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Generation of α-Diketones and 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone upon Coffee Roasting—Impact of Roast Degree on Reaction Pathways

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ABSTRACT: The formation pathways of α-diketones (2,3-butanedione and 2,3-pentanedione) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) upon coffee roasting were investigated in a kinetic study applying labeled and unlabeled sucrose (CAMOLA approach) in biomimetic in-bean experiments. The results highlighted that not only did the contribution of sucrose to the level of α-diketones in roasted coffee change with the roasting degree but also the portion of the individual reaction pathways. At early roasting stages, 2,3-butanedione was formed from sucrose mainly via the intact sugar skeleton, whereas from the middle of the roasting course, the formation foremost occurred from sugar fragments, primarily by C_1/C_3 and C_2/C_2 recombinations. In contrast, 2,3-pentanedione was generated from sucrose mainly via an intact sugar skeleton during the whole roasting cycle; nevertheless, the share of 2,3-pentanedione formed by recombination of fragments (mainly C_2/C_3) progressively increased with roasting time. HDMF was generated from sucrose almost exclusively via cyclization of an intact skeleton, irrespective of the roast time.

KEYWORDS: *coffee flavor formation, Maillard reaction, α-diketones, CAMOLA, kinetics, roasting*

INTRODUCTION

The roasting process unlocks the characteristic flavor of coffee by a complex interplay of chemical and physical transformations occurring in the green coffee bean at temperatures higher than 200 °C. In view of this very complex reaction environment, the so-called in-bean approach to study flavor formation in coffee was developed[1] with the basic idea to maintain the integrity of the coffee bean structure providing the most real reaction conditions with similar physical and chemical transformations during roasting in the recombined model bean, such as in the original coffee bean matrix. Applying this technique, the role of the water-extractable fraction, containing important precursors, such as free amino acids and free sugars, was assessed[1,2] and the formation pathways of specific Maillard-derived components could be elucidated.[3] The in-bean approach was also successfully applied to study the mechanism of coffee melanoidin formation[4] and to investigate the presence and nature of thiol-binding sites in raw coffee beans.[5,6]

The key role of soluble saccharides, mainly sucrose, in the formation of many volatile components was studied in detail by means of in-bean experiments, where the targeted precursor was enriched in the green coffee bean or omitted in the biomimetically recombined bean.[2] Spiking of sucrose into the green coffee bean resulted in an increase of 2,3-butanedione and 2,3-pentanedione in the final roasted beans. This finding is typically reflected by higher levels of α-diketones present in Arabica coffees, which are naturally higher in sucrose compared to Robusta coffee, whereas the omission of the free sugar fraction significantly decreased both diketones. Other free mono- and disaccharides present in the green coffee bean were shown to be irrelevant in the formation of α-diketones as a result of their low amounts.

Labeling studies using ^{13}C- and ^{15}N-labeled precursors and more specifically the carbon module labeling (CAMOLA)[1] technique were proven as very potent tools in gaining detailed insight into the formation pathways of flavor components. The CAMOLA technique, developed by Schieberle et al., allowed for the study of the formation of diketones, such as 2,3-butanedione.[7,8] Dry roasting of a glucose/proline model system at 180 °C generated 2,3-butanedione foremost via recombination of C_3/C_1 (e.g., 1-hydroxy-2-propanone and formaldehyde) and C_2/C_2 fragments (e.g., hydroxyacetaldehyde and acetaldehyde). Similarly, the formation of 2,3-pentanedione was reported through the recombination of different sugar fragments.[9−11]

Kinetic experiments describing the molecular composition as a function of time were highlighted as a valuable mean to understand the highly dynamic coffee roasting process. Baggenstoss et al.[12] applied time-resolved sampling during roasting and followed aroma formation by quantitative analysis. Online mass spectrometric techniques, such as proton transfer reaction−mass spectrometry (PTR−MS) or photon ionization

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Table 1. Amounts of Components Used for the Preparation of BRE[a,b]

component	amount (mg)	component	amount (mg)
phenols		amino acids (continued)	
chlorogenic acid	3229	L-glutamine	6.04
trigonelline	501	L-tryptophane	13.2
caffeine	412	L-valine	5.97
organic acids		L-isoleucine	4.03
D-(−)-quinic acid	347	L-leucine	5.71
L-(+)-lactic acid	1.0	L-phenylalanine	10.5
potassium acetate	32.9	L-lysine	6.82
malic acid	163	sugars	
potassium citrate	1049	D-(−)-arabinose	4.40
amino acids		L-rhamnose	1.44
L-alanine	19.4	D-(+)-galactose	79.2
L-threonine	15.9	D-(+)-sucrose	2003
L-serine	12.7	D-[$^{13}C_{12}$]sucrose	2590
L-aspartic acid	45.4	D-(+)-mannose	11.6
L-glutamic acid	82.9	metals	
glycine	1.62	iron(III) chloride	3.36
L-histidine	6.62	manganese(II) chloride	1.92
L-arginine	30.0	copper(II) sulfate	2.12
L-proline	10.3	zinc(II) sulfate, heptahydrate	0.320
L-tyrosine	8.83		
L-asparagine	39.5		

[a]For 50 g of EB. [b]Composition of BRE was based on the composition of the water extract of green coffee beans.

mass spectrometry, were also applied to investigate coffee aroma formation because they enable a continuous monitoring of this fast process.[13−16]

The investigation of flavor formation kinetics in recombined model beans applying labeled precursors allowed for a very detailed picture of the intermediate transformations during the roasting course.[3] Replacing natural sucrose by the fully labeled $^{13}C_{12}$ analogue revealed the importance of the free sugar and reaction pathways constantly shifted throughout the roasting course. Sucrose was shown to be more important in the formation of 2,3-pentanedione, with about 76% still originating from the original sucrose at the final roasting point, whereas in the formation of 2,3-butanedione, sucrose was found only relevant in the early stages of roasting but, later on bound sugars, namely, polysaccharides, seemed to take over relevancy because 65% did not stem from sucrose in the final roasted beans.

The same publication highlighted that sugar fragment recombination occurred for both molecules but seemed to be more important in 2,3-butanedione formation, whereas formation from the intact sugar skeleton is the major pathway generating 2,3-pentanedione from sucrose. 2,3-Butanedione was mainly generated by recombination of sucrose fragments.

The caramel smelling odorant 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone was shown by Schieberle[8] applying CAMOLA studies to be solely formed via the intact C_6-glucose skeleton under roasting conditions. In-bean experiments with fully labeled sucrose proved that this is also valid under coffee roasting conditions because only fully labeled and unlabeled molecules were formed.[2]

Despite the significant effort devoted to understand the generation of aroma-active compounds in the complex coffee bean system, the knowledge on the involved precursors and their mechanistic transformations during the roasting course is still fragmentary.

Therefore, the present study aimed to understand how the contribution of individual formation pathways of α-diketones and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone evolve during the coffee roasting course by applying the CAMOLA approach with labeled sucrose combined with a kinetic study.

MATERIALS AND METHODS

Raw Material. Green coffee beans (*Coffea arabica*, Costa Rica, La Giorgia, wet processed) were used.

Chemicals. The following chemicals were commercially available: caffeine (99.5%), copper(II) sulfate (99%), D-(−)-arabinose (99%), D-(+)-galactose (99%), L-rhamnose (99%), D-(+)-mannose (99%), D-(+)-sucrose (99.5%), D-(−)-quinic acid (98%), iron(III) chloride (97%), L-alanine (99.5%), L-arginine (99.5%), L-asparagine (98.5%), L-aspartic acid (99.5%), L-cysteine (99.5%), L-glutamic acid (99.5%), L-glutamine (99.9%), glycine (98.5%), L-histidine (99.5%), L-isoleucine (99.5%), L-(+)-lactic acid (99%), L-leucine (99.5%), L-lysine (98%), L-methionine (99.5%), L-phenylalanine (98%), L-proline (99.5%), L-serine (98.5%), L-threonine (98.5%), L-tyrosine (99%), L-tryptophane (99.5%), L-valine (99.5%), malic acid (99.5%), manganese(II) chloride (98%), potassium acetate (99%), potassium hydroxide (85%), trigonelline hydrochloride (98%), zinc(II) sulfate heptahydrate (99%), chlorogenic acid (95%), potassium citrate (99%) (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), and [$^{13}C_{12}$]-sucrose (98%) (Omicron Biochemicals, Inc., South Bend, IN, U.S.A.).

Water Extraction of Green Coffee Beans. The extraction of green coffee beans was performed according to the procedure described by Poisson et al.[3]

Green coffee beans were extracted with hot water applying the following conditions: 10 kg of green coffee beans was mixed with 20 L of water in a Scanima batch mixer (Tetra Pak Scanima, Denmark) and heated to 60 °C for 1 h. The obtained extract was drained, and the coffee beans were extracted another 4 times with 20 L of demineralized water at 60 °C for a total of 4 h to obtain the water-soluble substances (total 100 L of extract). The resulting extraction yield was found at 20.5% (23.2% on a dry matter basis). In a second step, the exhausted green coffee beans were dried in the Scanima batch mixer by increasing the temperature of the double jacket to 110 °C and applying a vacuum of 150 mbar. During the first 2 h of drying,

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Table 2. Roasting Profile for the Kinetic Study

time (s)	50	100	150	210	260	300	330	360	400
temperature (°C)	171	183	191	199	201	204	206	217	220

the product temperature was raised from 60 to 80 °C and then the final temperature was held constant for another 4 h (total 6 h of drying time). To prevent bean breakage, the agitator/mixer was regulated at the lowest possible rotation speed (24 rpm agitator, 100 rpm mixer). The obtained green coffee extracts were combined and freeze-dried in a LyoBeta 35 freeze dryer (Telstar, Terrassa, Spain) and stored at −40 °C until use.

Preparation of Biomimetically Recombined Beans (RB). The recombination of green coffee beans was performed according to the procedure described by Poisson et al.[3] Some adaptations were performed to obtain a higher batch size. For the preparation of the biomimetically recombined extract (BRE), the single components (see Table 1) were dissolved in 20 mL of demineralized water at 80 °C. The pH value of BRE was adjusted to 5.5 (corresponding to the pH of the natural extract) with a 16.5% (w/w) solution of KOH. The water exhausted green coffee beans (EB, 50 g) were soaked with the BRE at 50 °C for 5 h and then overnight at room temperature. During soaking, the beans were gently stirred using a Rotavapor (Büchi, Switzerland). To improve the incorporation of BRE from the bean surface into EB, 20 mL of demineralized water was added to incorporated coffee beans and absorbed using a Rotavapor (gentle stirring for 1 h at 50 °C and then 5 h at room temperature). After water absorption, the treated beans were washed with the same mass of water as beans during 10 s. The washing losses were controlled by analyzing the washing waters with an ATAGO PAL3 pocket refractometer (Atago, Japan), which measures the total solid content on a Brix scale. Finally, the washed coffee beans were frozen to −80 °C and then freeze-dried for 24 h at 0.1 mbar and about −80 °C on an Alpha 2-4 LSC freeze dryer (Christ, Germany) to reach a moisture content of 10 ± 0.5%.

Labeling Experiments (CAMOLA). The labeling trials were conducted according to the procedure described by Poisson et al.[3] For the kinetic study with [$^{13}C_{12}$]sucrose, the setup was scaled up by factor of 10. To reach an equimolar 1:1 ratio of unlabeled and labeled sucrose, the level of unlabeled sucrose in BRE was adjusted to compensate for the residual level of sucrose in EB (499 mg/50 g of EB). Consequently, the BRE omitted in all sugars was spiked with [$^{13}C_{12}$]sucrose (2590 mg) and unlabeled sucrose (2003 mg) prior its incorporation into 50 g of EB (Table 1).

Laboratory Roasting Trials. The roasting trials were conducted according to the procedure described by Poisson et al.[3]

After freeze drying, 5 g of recombined green beans were counted and then mixed with 10 g of reference coffee beans to increase the batch size to a critical amount needed (15 g) for the roasting using an IKAWA sample roaster (Ikawa, London, U.K.). The roasting temperature and air flow profiles were optimized for the roasting of 15 g of green coffee. This profile led to a color test number (CTN, Neuhaus-Neotec, Germany) of 65 for reference coffee beans after 400 s. The individual batches (15 g) were roasted under the same conditions at different roasting durations (temperature measured above the fluidized bed of coffee beans) as given in Table 2. The air cooling of the beans roasted for 400 s at a final temperature of 220 °C took 50 s to reach 100 °C and further 70 s to reach the end point of the cooling phase (60 °C). The color of the roasted coffee samples was not measured. After roasting, darker colored beans (recombined beans) were sorted out from the reference beans (lighter color) and counted to control their number against the initial number of provided beans.

Free Sugar Analysis. The free sugar analysis was performed according to Poisson et al.[3]

Aroma Analysis by Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometry (SPME−GC−MS). Roast and ground (R&G) coffee (500 ± 5 mg) was added to a 20 mL headspace vial, and the sample was equilibrated for 10 min at 40 °C. Aroma compounds were then extracted from the headspace by SPME at 40 °C for 10 min [2 cm fiber, 50/30 μm StableFlex, coated with polydimethylsiloxane (PDMS)/divinylbenzene (DVB)/carboxen, Supelco, Buchs, Switzerland] and thermally desorbed into the split−splitless injector (in split mode; split of 2) heated at 240 °C for 10 min. Separation was carried out on a 60 m × 0.25 mm × 0.25 μm polar DB-624 column (Phenomenex, Brechbühler, Switzerland) using an Agilent 7890B gas chromatograph (Agilent, Basel, Switzerland). Helium was used as the carrier gas with a constant flow of 1.2 mL/min. The following oven program was applied: initial temperature of 40 °C was held for 2 min and then raised to 240 °C at 5 °C/min, and the final temperature was held for 10 min. Mass spectrometry was performed on a 7200 accurate mass Q-TOF mass spectrometer (Agilent, Basel, Switzerland). Electron impact ionization was applied, and the mass spectrometer was operated in the full-scan mode (m/z 30−250) at a spectra acquisition rate of 5 spectra/s. Chromatograms were processed using the Agilent MassHunter software. All results were corrected for the ^{13}C content of the natural isotope. The obtained percentage after correction lower than 0.5% was set to 0% by definition. All following discussions are based on the nominal masses.

RESULTS AND DISCUSSION

CAMOLA Experiments with Sucrose. On the basis of the model systems, several pathways involving either the intact sugar skeleton or recombination of sugar fragments have been reported to explain the formation of 2,3-butanedione and 2,3-pentanedione.[7,9−11,17] However, the results obtained from simplified model systems do not always reflect the reality of the complex food system. Therefore, to gain deeper insight into reaction mechanisms responsible for the formation of both α-diketones from sucrose upon coffee roasting, the biomimetic in-bean experiments combined with labeling experiments were recently performed.[3] Among others, the results showed that the contribution of sucrose to α-diketones changes substantially with the roast degree, and consequently, the contribution of the individual reaction pathways generating α-diketones from sucrose may change as well. To substantiate this hypothesis, the kinetics study was performed in combination with the CAMOLA technique.[7] The biomimetic recombinate containing a mix of unlabeled and fully carbon-labeled sucrose ([$^{13}C_{12}$]sucrose), instead of sucrose, was prepared and soaked into the exhausted green coffee beans. Because the exhausted beans contained 10% of residual sucrose, the amount of non-

Table 3. Relative Distribution (%) of 2,3-Butanedione Isotopologues Generated in the CAMOLA Experiment with [$^{13}C_{12}$]Sucrose at Different Roasting Times

	ion (m/z)				
roasting time (s)	$[M]^+$ (86)	$[M+1]^+$ (87)	$[M+2]^+$ (88)	$[M+3]^+$ (89)	$[M+4]^+$ (90)
50	89.2	1.0	1.2	2.0	6.6
100	71.0	3.3	2.7	5.6	17.4
150	56.4	7.0	5.7	9.7	21.2
210	60.6	6.3	7.4	9.0	16.7
260	72.5	4.1	6.0	6.4	11.0
300	78.3	3.2	5.1	5.1	8.3
330	84.8	1.3	4.2	4.2	5.5
360	91.1	1.1	2.6	2.2	3.0
400	93.0	1.0	2.0	1.8	2.2

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Table 4. Interpretation of the 2,3-Butanedione Labeling Pattern Obtained at 210 s

		relative distribution of isotopologues (%)					
			calculated contribution of individual pathways				
ion	m/z	measured[a]	C_1/C_3[b]	C_2/C_2[c]	$C_1(o)/C_3$[d]	intact skeleton[e]	other sources[f]
$[M]^+$	86	60.6	6.3	3.7	2.7	6.7	41.2
$[M + 1]^+$	87	6.3	6.3				
$[M + 2]^+$	88	7.4		7.4			
$[M + 3]^+$	89	9	6.3		2.7		
$[M + 4]^+$	90	16.7	6.3	3.7		6.7	
total			25.2	14.8	5.4	13.4	41.2

[a]Isotopic patterns determined from the cluster of the molecular ions of 2,3-butanedione. [b]C_1/C_3 = recombination of C_3 and C_1 sucrose fragments. [c]C_2/C_2 = recombination of two C_2 sucrose fragments. [d]$C_1(o)/C_3$ = recombination of C_3 and sucrose fragment with C_1 fragment origination from other precursors. [e]Formation from the intact C_4 sugar backbone. [f]Formation from other precursors present in coffee bean than sucrose.

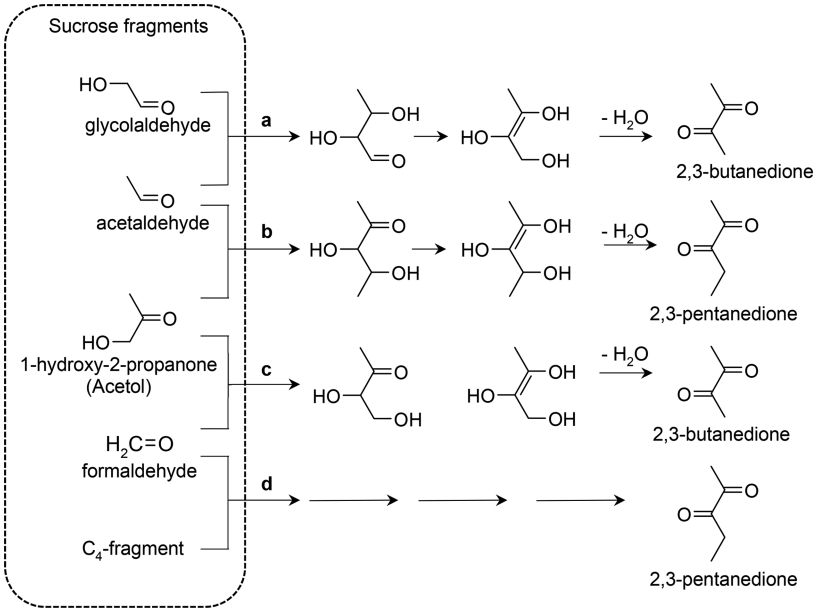

Figure 1. Schematic presentation of different pathways generating α-diketones by recombination of sucrose fragments: (a) recombination of glycolaldehyde and acetaldehyde as proposed by Schieberle et al.,[7] (b) recombination of 1-hydroxy-2-propanone and acetaldehyde as proposed by Hofmann,[11] (c) recombination of 1-hydroxy-2-propanone and formaldehyde as proposed by Schieberle et al.,[7] and (d) recombination of C_4 fragment and formaldehyde as proposed by Poisson et al.[3]

labeled sucrose in the CAMOLA mix was adjusted in such a way to obtain an exact 1:1 mix of non-labeled and labeled sucrose in a recombined bean. To perform a kinetic study, a larger batch of recombined green coffee beans (50 g) was prepared, divided in 10 portions, and roasted for different periods of time varying between 0 and 400 s.

Formation of 2,3-Butanedione. The relative distribution of the isotopologues obtained at different roasting times is summarized in Table 3. Irrespective of the roasting time, the major isotopologue formed was unlabeled isotopologue ($[M]^+$; m/z 86, 56.4−93%), followed by the fully labeled isotopologue ($[M + 4]^+$; m/z 90, 2.2−21.2%), Smaller amounts of singly (1.0−7.0%), doubly (1.2−7.4%), and triply (1.8−9.7%) labeled isotopologues were detected.

The isotopologue distribution served to evaluate the relative contribution of different reaction pathways. The interpretation of the pattern was discussed in detail in a former publication[3] and is briefly explained on the relative isotopologue distribution obtained at 210 s, as illustrated in Table 4.

The singly labeled isotopologue (m/z 87) can be explained by the recombination of a labeled C_1 fragment with a non-labeled C_3 fragment. Such C_1/C_3 recombination must also generate the triply labeled (m/z 89), fully labeled (m/z 90), and unlabeled isotopologues (m/z 86), each one at the same level (i.e., at 6.3%). In total, the recombination of C_1 with C_3 sucrose fragments contributes to 25.2% of formed 2,3-butanedione (pathway c in Figure 1).

The recombination of two C_2 sugar fragments represents another formation pathway of 2,3-butanedione as reported in model systems.[7] The doubly labeled isotopologue (m/z 88) was formed at 7.4%, and consequently per statistical rule, this isotopologue has to be formed in double amount compared to the unlabeled (m/z 86) and fully labeled (m/z 90; 3.7% each) isotopologues, summing up to a total contribution of the C_2/C_2 recombination to 14.8% (7.4% + 2 × 3.7%) of the total 2,3-butanedione formed (pathway a in Figure 1). This confirms our previous finding that the recombination of acetaldehyde with hydroxyacetaldehyde by aldol condensation[7,18] also occurs under coffee roasting conditions.[3]

Actually, the formation of 2,3-butanedione by recombination of C_1/C_3 and C_2/C_2 sucrose fragments may be a bit lower than shown in Table 3 because the singly labeled isotopologue

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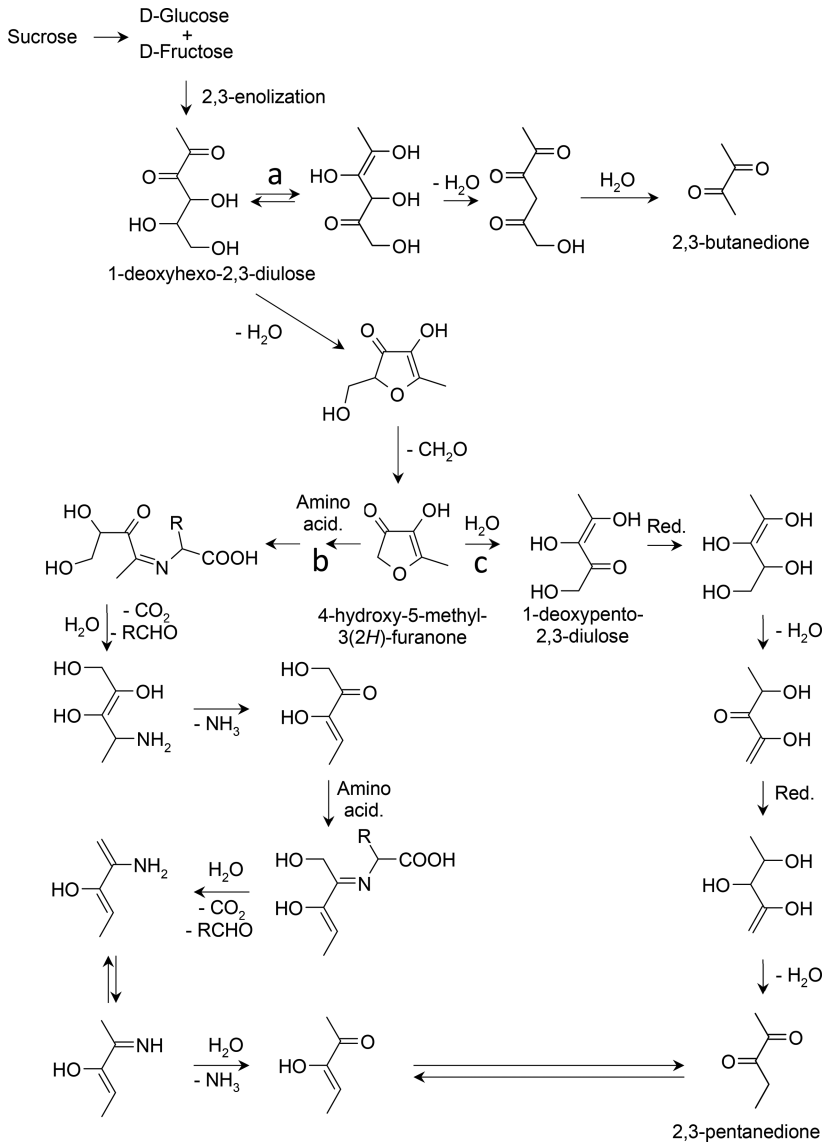

Figure 2. Generation of α-diketones from the intact sucrose skeleton: (a) generation of 2,3-butanedione from 1-deoxyhexo-2,3-diulose via dehydration, followed by β-dicarbonyl cleavage. Hypothetical formation pathways of 2,3-pentanedione from 4-hydroxy-5-methyl-3(2*H*)-furanone: (b) via reduction and acid-catalyzed dehydration of 1-deoxypento-2,3-diulose as proposed by Whitfield and Mottram[20] or (c) via successive Strecker degradations as proposed by Cerny and Davidek.[21]

could potentially also be formed by recombination of the labeled C_1 fragment of sucrose with either the non-labeled C_3 fragment from the coffee matrix or with two non-labeled fragments (C_2 and C_1) from sucrose or the coffee matrix. The latter recombination has been described in the glucose/glycine model system under pyrolytic conditions.[9] Similarly, doubly labeled isotopologue, apart from being formed by recombination of non-labeled and labeled C_2 fragments, could also be formed by $C_1/C_1/C_2$ recombination. However, the obtained data do not allow for more detailed calculations without speculation.

Not all of the triply labeled isotopologue (m/z 89) ratio can be explained by the recombination of C_1 and C_3 sucrose fragments. Hence, the remaining portion (9.0% − 6.3% = 2.7%) should stem mainly from a C_3-labeled fragment of sucrose and an unlabeled C_1 fragment, originating from another precursor in the green coffee matrix. The unlabeled isotopologue is equally formed at 2.7%, and therefore, this pathway contributes to 5.4% of the total 2,3-butanedione formed.

As discussed before, C_1/C_3 and C_2/C_2 sucrose fragment recombinations form the fully labeled isotopologue (m/z 90). However, both combinations together only make up for 10%; thus, the remaining portion of 6.7% must stem from the intact skeleton of labeled sucrose, e.g., from 1-deoxyhexo-2,3-diulose, via dehydration, followed by β-dicarbonyl cleavage (pathway a in Figure 2). Because this pathway forms only two isotopologues (unlabeled and fully labeled) at the same level, it contributes to 13.4% of the total 2,3-butanedione formed.

With a ratio of 60.6%, the unlabeled isotopologue represented the biggest part. However, the formation pathways involving sucrose only explain 19.4% of the unlabeled isotopologue. Thus, the remaining 41.2% had to be formed from other green coffee constituents than sucrose, most likely non-water-soluble polysaccharides. As discussed previously,[3] it seems that they are much more relevant in the formation of 2,3-butanedione than expected.

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Table 5. Relative Contribution (%) of Individual Pathways Generating 2,3-Butanedione Calculated from the Isotopologue Distribution of the CAMOLA Experiment at Different Roasting Times

	relative contribution of individual pathways (%)				
roasting time (s)	C_1/C_3[a]	C_2/C_2[b]	$C_1(o)/C_3$[c]	intact skeleton[d]	other sources[e]
50	4.0	2.4	2.0	10.0	81.6
100	13.2	5.5	4.6	25.4	51.3
150	28.0	11.5	5.4	22.6	32.5
210	25.2	14.8	5.4	13.4	41.2
260	16.4	12.0	4.6	7.8	59.2
300	12.8	10.3	3.8	5.0	68.1
330	5.2	8.4	5.8	4.2	76.4
360	4.4	5.2	2.2	1.2	87.0
400	4.0	4.0	1.6	0.4	90.0

[a]C_1/C_3 = recombination of C_3 and C_1 sucrose fragments. [b]C_2/C_2 = recombination of two C_2 sucrose fragments. [c]$C_1(o)/C_3$ = recombination of C_3 and sucrose fragment with C_1 fragment origination from other precursors. [d]Formation from the intact C_4 sugar backbone. [e]Formation from other precursors present in coffee bean than sucrose.

The labeling patterns obtained at other roasting times were interpreted analogously, as shown for 210 s, and the relative contribution of the individual pathways obtained are presented in Table 5. The contribution of sucrose in the generation of 2,3-butanedione at different roasting times is shown in Figure 3. In line with our previous study,[3] the results point out that the importance of sucrose in the generation of 2,3-butanedione depends strongly upon the roast degree. The maximum contribution (67.5%) was obtained at 150 s, demonstrating that sucrose is an important precursor at early roasting stages; however, its importance progressively drops in favor of other precursors present in the coffee matrix as the roasting advances. At the end of the roasting cycle, the contribution of sucrose to 2,3-butanedione was only 10%.

The data further demonstrate that not only did the contribution of sucrose to 2,3-butanedione change with the roast degree but the contributions of the individual reaction pathways generating 2,3-butanedione from sucrose change as well. The relative contributions of the individual pathways are shown in Figure 3. At very early roasting stages (until 100 s), more than half of 2,3-butanedione was formed from the intact sucrose skeleton. As the roast cycle advanced, the share of 2,3-butanedione formed from the intact sucrose skeleton rapidly decreased in favor of other pathways, and at the end of the roast cycle, the formation from the intact sucrose skeleton was negligible (4%). This must be due to the increased fragmentation of the sucrose molecule at high temperatures, which leads to the higher recombination rates of the fragments. The recombination of C_1/C_3 sucrose fragments was the major pathway between 150 and 300 s and contributed to about 40% of 2,3-butanedione. Afterward, the formation of 2,3-butanedione by recombination of C_2/C_2 sucrose fragments becomes more or equally important. Interestingly, the importance of this pathway increased progressively throughout the roast cycle.

Formation of 2,3-Pentanedione. The relative distribution of 2,3-pentanedione isotopologues obtained at different roasting times is presented in Table 6. Irrespective of the roasting time and similar to 2,3-butanedione, the major isotopologue formed was unlabeled isotopologue ($[M]^+$; m/z 100, 49.4−77.7%), followed by fully labeled isotopologue ($[M + 5]^+$; m/z 105, 15.3−33.8%). Nevertheless, the relative intensity of the fully labeled isotopologue of 2,3-pentanedione was higher throughout the whole roast cycle compared to 2,3-butanedione.

The interpretation of the labeling pattern permitting calculation of the relative contribution of the individual reaction pathways generating 2,3-pentanedione at 210 s is illustrated in Table 7.

Upon the coffee roasting, 2,3-pentanedione has been shown to be formed via six different pathways: the recombination of C_1/C_4 sucrose fragments, the recombination of C_2/C_3 sucrose fragments, the recombination of C_1 fragment originating from other green coffee precursors with the C_4 sucrose fragment, the recombination of C_2 fragment originating from other green coffee precursors with the C_3 sucrose fragment, the formation from the intact sucrose skeleton, and finally the formation from other green coffee precursors.[3] In a CAMOLA experiment, the first pathway generates four isotopologues ($[M]^+$, $[M + 1]^+$, $[M + 4]^+$, and $[M + 5]^+$) in equimolar ratio. Consequently, 5.2% of 2,3-pentanedione was formed via the recombination of C_1/C_4 sucrose fragments because singly labeled isotopologue

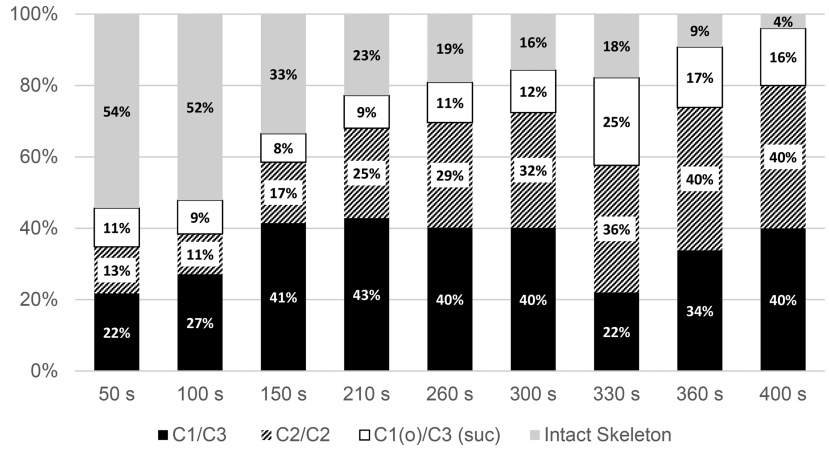

Figure 3. Relative contribution of individual pathways generating 2,3-butanedione from sucrose as a function of the roasting time: C_1/C_3, recombination of C_1 and C_3 sucrose fragments; C_2/C_2, recombination of two C_2 sucrose fragments; $C_1(o)/C_3$, recombination of C_3 and sucrose fragment with C_1 fragment originating from other precursors; and formation from the intact C_4 sugar backbone.

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Table 6. Relative Distribution (%) of 2,3-Pentanedione Isotopologues Generated in the CAMOLA Experiment with [$^{13}C_{12}$]Sucrose at Different Roasting Times

	ion (m/z)					
roasting time (s)	$[M]^+$ (100)	$[M + 1]^+$ (101)	$[M + 2]^+$ (102)	$[M + 3]^+$ (103)	$[M + 4]^+$ (104)	$[M + 5]^+$ (105)
50	77.7	0	1.2	4.8	1.0	15.3
100	60.3	0.6	2.3	9.3	1.7	25.6
150	50.7	1.0	3.3	8.4	2.8	33.8
210	49.4	1.3	4.6	7.2	3.0	34.5
260	54.8	1.2	4.7	7.0	2.7	29.6
300	59.3	1.1	4.4	6.8	2.4	26.0
330	62.0	1.1	4.7	7.2	2.1	22.9
360	69.1	0.9	4.4	5.9	1.7	18.0
400	71.5	1.0	4.2	5.4	1.5	16.4

Table 7. Interpretation of the 2,3-Pentanedione Labeling Pattern Obtained at 210 s

		relative distribution of isotopologues (%)						
				calculated contribution of individual pathways				
ion	m/z	measured[a]	C_1/C_4[b]	$C_1(o)/C_4$[c]	C_2/C_3[d]	$C_2(o)/C_3$[e]	intact skeleton[f]	other sources[g]
$[M]^+$	100	49.4	1.3	1.7	4.6	2.6	28.6	10.6
$[M + 1]^+$	101	1.3	1.3					
$[M + 2]^+$	102	4.6			4.6			
$[M + 3]^+$	103	7.2			4.6	2.6		
$[M + 4]^+$	104	3	1.3	1.7				
$[M + 5]^+$	105	34.5	1.3		4.6		28.6	
total			5.2	3.4	18.4	5.2	57.2	10.6

[a]Isotopic patterns determined from the cluster of the molecular ions of 2,3-pentanedione. [b]C_1/C_4 = recombination of C_4 and C_1 sucrose fragments. [c]$C_1(o)/C_4$ = recombination of the C_4 sucrose fragment with C_1 fragment origination from other precursors. [d]C_2/C_3 = recombination of C_2 and C_3 sucrose fragments. [e]$C_2(o)/C_3$ = recombination of the C_3 sucrose fragment with C_2 fragment origination from other precursors. [f]Formation from the intact C_4 sugar backbone. [g]Formation from other precursors present in coffee bean than sucrose.

Table 8. Relative Contribution (%) of Individual Pathways Generating 2,3-Pentanedione Calculated from the Isotopologue Distribution of the CAMOLA Experiment at Different Roasting Times

		relative contribution of individual pathways (%)				
roasting time (s)	C_1/C_4[a]	$C_1(o)/C_4$[b]	C_2/C_3[c]	$C_2(o)/C_3$[d]	intact skeleton[e]	other sources[f]
50	0	2	4.8	7.2	28.2	57.8
100	2.4	2.2	9.2	14.0	45.4	26.8
150	4.0	3.6	13.2	10.2	59.0	10.0
210	5.2	3.4	18.4	5.2	57.2	10.6
260	4.8	3.0	18.8	4.6	47.4	21.4
300	4.4	2.6	17.6	4.8	41.0	29.6
330	4.4	2.0	18.8	5.0	34.2	35.6
360	3.6	1.6	17.6	3.0	25.4	48.8
400	4.0	1.0	16.8	2.4	22.4	53.4

[a]C_1/C_4 = recombination of C_4 and C_1 sucrose fragments. [b]$C_1(o)/C_4$ = recombination of the C_4 sucrose fragment with C_1 fragment origination from other precursors. [c]C_2/C_3 = recombination of C_2 and C_3 sucrose fragments. [d]$C_2(o)/C_3$ = recombination of the C_3 sucrose fragment with C_2 fragment origination from other precursors. [e]Formation from the intact C_4 sugar backbone. [f]Formation from other precursors present in coffee bean than sucrose.

($[M + 1]^+$, m/z 101) was formed at 1.3%. The second pathway, the recombination of C_2/C_3 sucrose fragments, such as acetaldehyde and 1-hydroxy-2-propanone[11] (pathway b in Figure 1), must also generate four isotopologues ($[M]^+$, $[M + 2]^+$, $[M + 3]^+$, and $[M + 5]^+$) in equimolar ratio. The presence of doubly labeled isotopologue ($[M + 2]^+$, m/z 102) at 4.6% thus indicates that this pathway contributed to 18.4% of 2,3-pentanedione. In reality, the contribution of the first two pathways (recombination of C_1/C_4 and C_2/C_3 sucrose fragments) could be a bit lower because the singly labeled isotopologue could potentially also be formed by recombination of the labeled C_1 sucrose fragment with the unlabeled C_4 fragment from the coffee matrix, while the doubly labeled isotopologue could be formed by recombination of the doubly labeled C_2 sucrose fragment with the unlabeled C_3 fragment from the coffee matrix. However, similar to 2,3-butanedione, the obtained data do not allow for more detailed calculation without speculation. Next, two pathways that generate 2,3-pentanedione via recombination of the sucrose fragment (C_1 or C_2) with the fragment originating from other green coffee precursors (C_4 or C_3) can generate only two isotopologues in equimolar ratio. Because the remaining part of the quadruple labeled isotopologue ($[M + 4]^+$, m/z 104) after subtraction of the contribution of the pathway involving C_1/C_4 sucrose

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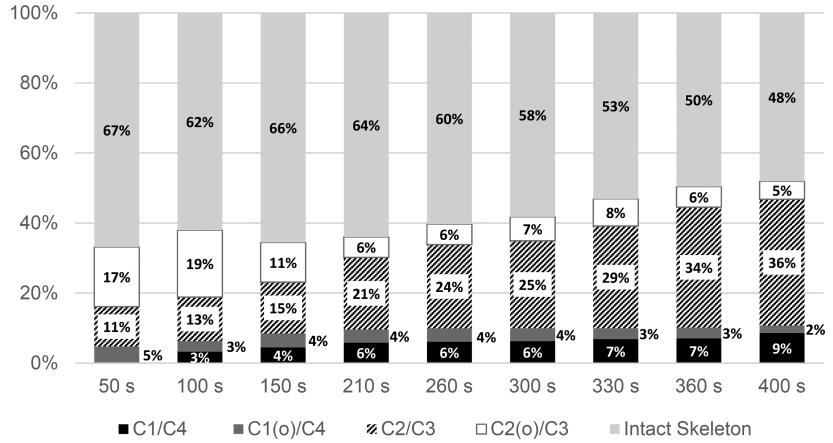

Figure 4. Relative contribution of individual pathways generating 2,3-pentanedione from sucrose as a function of the roasting time: C_1/C_4, recombination of C_1 and C_4 sucrose fragments; $C_1(o)/C_4$, recombination of the C_4 sucrose fragment with C_1 fragment originating from other precursors; C_2/C_3, recombination of C_2 and C_3 sucrose fragments; $C_2(o)/C_3$, recombination of the C_3 sucrose fragment with C_2 fragments originating from other precursors; and formation from the intact C_4 sugar backbone.

Table 9. Relative Distribution (%) of 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone Isotopologues Generated in the CAMOLA Experiment with [$^{13}C_{12}$]Sucrose at Different Roasting Times

	ion (m/z)						
roasting time (s)	$[M]^+$ (128)	$[M + 1]^+$ (129)	$[M + 2]^+$ (130)	$[M + 3]^+$ (131)	$[M + 4]^+$ (132)	$[M + 5]^+$ (133)	$[M + 6]^+$ (134)
50	96.6						3.4
100	69						31
150	57.3						42.7
210	55.5			3.5			41.0
260	56			5			39
300	66.6			3.6			29.8
330	82.2						17.8
360	85.1						14.9
400	89.9						10.1

Table 10. Relative Contribution (%) of Individual Pathways Generating 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone Calculated from Isotopologue Distribution of the CAMOLA Experiment at Different Roasting Times

	relative contribution of individual pathways (%)		
roasting time (s)	C_3/C_3[a]	intact skeleton[b]	other sources[c]
50		7.0	93.0
100		61.8	38.2
150		85.4	14.6
210	7.0	78.5	14.5
260	10.6	72.9	16.5
300	7.2	56.0	36.8
330		35.6	64.4
360		29.8	70.2
400		20.2	79.8

[a]C_3/C_3 = recombination of two C_3 sucrose fragments. [b]Formation from the intact C_6 sugar backbone. [c]Formation from other precursors present in coffee bean than sucrose.

fragments corresponds to 1.7% (3% − 1.3%), the recombination of the C_1 fragment originating from other green coffee precursors with the C_4 sucrose fragment contributes to 3.4% of 2,3-pentanedione. Similarly, the remaining part of the triply labeled isotopologue ($[M + 3]^+$, m/z 103) after subtraction of the contribution of the pathway involving C_2/C_3 sucrose fragments corresponds to 2.6% (7.2% − 4.6%), and consequently, the recombination of the C_2 fragment originating from other green coffee precursors with the C_3 sucrose fragment contributes to 5.2% of 2,3-pentanedione. Such an unlabeled C_2 fragment could potentially stem from alanine because, under pyrolytic conditions, the majority (90%) of 2,3-pentanedione formed in the glucose/alanine model system and 100% of 2,3-pentanedione formed in glyceraldehyde/alanine or methylglyoxal/alanine model systems incorporated C-2 and C-3 carbon of alanine.[9] The residual part (28.2%) of fully labeled isotopologue ($[M + 5]^+$, m/z 105), which does not stem from recombination of sucrose fragments (C_1/C_4 or C_2/C_3), must be formed from the intact sucrose skeleton (pathways b and c in Figure 2). Thus, this pathway generated 57.2% of 2,3-pentanedione. The rest of the unlabeled isotopologue (10.6%), which does not stem from any above-mentioned pathways, must originate from other green coffee precursors present in the bean.

The relative contribution of the individual pathways at different roasting times was calculated analogously and shown in Table 8. Contrary to 2,3-butanedione, sucrose was an important precursor of 2,3-pentanedione not only at the early roasting stages but during the whole roasting cycle. Maximum contribution was reached between 150 and 210 s, where about 90% of 2,3-pentanedione originated from sucrose. However, even at the end of the roast cycle, sucrose still contributed to almost half of 2,3-pentanedione present.

The data indicate that the generation of 2,3-pentanedione from sucrose is much less dependent upon the roast cycle compared to 2,3-butanedione, but interestingly, also, the

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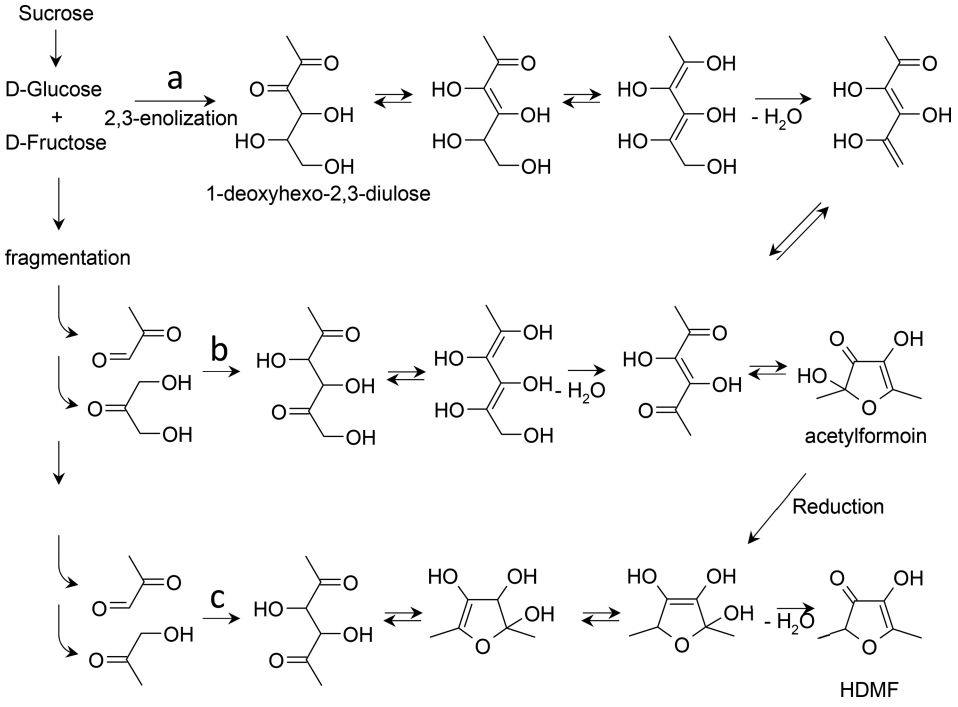

Figure 5. Generation of HDMF: (a) generation from the intact sucrose skeleton via reduction and dehydration of acetylformoin as proposed by Schieberle,[8] (b) recombination of dihydroxyacetone and 2-oxopropanal as proposed by Hofmann and Schieberle,[18] and (c) recombination of 1-hydroxy-2-propanone and 2-oxopropanal as proposed by Schieberle.[8]

contribution of the individual reaction pathways generating 2,3-pentanedione from sucrose depends much less upon the roast cycle. The relative contribution of the individual pathways is shown in Figure 4. Irrespective of the roasting time, the formation of 2,3-pentanedione via the intact sucrose skeleton was the major pathway (66.8−48.1%), despite a slight decrease of the importance in favor of fragmentation pathways toward the end of the roasting cycle. Interestingly, the contribution of the pathway involving recombination of the C_2/C_3 sucrose fragments steadily increased during the roasting cycle from about 11% to about 36%. Possibly one of the reaction fragments requires a higher temperature to be formed in sufficient levels from sucrose. When considering acetaldehyde and 1-hydroxy-2-propanone as proposed by Hofmann,[11] it is most likely acetaldehyde that requires higher temperatures because 1-hydroxy-2-propanone is easily formed, e.g., by retroaldolization of deoxyosones already under mild conditions. This hypothesis is further supported by the relatively high portion of 2,3-pentanedione (up to 19%) formed by recombination of the C_2 fragment originating from other green coffee precursors (e.g., acetaldehyde from free alanine) with the C_3 sucrose fragment (e.g., 1-hydroxy-2-propanone). In addition, a similar behavior was also observed for the formation of 2,3-butanedione via the pathway involving recombination of C_2/C_2 sucrose fragments (Table 5). The importance of this pathway also continuously increased during the whole roasting cycle, and acetaldehyde together with glycolaldehyde are considered as the reacting fragments (pathway a in Figure 1).

Formation of 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF). The relative distribution of HDMF isotopologues obtained at different roasting times is presented in Table 9. Irrespective of the roasting time and similar to α-diketones, the major isotopologue formed was unlabeled isotopologue ($[M]^+$; m/z 128, 55.5−96.6%), followed by fully labeled isotopologue ($[M + 6]^+$; m/z 134, 3.4−42.7%). Contrary to α-diketones, which formed all isotopologues, HDMF formed only unlabeled and fully labeled isotopologues, apart from the roasting time between 210 and 300 s, where small amounts of triply labeled isotopologue ($[M + 3]^+$; m/z 131, 3.5−5%) were formed as well.

The relative contribution of the individual pathways at different roasting times were calculated analogously as for α-diketones and are shown in Table 10. The results indicate that, similar to 2,3-butanedione, the importance of sucrose in the generation of HDMF depends strongly upon the roast degree. Sucrose was a major precursor of HDMF between 100 and 300 s, with a maximum contribution (83.5−85.5%) between 150 and 260 s. This demonstrates that sucrose is a key precursor at the early and middle roasting stages. After 300 s, its importance progressively drops in favor of other green coffee bean precursors. At the end of the roasting cycle, the contribution of sucrose to HDMF was only about 20%. On the other hand, the contribution of the individual reaction pathways generating HDMF from sucrose was almost independent of the roast cycle. HDMF was formed nearly exclusively from the intact sucrose skeleton. Only in the middle of the roasting cycle (between 150 and 300 s), small amounts of HDMF (8.2−12.7%, considering sucrose contribution only) were formed by recombination of two C_3 sucrose fragments. These data are in line with those obtained by Schieberle,[8] who reported exclusive generation of HDMF via the intact sugar skeleton when reacting with proline and glucose under roasting conditions as well as with our previously reported data[17] concerning the proline and glucose model system. Formation from the intact skeleton or by recombination of two C_3 sucrose fragments is schematically shown in Figure 5. Acetylformoin has been proposed as a key intermediate forming HDMF after its reduction and dehydration. Acetylformoin can be formed via either the intact sugar skeleton (pathway a) or recombination

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of dihydroxyacetone and 2-oxopropanal (pathway b) as proposed by Hofmann and Schieberle.[19] Alternatively, HDMF could also be formed via recombination of 1-hydroxy-2-propanone and 2-oxopropanal as proposed by Schieberle (pathway c).[8]

In summary, the results highlighted that not only did the contribution of sucrose to the level of α-diketones in roasted coffee change with the roasting degree but also the portion of the individual reaction pathways. Whereas sucrose is an important precursor of 2,3-butanedione at early roasting stages, with the main formation pathway from the intact sugar skeleton, non-water extractable sources (i.e., polysaccharides) take over the role of the main precursor from the middle of the roasting course and the formation foremost occurs from sugar fragments, primarily by C_1/C_3 and C_2/C_2 recombinations. In contrast, 2,3-pentanedione is generated from sucrose mainly via the intact sugar skeleton during the whole roasting cycle. Nevertheless, the share of 2,3-pentanedione formed by recombination of sucrose fragments (mainly C_2/C_3) progressively increases with the roasting time, while the share of 2,3-pentanedione formed from sucrose gradually decreases in favor of non-water extractable precursors. Similar to diketones, the importance of sucrose in the generation of HDMF depends strongly upon the roast degree. Sucrose is the major precursor of HDMF at the early and middle roasting stages, and its importance progressively drops in favor of other green coffee bean precursors toward the end of the roasting cycle. On the other hand, the contribution of the individual reaction pathways generating HDMF from sucrose was almost independent of the roast cycle, and HDMF was generated almost exclusively via cyclization of the intact skeleton, irrespective of the roast time.

The combination of the kinetics study with CAMOLA experiments in a real food matrix was shown as a very powerful tool to elucidate the origin of odorants upon food processing. To the best of our knowledge, this is the first time such an experimental setup was employed to study the generation of coffee odorants upon roasting and, in general, to study the generation of key odorants upon food processing. The newly obtained data will enable modulation of the coffee aroma through better control of the formation of key odorants.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

EB, exhausted beans; BRE, biomimetically recombined extract; RB, exhausted beans reconstituted with the biomimetically recombined extract; CAMOLA, carbon module labeling

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