to ambient temperature and dried under a nitrogen evaporator at 45 °C to remove TFA. Dried samples were dissolved in 400 μL of ultrapure water and filtered (0.45 μm, hydrophilic) into dark brown vials with glass inserts for the analysis. The hydrolysis procedure was performed in duplicate.

The determination of monosaccharides was performed on a HPLC (Dionex, Sunnyvale, CA, U.S.A.) equipped with a CarboPacTM PA20 column, a CarboPac PA20TM guard column, and an ED50 electrochemical detector. The system was equilibrated for 5 min using 100 mM NaOH and for additional 5 min using 0.5 or 25 mM NaOH depending on the eluent concentration used during sample elution. Samples (10 μL) were injected and eluted at a flow rate of 0.5 mL min^{-1} with eluent concentration of 0.5 mM in order to detect fucose, rhamnose, arabinose, galactose, glucose, xylose, mannose, and galacturonic acid. The analysis of the samples was repeated using a 25 mM eluent concentration to obtain a better peak resolution for rhamnose and arabinose. Commercial neutral sugar standards at different concentrations were used as external standards for identification and quantification.

Protein Analysis. Total nitrogen content of the samples was determined by the Dumas method using an EA 1108 elemental analyzer. Protein content in the sample was calculated from the nitrogen content using a nitrogen-to-protein conversion factor of 6.25.[31] All samples were analyzed in duplicate.

Analysis of the Soluble Compounds Related to Non-enzymatic Browning. *Determination of the Ascorbic Acid and Dehydroascorbic Acid Content.* The determination of the ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents was performed following a method proposed by Wibowo et al.[13] Sample was mixed with extraction buffer (1% (w/v) *m*-phosphoric acid with 0.5% (w/v) oxalic acid, pH 2.0) in a ratio of 1:3 (v/v). Subsequently, the mixture was centrifuged (24000xg, 4 °C, and 15 min) (J2-HS centrifuge, Beckman, Brea, CA, U.S.A.). The pH of the obtained supernatant was adjusted to 3.5 using 1 M NaOH or 1 M HCl. To determine the AA content, phosphate buffer (20 mM NaH_2PO_4+ 1 mM Na_2EDTA, pH 3.5) was added to part of the pH-adjusted supernatant in a ratio of 2:1 and the mixture was filtered (0.45 μm, hydrophilic) prior to the HPLC analysis. The DHAA content was quantified indirectly after reduction to AA by adding a reducing agent (TCEP 2.5 mM tris (2-carboxyl-ethyl) phosphine in phosphate buffer, pH 3.5) to the other part of the pH-adjusted supernatant in a ratio of 2:1 (v/v). The mixture was centrifuged (19900xg, 23 °C, and 15 min) (Microfuge 22R, Beckman Coulter) and filtered (0.45 μm, hydrophilic).

The analysis of AA was performed in a HPLC (Dionex, Sunnyvale, CA, U.S.A.) with an AD25 UV−vis detection at 245 nm. A prevail C18 column (250 × 4.6 mm, 5 μm particle size) with a corresponding guard column was used for chromatographic separation. The mobile phase was a solution of 1 mM Na_2EDTA and 10 mM CH_3COONH_4, and an isocratic elution at 0.8 mL min^{-1} and 25 °C was performed. The injection volume was 25 μL. Quantification was carried out using a calibration curve of AA in extraction buffer.

Determination of the Sugar Content. Sugar extraction and HPLC analysis were performed following the method of Vervoort et al.[32] with slight modifications. The sugar extraction was carried out in triplicate adding 50 μL of Carrez I (15% w/v $K_4[Fe(CN)_6]$) and Carrez II (30% w/v $ZnSO_4$) to 1 mL of sample. The mixture was vortexed, kept for 30 min (room temperature), and centrifuged (19900xg, 4 °C, and 15 min) (Microfuge 22R, Beckman Coulter). The supernatant was filtered (0.45 μm, hydrophilic), and 2 μL of a 10-fold dilution of the filtrate was injected into the HPLC system. The analysis was performed using a HPLC (Agilent 1200 series, Santa Clare, CA, U.S.A.) with evaporative light scattering detection. Separation was carried out on a Prevail C18 column (250 × 4.6 mm, 5 μm particle size), coupled to a Prevail C18 guard cartridge (7.5 × 4.6 mm, 5 μm particle size) at 30 °C using isocratic elution with 75% (v/v) acetonitrile/water at 1 mL min^{-1}. Sugar standards (fructose, glucose, and sucrose) were used for identification based on retention time, and standard curves were prepared for quantification.

Determination of Furfural and HMF Content. Furfural and HMF were analyzed based on the previously described method[13,32] with some modifications. The extraction of furfural and HMF was performed in triplicate mixing 500 μL each of the Carrez I reagent and the Carrez II reagent with 10 mL of the sample. Subsequently, the sample was kept at room temperature for 30 min and centrifuged (24000xg, 4 °C and 15 min) (J2-HS centrifuge, Beckman, Brea, CA, U.S.A.). After that, 1 mL of the supernatant was applied on a C18 SPE precolumn (Sep-PAK Water, Milford, U.S.A.), preconditioned with 2 mL methanol and 5 mL 0.5% acetic acid. Subsequently, 2 mL of ultrapure water was used to wash the SPE column; 4.5 mL ethyl acetate was applied on the column to elute furfural and HMF, and the resulting mixture was dried with anhydrous sodium sulfate. The eluate was filtered (0.45 μm, hydrophilic), and the volume was adjusted to 5 mL with ethyl acetate. The chromatographic analysis was carried out in a HPLC system (Agilent 1200 series, Santa Clara, CA, U.S.A.) with a UV−vis detection. The separation was done using a Zorbax Eclipse XDB C18 column (150 × 4.6 mm, 5 μm particle size) coupled to a Prevail C18 guard cartridge, at 25 °C and acetonitrile/water 5/95 (v/v) as mobile phase at 1 mL min^{-1} isocratic elution. Furfural was detected at 277 nm, and HMF was detected at 285 nm. Furfural and HMF standards were used for identification, and standard curves were established for quantification.

Data Analysis. One-way ANOVA using JMP statistical software (JMP Pro 14.0.0, SAS Institute Inc.) was performed to determine the statistical significance of duplicate means. When the mean values of two groups (i.e., between the nonstored sample and 15 week-stored samples) were compared, a student's *t* test was applied, while a posthoc test using Tukey's honest significant difference (HSD) was used to compare the mean values of more than two groups (i.e., storage times).

RESULTS AND DISCUSSION

Color Changes and Browning Development of Orange Juice during Storage. Overall color changes of the orange juice during storage were assessed objectively using a colorimeter. The total color difference (ΔE^*) was calculated based on the differences in the color parameters between the nonstored and stored juice using eq 1. In addition, the development of browning was specifically evaluated based on the browning index (BI) which is the measurement of the absorbance at 420 nm after ethanol extraction. The changes in ΔE^* and BI of the pasteurized shelf-stable orange juice as a function of storage time are described in Figure 1A,B, respectively.

It is clear from the results that both ΔE^* and BI increased significantly during storage. The increase in ΔE^* during storage is an indicator of the overall color changes of the juice,

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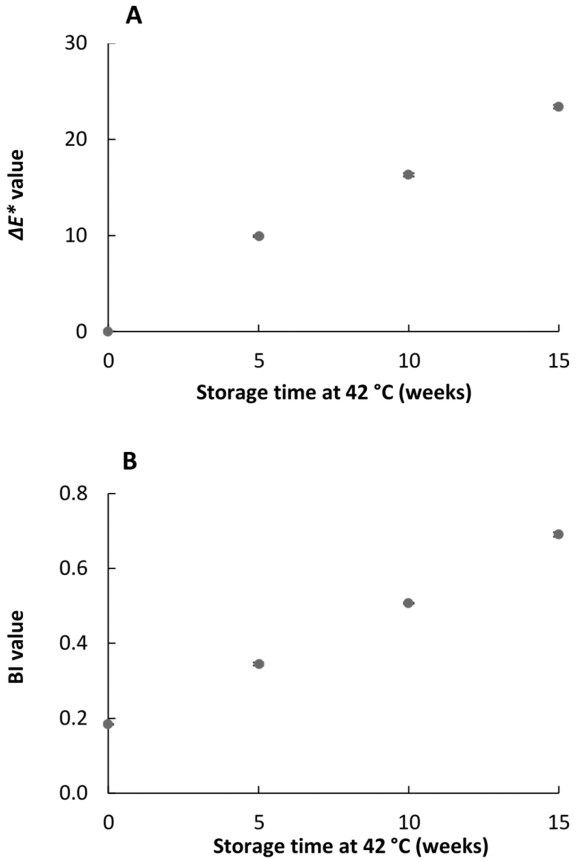

Figure 1. Changes in (A) total color difference (ΔE^*) and (B) browning index (BI) of orange juice during storage at 42 °C.

which can be caused by NEB reactions and other reactions such as the degradation of natural pigments (i.e., carotenoids). The rise in BI is mainly due to the formation and accumulation of brown compounds during storage. A strong correlation (r = 0.99) was found between BI and ΔE^* verifying the fact that the formation of brown compounds has an important contribution to the overall color changes of the pasteurized shelf-stable orange juice during storage.

Fractionation of Orange Juice into Pulp, Cloud, and Serum. After centrifugation and 70% ethanol precipitation, three different fractions, namely, the pellet, the ethanol precipitated fraction (EP), and the ethanol soluble fraction (ES) were obtained and carotenoids were removed from these fractions (Figure 2A). Freeze-dried and washed pellet and EP represented 267 and 363 mg per 100 mL of the non- stored orange juice, respectively, which was similar to the results of these two fractions (247 and 361 mg per 100 mL of the juice, respectively) obtained from the 15 week-stored juice. This indicates that the amounts of precipitated pulp and cloud did not change with storage time.

From Figure 2A, it can be seen clearly that the pellet, EP, and ES fractions of the 15 week-stored orange juice after removing carotenoids were brown compared to the white or yellow-like color of those from the nonstored juice. This observation reveals that the brown compounds formed during storage of the orange juice were distributed over the water/ethanol insoluble and ethanol soluble fractions. It has been stated that orange juice pulp and cloud are rich in cell wall polysaccharides and proteins.[18,19,23] Hence, the brown compounds present in the pellet and EP fractions might be linked to water/ethanol insoluble polysaccharides and proteins, while in the ES fraction, the brown compounds are possibly present in a free state. However, it cannot be excluded that some of the brown compounds are long chain polymers that were also precipitated together with natural occurring polymers of the juice during the ethanol precipitation step.

It has been intensively reported in literature that the degradation products from ascorbic acid and/or sugars may polymerize in the presence or absence of amino acids to form brown compounds during storage of shelf-stable citrus juices including orange juice.[3,4] However, to the best of our knowledge, none of the published data reveals the participation of the endogenous polymeric components of orange juice pulp and cloud toward the juice browning or their association with the brown compounds. As the EP fraction (after removing carotenoids) is mainly composed of polymers such as polysaccharides and proteins which may have different solubilities in ethanol, we intended to further fractionate this fraction using stepwise ethanol precipitation with increasing concentrations of ethanol to verify the fractions in which brown compounds are mainly present.

Further Fractionation of the Ethanol Insoluble Fraction. The ethanol precipitated fractions (EP) of the 15 week-stored and nonstored orange juice samples were subfractionated with stepwise ethanol precipitation in which the concentrations of ethanol were increased from 20% to 40% and then to 60% (Figure 2B). When this method was applied, four fractions were obtained: 20% ethanol precipitated material (EP20), 40% ethanol precipitated material (EP40), 60% ethanol precipitated material (EP60), and 60% ethanol soluble material (ES60).

For both the nonstored and 15 week-stored samples, most of the fractions were obtained in a significant amount (higher than 15% w/w of starting material), except for the EP60 which only accounted for around 5% w/w of the starting material (EP). Although it can be noticed that all subfractions of the EP of 15 week-stored juice had a brown color, the EP20 and EP40 fractions had a much darker brown color, compared to that of EP60 and ES60 revealing that almost all the brown compounds from the EP fraction stayed in the EP20 and EP40 fractions which were precipitated at low ethanol concentrations (20 and 40%, respectively).

Characterization of the Ethanol Insoluble Fraction and Subfractions. The ethanol insoluble fraction (EP) (i.e., cloud) of orange juice, which contains mainly polysaccharides and proteins,[19,22,23] also turned brown during storage. Therefore, it was of interest to verify whether the presence of brown compounds in this fraction would change its physicochemical characteristics such as molecular weight distribution, monosaccharide composition, and protein content. The characterization was made for the EP and its subfractions (EP20, EP40, EP60, and ES60) of the 15 week-stored orange juice in comparison to those of the nonstored juice.

Molecular Weight Distribution. To investigate the effect of the presence of brown compounds to the EP fraction on the size of polymers in these fractions, the molecular weight distributions of the EP from the 15 week-stored (EP 15W) and nonstored (EP 0W) juice were compared in Figure 3. On the basis of the concentration profile (dot-dash-dot lines), the EP 15W fraction, which had a brown color, consisted of two polymer populations with retention times from 35.5 to 47.5 min and from 47.5 to 60.0 min having a weight-average

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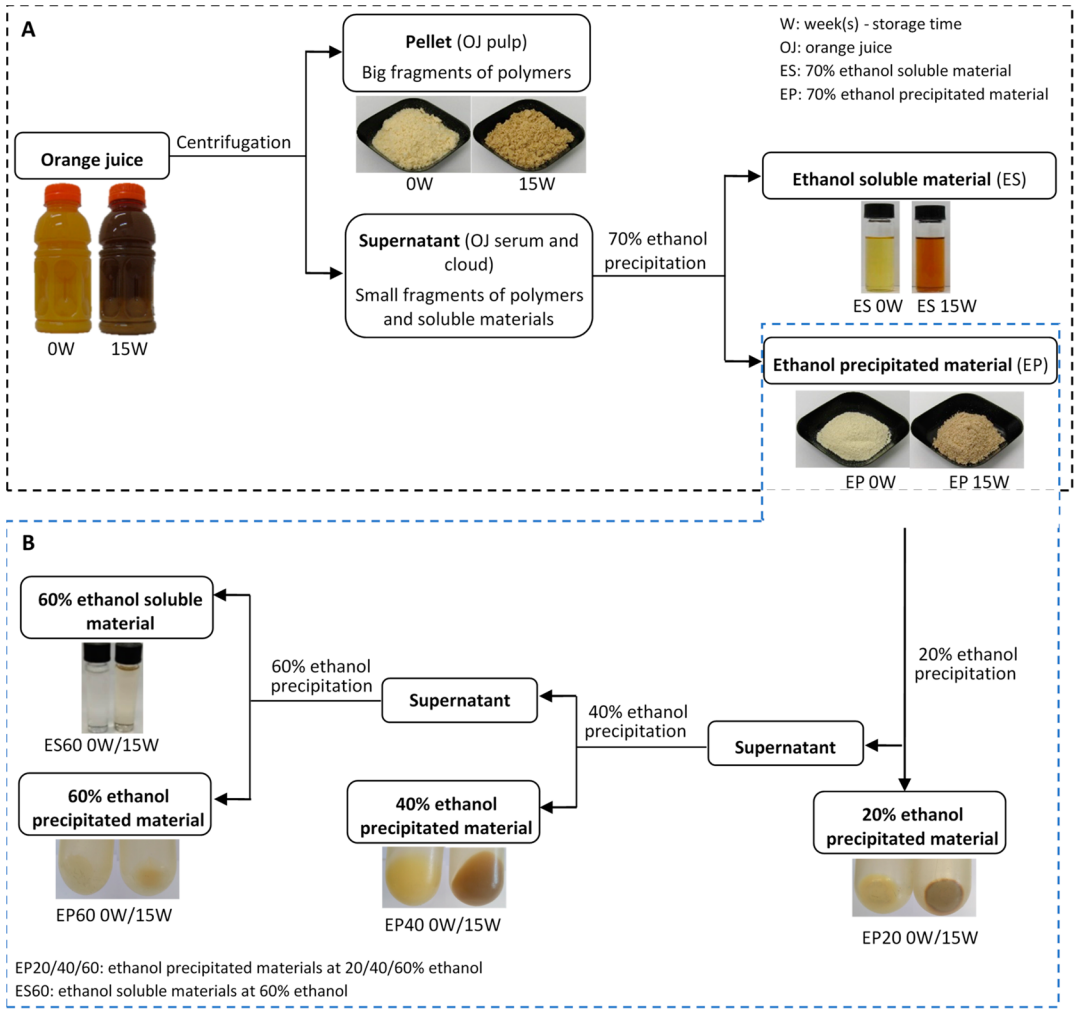

Figure 2. Schematic overview of (A) ethanol precipitation of the nonstored orange juice and 15 week-stored orange juice at 42 °C and (B) stepwise ethanol precipitation of the total ethanol precipitated materials.

molecular weight (M_w) of 2670 and 139 kDa, respectively. The materials that were eluted after 60.0 min were considered as low molecular weight population. Similarly, the EP 0W fraction, which had a white color, also contained two polymer populations with comparable retention times and M_w, and had similar concentration profiles. These results revealed that the presence of brown compounds in the EP 15W fraction did not influence its molecular weight distribution. Possible explanations could be that the brown compounds are small in size or that they account for a very small portion in the EP 15W fraction. Hence, their association to the large polymers in this fraction does not significantly affect the overall polymer size.

For the subfractions (EP20, EP40, EP60, and ES60) obtained from the EP 15W fraction, it was observed that the EP20 and EP40 fractions which precipitated at 20 and 40% ethanol, respectively, contained the large polymer populations of the EP 15W fraction (Figure 4). The EP60 and ES60 fractions possessed the small polymer population with a high concentration, especially the EP60. From the results, it can be concluded that the large polymers in orange juice cloud seem to precipitate at low ethanol concentrations while higher ethanol concentrations were required to precipitate the smaller polymers. In addition, there was insignificant difference in between subfractions of the EP 15W and EP 0W fractions (results not shown).

Monosaccharide Composition. In Table 1, the monosaccharide compositions of the ethanol insoluble fraction (EP) and its subfractions (EP20, EP40, EP60, and ES60) obtained from the 15 week-stored (15W) and nonstored (0W) orange juice are given. The main monosaccharide found in the EP fractions of both the nonstored and stored samples (EP 0W and EP 15W) was galacturonic acid followed by galactose, arabinose, and glucose. As galacturonic acid is the main monomer in pectin, the abundance of this uronic acid indicates that pectin is a predominant polysaccharide in EP 0W and EP 15W. According to Klavons et al.[19] pectin accounted for approximately 4.5% of the orange juice cloud. These authors found that around 60% of the cloud pectin exists as previously soluble pectin that is associated with insoluble proteins, 25–30% as calcium pectate, and 15% as protopectin.[19] The presence of galactose and arabinose might be related to the hydrolysis of arabinogalactan and/or the side chains of pectin. The glucose residues might be a breakdown product of nonpectic polymers such as celluloses and hemicelluloses or residues of the soluble sugar in orange juice that is trapped in the ethanol insoluble material. According to the results, no significant difference ($p > 0.05$) can be observed for the monosaccharide composition of the EP fraction from the nonstored and 15 week-stored juice. This means that the

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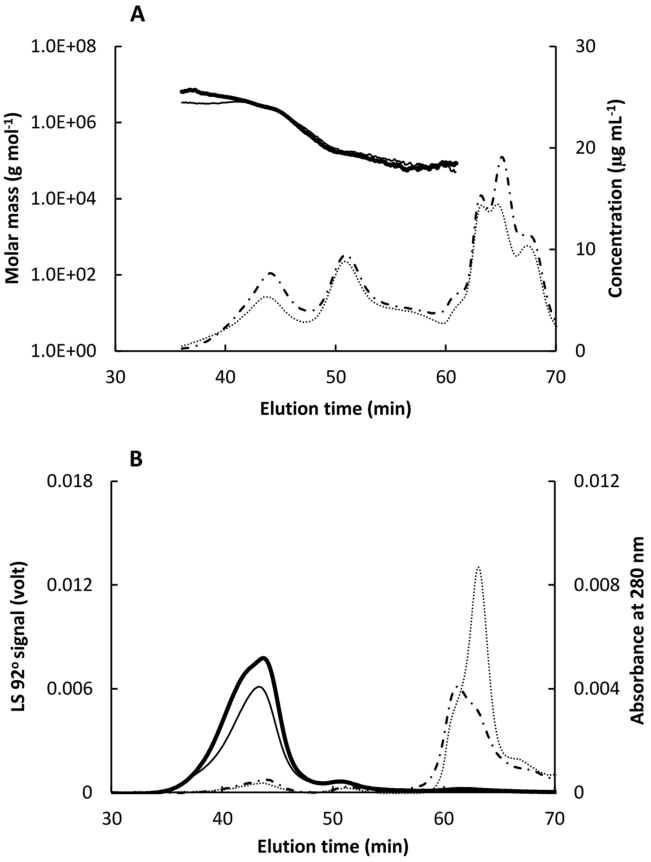

Figure 3. Size exclusion profile of the total ethanol precipitated material (EP) from the nonstored (0W) and 15 week- stored (15W) orange juice at 42 °C: (A) Log molar mass distribution (solid lines) of EP 0W (thin) and EP 15W (thick) and concentration of EP 0W (dotted line) and EP 15W (dot-dash-dot) as a function of elution time (min). (B) Light scattering signal at 92° angle (solid line) of EP 0W (thin) and EP 15W (thick) and UV absorbance chromatogram at 280 nm of EP 0W (dotted line) and EP 15W (dot-dash-dot).

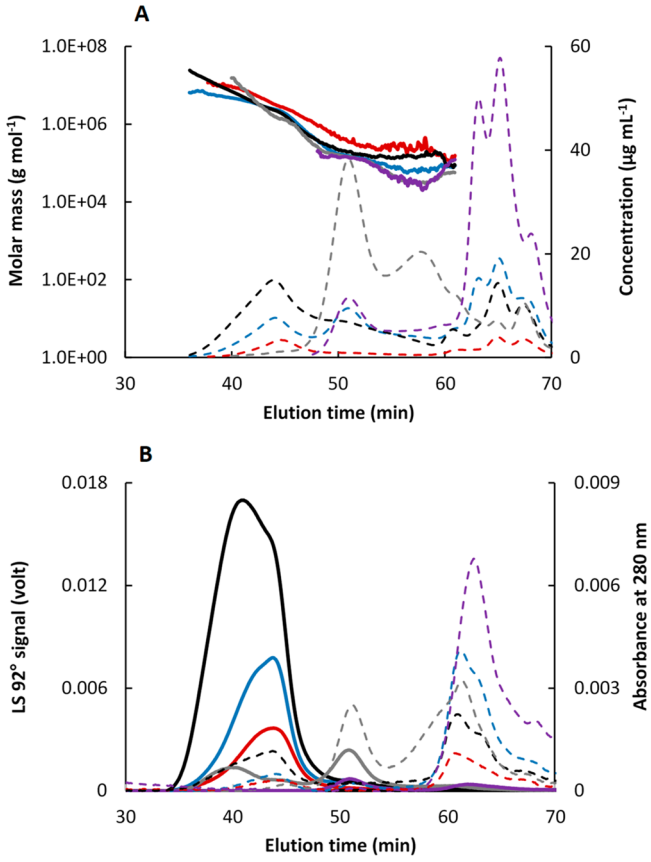

Figure 4. Size exclusion profile of the total ethanol precipitated material (EP) and its subfractions from the 15 week-stored orange juice at 42 °C: (A) Log molar mass distribution (solid line) and concentration (dashed line) as a function of elution time (min). (B) Light scattering signal at 92° angle (solid line) and UV absorbance chromatogram at 280 nm (dashed line). EP, blue; EP20, red; EP40, black; EP60, gray; ES60, purple.

presence of brown compounds in the EP fraction does not significantly alter its monosaccharide composition.

Among the subfractions of the EP material, monosaccharide compositions did vary considerably for both the nonstored and 15 week-stored samples (Table 1). Generally, the subfraction EP20 0W/15W contained the lowest total carbohydrate content (22−23%) and possessed only about 4% of galacturonic acid while this monosaccharide accounted for more than 20% of other subfractions precipitated at higher ethanol concentrations (40 and 60%). This observation revealed that for both 0W and 15W samples, pectin is more predominant among the polysaccharides in EP40 and EP60 while arabinogalactan is the most important polysaccharide in EP20. Bekedam et al.[20] reported that arabinogalactan might be linked to the brown compounds in coffee brew in the form of arabinogalactan proteins. This possible linkage can also be valid for the brown compounds formed in orange juice during storage based on the intensive brown color of the EP20 fraction in 15 week-stored orange juice and its considerable content of arabinogalactan. However, the presence of brown compounds did not significantly alter the monosaccharide compositions of the subfractions when a comparison was made between the nonstored and 15 week-stored samples. The most possible reasons could be that those monosaccharides studied are not present in the structure of the brown compounds or the brown compounds just account for a small part of the polymer material.

Protein Content. The protein content of the ethanol insoluble fraction (EP) and its subfractions is considered as a crude protein content (Table 1) since no corrections were made for nitrogen from the nonprotein nitrogen containing compounds. The EP 0W possessed around 29% (w/w) of proteins, which was significantly higher than the protein content of the EP 15W. These results are in accordance with the observation of UV absorbance at 280 nm (Figure 3B) which showed a higher absorbance peak of the small polymer population for the EP 0W fraction compared to that of the EP 15W fraction. According to Brat et al.,[18] 50% of the orange juice cloud's alcohol insoluble residues is made of proteins, and the rest is made of cell wall materials. Since proteins constitute less than 10% of primary cell walls, most of the proteins found in the orange juice cloud are of intracellular rather than cell wall origin.[18] In addition, the authors also reported that proteins were mainly responsible for the nitrogen content of orange juice cloud.

After the subfractionation of the EP 15W fraction through the stepwise ethanol precipitation, it can be seen that the EP20 15W fraction had the highest protein content with almost 50% w/w of the lyophilized material, while the EP60 15W had the lowest protein content (only around 5% w/w). A similar distribution of proteins was observed for the subfractions from

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Table 1. Average Monosaccharide Composition and Crude Protein Content of the Ethanol Precipitated Material and Their Sub-Fractions from the Non-Stored and 15 Week-Stored Orange Juice at 42 °C Expressed in g/100g Lyophilized Sample

Sample	Fuc[P]	Rha[P]	Ara[P]	Gal[P]	Glu[P]	Xyl[P]	Man[P]	GalA[P]	Crude protein[P]
EP 0W	0.18^{A}	1.60^{A}	6.13^{A}	9.13^{A}	3.58^{A}	0.24^{A}	0.26^{A}	11.62^{A}	29.23^{B}
EP20 0W	0.15^{Aa}	3.17^{Ab}	4.05^{Aa}	6.88^{Aa}	4.53^{Ab}	0.18^{Aa}	0.22^{Aa}	4.18^{Aa}	49.59^{Ad}
EP40 0W	0.24^{Ab}	0.99^{Ba}	4.75^{Aa}	7.82^{Aa}	1.75^{Aa}	0.20^{Aa}	0.20^{Aa}	22.34^{Ab}	25.78^{Bc}
EP60 0W	0.50^{Bc}	1.34^{Aa}	13.62^{Ac}	23.01^{Bc}	2.35^{Aa}	0.73^{Aa}	0.19^{Aa}	21.98^{Ab}	4.88^{Aa}
ES60 0W	0.09^{Ba}	1.07^{Aa}	8.19^{Bb}	10.33^{Aa}	10.46^{Ac}	0.64^{Aa}	1.21^{Aa}	6.71^{Ba}	9.95^{Ab}
EP 15W	0.19^{A}	1.76^{A}	6.29^{A}	10.11^{A}	5.69^{A}	0.54^{A}	0.25^{A}	15.06^{A}	25.61^{A}
EP20 15W	0.17^{Aa}	3.27^{Ab}	3.84^{Aa}	7.25^{Aa}	4.17^{Ab}	0.16^{Aa}	0.25^{Aa}	4.35^{Aa}	48.22^{Ac}
EP40 15W	0.25^{Ab}	0.76^{Aa}	4.22^{Aa}	7.70^{Aa}	3.36^{Bb}	0.20^{Aa}	0.18^{Aa}	28.86^{Ba}	19.69^{Ab}
EP60 15W	0.27^{Ac}	1.25^{Aa}	12.66^{Ac}	20.54^{Ac}	0.95^{Aa}	0.39^{Aa}	0.10^{Aa}	22.48^{Ab}	6.97^{Aa}
ES60 15W	0.05^{Aa}	0.77^{Aa}	7.23^{Ab}	10.05^{Ab}	11.61^{Ac}	0.91^{Ab}	0.97^{Ab}	4.70^{Aa}	10.08^{Aa}

[P]Different capital letters (in superscript) indicate statistical difference ($p < 0.05$) between the nonstored sample and 15 week-stored sample, while small letters indicate statistical differences among the subfractions of the nonstored sample or 15 week-stored sample.

Table 2. Changes in Different Attributes Related to Browning of Shelf-Stable Orange Juice during Storage at 42 °C

Storage time (weeks)	AA (mg L^{-1})[P]	DHAA (mg L^{-1})[P]	Suc (g L^{-1})[P]	Glu (g L^{-1})[P]	Fru (g L^{-1})[P]	Furfural (mg L^{-1})[P]	HMF (mg L^{-1})[P]
0	234.40^{d}	11.28^{a}	39.90^{d}	17.93^{a}	21.49^{a}	0.00^{a}	0.00^{a}
5	111.76^{c}	17.26^{b}	21.00^{c}	27.25^{b}	32.43^{b}	0.99^{b}	3.61^{b}
10	42.88^{b}	24.85^{c}	13.41^{b}	35.05^{c}	40.64^{c}	1.50^{c}	10.62^{c}
15	0.55^{a}	8.14^{a}	7.34^{a}	39.93^{d}	45.86^{d}	2.21^{d}	25.91^{d}

[P]For each attribute listed in the same column, different letters (in superscript) indicate statistical difference ($p < 0.05$) among storage moment.

the nonstored samples (0W). The intensive brown color of the high protein containing fraction (EP20 15W) could probably be due to the linkage of brown compounds to proteins or protein-pectin complexes in this fraction.[21] A strong correlation between the melanoidin content and protein content has been reported in coffee brew, and the authors have concluded that proteins are incorporated into the melanoidins.[28]

From the results of monosaccharide composition and protein content, it seems that the material which is rich in proteins tends to precipitate at a low concentration of ethanol (20%) while a higher ethanol concentration (e.g., 40%) is required to precipitate the material that is rich in cell wall polysaccharides. The presence of the brown compounds in the ethanol insoluble material seems not to have any significant influence on its monosaccharide composition and protein content. Possibly the ratio of brown compounds to the insoluble material was probably relatively small, so that their presence did not make any significant change in these attributes as was also observed previously for the molecular weight distribution (Figure 4B). The EP20 fraction which has the highest protein content seems to possess a high amount of brown compounds as it has the most intensive brown color compared to the other fractions. On the basis of this observation, it can be hypothesized that the brown compounds are linked to proteins, arabinogalactan proteins, or protein-pectin complexes in this fraction.

Changes in Different Soluble Compounds Related to Nonenzymatic Browning. It has been proposed in literature that NEB in citrus juices including orange juice involves the degradation of several reaction precursors such as AA and sugars, and the formation of reaction intermediates, namely, furfural and HMF.[3,10,13,16,33] Therefore, the changes of the aforementioned NEB precursors and intermediates during storage were investigated and the obtained results were summarized in Table 2.

Ascorbic Acid and Dehydroascorbic Acid Content. During the accelerated storage at 42 °C, the AA content decreased by 52% after 5 weeks of storage and was almost zero at the end of storage (Table 2). It was reported in literature that the storage of commercial orange juice at room temperature for 4 months caused a loss of 29% in the AA content.[34] The faster drop in the AA content observed in this study could be due to the high storage temperature used.[13,35] In addition, Gómez Ruiz and co-workers[36] stated that AA degradation increased with temperature and oxygen concentration. The orange juice used in this study was packed in PET bottles which have a relatively high oxygen permeability resulting in a continuous diffusion of oxygen during storage thus affecting the degradation of AA.[37] The first-order kinetic model is the most widely used and cited model in literature descibing the degradation of AA in model systems and orange juice systems.[13,36,38,39] The changes in AA content in this work was not kinetically modeled since the data points were not sufficient.

In the presence of oxygen, AA is primarily oxidized to DHAA. The DHAA content increased significantly ($p < 0.05$) during the first 10 weeks of storage, followed by a decrease to the end of storage (Table 2). The increase, followed by a decrease in the DHAA content, observed in this study, is consistent with the results reported previously[40] for the changes in DHAA content of orange juice packed in materials with different oxygen permeabilities. Furthermore, it could be observed that the increase in the DHAA content was much smaller than the decrease in AA content. For instance, while the AA content decreased from 234.40 to 111.76 mg L^{-1} after 5 weeks of storage, the DHAA increased from 11.28 to 17.26 mg L^{-1}. There are two possible reasons for the imbalances in AA degradation and DHAA formation. First, DHAA is a very unstable compound which can be irreversibly hydrolyzed to 2,3-diketogulonic acid. Therefore, the observed change in DHAA content is the net result of formation and degradation reactions. The second reason could be that part of the AA

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content might decrease through the anaerobic pathway in which DHAA is not formed. Consequently, the total vitamin C (AA plus DHAA) content (results not shown) decreased over storage time and the overall nutritional quality of orange juice is negatively affected upon storage.

Sugar Content. During storage, the sucrose content significantly decreased ($p < 0.05$) while the glucose and fructose contents increased (Table 2). After 15 weeks of storage at 42 °C, approximately 82% of sucrose was hydrolyzed while the glucose and fructose contents increased more than double the initial levels. The observed results in sugar changes in the current work are in agreement with the literature data with the main mechanism being the hydrolysis of sucrose to glucose and fructose under acidic conditions.[13,33] However, it can be noticed that the increase of glucose and fructose contents did not coincide with the decrease of sucrose content. It is possible that glucose and fructose were also formed from the hydrolysis of other carbohydrates like orange juice fibers (e.g., pectin, hemicelluloses) during storage. The reducing sugars, including fructose and glucose, can undergo acid-catalyzed decomposition to form reactive intermediates (e.g., HMF and/or 3-deoxyglucosone), which are important in NEB of citrus juices and orange juice.[5,41]

Furfural and 5-Hydroxymethylfurfural Content. The results (Table 2) show that both furfural and HMF were absent in the pasteurized shelf-stable orange juice prior to storage and their concentrations increased significantly with storage time. After 15 weeks storage at 42 °C, the concentration of furfural was 2.21 mg L^{-1} while that of HMF was 25.91 mg L^{-1}. It is interesting to note that there was higher formation of HMF than furfural during storage, which is consistent with the literature reported data for orange juice[13] and for grapefruit juice.[10]

It is well established that furfural is primarily generated from AA via an anaerobic degradation pathway[10,42,43] while HMF is a breakdown product during the dehydration of glucose and fructose.[10,41] The formation of furfural and HMF in citrus juices is generally agreed to be related to the darkening of the juices during storage.[3,10] While furfural is a known reactive compound, HMF has been regarded as a byproduct which only accumulates because of its low reactivity in browning reactions.[10]

The changes in AA and sugar content and the formation of furfural and HMF are consistent with the results of the browning development (BI). This is in agreement with literature which has demonstrated that AA and sugars are precursors of the NEB reactions and their degradation causes the formation of furfural and HMF which are considered as NEB reaction intermediates.[3] In conclusion, the changes in soluble compounds observed in this study confirm the documented literature on NEB, while the participation of polymeric components (e.g., proteins, arabinogalactan proteins, protein-pectin complexes) of orange juice cloud and pulp during juice browning is demonstrated for the first time in literature.

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ABBREVIATIONS USED

AA, ascorbic acid; AIJN, Association of the Industry of Juices and Nectars from fruits and vegetables of the European Union; BI, browning index; DHAA, dehydroascorbic acid; EP, material precipitated in 70% ethanol solution; EP20, material precipitated in 20% ethanol solution; EP40, material precipitated in 40% ethanol solution; EP60, material precipitated in 60% ethanol solution; ES, material soluble in 70% ethanol solution; ES60, material soluble in 60% ethanol solution; Fuc, fucose; Fru, fructose; GalA, galacturonic acid; Gal, galactose; Glu, glucose; HMF, 5-hydroxymethylfurfural; HPLC, high-performance liquid chromatography; Man, mannose; M_w, weight-average molecular weight; NEB, non-enzymatic browning; PET, polyethylene terephthalate; PPO, polyphenol oxidase; Rha, rhamnose; Suc, sucrose; W, week(s); Xyl, xylose

REFERENCES

(1) Álvarez, J.; Pastoriza, S.; Alonso-Olalla, R.; Delgado-Andrade, C.; Rufián-Henares, J. A. Nutritional and Physicochemical Characteristic of Commercial Spanish Citrus Juices. *Food Chem.* **2014**, *164*, 396−405.

(2) Bacigalupi, C.; Lemaistre, M. H.; Boutroy, N.; Bunel, C.; Peyron, S.; Guillard, V.; Chalier, P. Changes in Nutritional and Sensory Properties of Orange Juice Packed in PET Bottles: An Experimental and Modelling Approach. *Food Chem.* **2013**, *141* (4), 3827−3836.

(3) Bharate, S. S.; Bharate, S. B. Non-Enzymatic Browning in Citrus Juice: Chemical Markers, Their Detection and Ways to Improve Product Quality. *J. Food Sci. Technol.* **2014**, *51* (10), 2271−2288.

(4) Handwerk, R. L.; Coleman, R. L. Approaches to the Citrus Browning Problem. A Review. *J. Agric. Food Chem.* **1988**, *36* (1), 231−236.

(5) Paravisini, L.; Peterson, D. G. Mechanisms Non-Enzymatic Browning in Orange Juice During Storage. *Food Chem.* **2019**, *289*, 320−327.

(6) Buvé, C.; Kebede, B. T.; De Batselier, C.; Carrillo, C.; Pham, H. T. T.; Hendrickx, M.; Grauwet, T.; Van Loey, A. Kinetics of Colour Changes in Pasteurised Strawberry Juice during Storage. *J. Food Eng.* **2018**, *216*, 42−51.

(7) Wibowo, S.; Grauwet, T.; Gedefa, G. B.; Hendrickx, M.; Van Loey, A. Quality Changes of Pasteurised Mango Juice during Storage. Part II: Kinetic Modelling of the Shelf-Life Markers. *Food Res. Int.* **2015**, *78*, 410−423.

(8) Paravisini, L.; Peterson, D. G. Role of Reactive Carbonyl Species in Non-Enzymatic Browning of Apple Juice during Storage. *Food Chem.* **2018**, *245*, 1010−1017.

(9) Clegg, K. M. Non-enzymic Browning of Lemon Juice. *J. Sci. Food Agric.* **1964**, *15* (12), 878−885.

DOI: 10.1021/acs.jafc.9b05014
J. Agric. Food Chem. 2019, 67, 12854−12862

(10) Lee, H. S.; Nagy, S. Quality Changes and Nonenzymic Browning Intermediates in Grapefruit Juice During Storage. *J. Food Sci.* **1988**, *53* (1), 168−172.
(11) Clegg, K. M.; Morton, A. D. Carbonyl Compounds and the Non-Enzymic Browning of Lemon Juice. *J. Sci. Food Agric.* **1965**, *16*, 191−198.
(12) Paravisini, L.; Peterson, D. G. Characterization of Browning Formation in Orange Juice during Storage. *ACS Symp. Ser.* **2016**, *1237*, 55−65.
(13) Wibowo, S.; Grauwet, T.; Santiago, J. S.; Tomic, J.; Vervoort, L.; Hendrickx, M.; Van Loey, A. Quality Changes of Pasteurised Orange Juice during Storage: A Kinetic Study of Specific Parameters and Their Relation to Colour Instability. *Food Chem.* **2015**, *187*, 140−151.
(14) Stadtman, E. R. Nonenzymatic Browning in Fruit Products. *Adv. Food Res.* **1948**, *1* (C), 325−372.
(15) Roig, M. G.; Bello, J. F.; Rivera, Z. S.; Kennedy, J. F. Studies on the Occurrence of Non-Enzymatic Browning during Storage of Citrus Juice. *Food Res. Int.* **1999**, *32* (9), 609−619.
(16) Shinoda, Y.; Murata, M.; Homma, S.; Komura, H. Browning and Decomposed Products of Model Orange Juice. *Biosci., Biotechnol., Biochem.* **2004**, *68* (3), 529−536.
(17) Hofmann, T. Structure, Color, and Formation of Low- and High-Molecular Weight Products Formed by Food-Related Maillard-Type Reactions. In *Chemistry and Physiology of Selected Food Colorants; ACS Symposium Series*; American Chemical Society, 2001; Vol. *775*, pp 134−151 SE − 9. DOI: 10.1021/bk-2001-0775.ch009.
(18) Brat, P.; Rega, B.; Alter, P.; Reynes, M.; Brillouet, J. M. Distribution of Volatile Compounds in the Pulp, Cloud, and Serum of Freshly Squeezed Orange Juice. *J. Agric. Food Chem.* **2003**, *51* (11), 3442−3447.
(19) Klavons, J. A.; Bennett, R. D.; Vannier, S. H. Physical/Chemical Nature of Pectin Associated with Commercial Orange Juice Cloud. *J. Food Sci.* **1994**, *59* (2), 399−401.
(20) Bekedam, E. K.; De Laat, M. P. F. C.; Schols, H. A.; Van Boekel, M. A. J. S.; Smit, G. Arabinogalactan Proteins Are Incorporated in Negatively Charged Coffee Brew Melanoidins. *J. Agric. Food Chem.* **2007**, *55* (3), 761−768.
(21) Hofmann, T. Studies on Melanoidin-Type Colorants Generated from the Maillard Reaction of Protein-Bound Lysine and Furan-2-Carboxaldehyde – Chemical Characterisation of a Red Coloured Domaine. *Zeitschrift für Leb. und -forsch. A* **1998**, *206* (4), 251−258.
(22) Scott, W. C.; Kew, T. J.; Veldhuis, M. K. Composition of Orange Juice Cloud. *J. Food Sci.* **1965**, *30* (5), 833−837.
(23) Klavons, J. A.; Bennett, R. D.; Vannier, S. H. Nature of the Protein Constituent of Commercial Orange Juice Cloud. *J. Agric. Food Chem.* **1991**, *39* (9), 1545−1548.
(24) Klim, M.; Nagy, S. An Improved Method to Determine Nonenzymic Browning in Citrus Juices. *J. Agric. Food Chem.* **1988**, *36* (6), 1271−1274.
(25) Ognyanov, M.; Remoroza, C.; Schols, H. A.; Georgiev, Y.; Kratchanova, M.; Kratchanov, C. Isolation and Structure Elucidation of Pectic Polysaccharide from Rose Hip Fruits (Rosa Canina L.). *Carbohydr. Polym.* **2016**, *151*, 803−811.
(26) Bian, J.; Peng, F.; Peng, P.; Xu, F.; Sun, R.-C. Isolation and Fractionation of Hemicelluloses by Graded Ethanol Precipitation from Caragana Korshinskii. *Carbohydr. Res.* **2010**, *345*, 802−809 Contents. .
(27) Ting, S. V.; Rouseff, R. L. *Citrus Fruits and Their Products: Analysis, Technology*; Dekker: 1986.
(28) Bekedam, E. K.; Schols, H.; van Boekel, M.; Smit, G. High Molecular Weight Melanoidins from Coffee Brew. *J. Agric. Food Chem.* **2006**, *54*, 7658−7666.
(29) Shpigelman, A.; Kyomugasho, C.; Christiaens, S.; Van Loey, A. M.; Hendrickx, M. E. Thermal and High Pressure High Temperature Processes Result in Distinctly Different Pectin Non-Enzymatic Conversions. *Food Hydrocolloids* **2014**, *39*, 251−263.
(30) Santiago, J. S. J.; Kyomugasho, C.; Maheshwari, S.; Jamsazzadeh Kermani, Z.; Van de Walle, D.; Van Loey, A. M.; Dewettinck, K.; Hendrickx, M. E. Unravelling the Structure of Serum Pectin Originating from Thermally and Mechanically Processed Carrot-Based Suspensions. *Food Hydrocolloids* **2018**, *77*, 482−493.
(31) Chang, S. K. C.; Zhang, Y. Protein Analysis. In *Food Analysis*; Nielsen, S. S., Ed.; Springer International Publishing: Cham, 2017; pp 315−331. DOI: 10.1007/978-3-319-45776-5_18.
(32) Vervoort, L.; Van Der Plancken, I.; Grauwet, T.; Timmermans, R. A. H.; Mastwijk, H. C.; Matser, A. M.; Hendrickx, M. E.; Van Loey, A. Comparing Equivalent Thermal, High Pressure and Pulsed Electric Field Processes for Mild Pasteurization of Orange Juice: Part II: Impact on Specific Chemical and Biochemical Quality Parameters. *Innovative Food Sci. Emerging Technol.* **2011**, *12* (4), 466−477.
(33) Kennedy, J. F.; Rivera, Z. S.; Lloyd, L. L.; Warner, F. P.; Jumel, K. Studies on Non-enzymic Browning in Orange Juice Using a Model System Based on Freshly Squeezed Orange Juice. *J. Sci. Food Agric.* **1990**, *52* (1), 85−95.
(34) Kabasakalis, V.; Siopidou, D.; Moshatou, E. Ascorbic Acid Content of Commercial Fruit Juices and Its Rate of Loss upon Storage. *Food Chem.* **2000**, *70* (3), 325−328.
(35) Klimczak, I.; Małecka, M.; Szlachta, M.; Gliszczyńska-Świgło, A. Effect of Storage on the Content of Polyphenols, Vitamin C and the Antioxidant Activity of Orange Juices. *J. Food Compos. Anal.* **2007**, *20* (3−4), 313−322.
(36) Gómez Ruiz, B.; Roux, S.; Courtois, F.; Bonazzi, C. Kinetic Modelling of Ascorbic and Dehydroascorbic Acids Concentrations in a Model Solution at Different Temperatures and Oxygen Contents. *Food Res. Int.* **2018**, *106*, 901−908.
(37) Berlinet, C.; Brat, P.; Brillouet, J.-M.; Ducruet, V. Ascorbic Acid, Aroma Compounds and Browning of Orange Juices Related to PET Packaging Materials and PH. *J. Sci. Food Agric.* **2006**, *86* (13), 2206−2212.
(38) Van Bree, I.; Baetens, J. M.; Samapundo, S.; Devlieghere, F.; Laleman, R.; Vandekinderen, I.; Noseda, B.; Xhaferi, R.; De Baets, B.; De Meulenaer, B. Modelling the Degradation Kinetics of Vitamin C in Fruit Juice in Relation to the Initial Headspace Oxygen Concentration. *Food Chem.* **2012**, *134* (1), 207−214.
(39) Polydera, A. C.; Stoforos, N. G.; Taoukis, P. S. Comparative Shelf Life Study and Vitamin C Loss Kinetics in Pasteurised and High Pressure Processed Reconstituted Orange Juice. *J. Food Eng.* **2003**, *60* (1), 21−29.
(40) Zerdin, K.; Rooney, M. L.; Vermuë, J. The Vitamin C Content of Orange Juice Packed in an Oxygen Scavenger Material. *Food Chem.* **2003**, *82* (3), 387−395.
(41) Arena, E.; Fallico, B.; Maccarone, E. Thermal Damage in Blood Orange Juice: Kinetics of 5-Hydroxymethyl-2-Furcarboxaldehyde Formation. *Int. J. Food Sci. Technol.* **2001**, *36* (1995), 145−151.
(42) Robertson, G.; Samaniego, C. Effect of Initial Dissolved Oxygen Levels on the De- Gradation of Ascorbic Acid and the Browning of Lemon Juice during Storage. *J. Food Sci.* **1986**, *51* (1), 184−187.
(43) Yuan, J.-P.; Chen, F. Degradation of Ascorbic Acid in Aqueous Solution. *J. Agric. Food Chem.* **1998**, *46*, 5078−5082.

DOI: 10.1021/acs.jafc.9b05014
J. Agric. Food Chem. 2019, 67, 12854−12862