JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Probing Free Radical Processes during Storage of Extracts from Whole Roasted Coffee Beans: Impact of O_2 Exposure during Extraction and Storage

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Supporting Information

ABSTRACT: Development of liquid coffee products of good quality with extended shelf lives is hampered by their rapid quality degradation as a result of both physical and flavor instability. One approach that is being considered for extending the shelf lives of liquid coffees is that of supplementing the beverage with a very mild and slow continuous extraction from intact roasted beans that are held within an aluminum can. This paper reports the use of electron paramagnetic resonance (EPR) spectroscopy to examine the effects of key parameters that affect the stability of liquid coffee prepared from aqueous extracts from whole roasted coffee beans, namely, the O_2 content of the water and headspace during extraction and the temperature during storage. It was found that the magnitude of the free radical signal was sensitive to the O_2 content of the water used for extraction and storage time and temperature, whereas the intensity of the Fe(III) ($g = 4.3$) signal was affected only by the O_2 content of the water and the Mn signal was insensitive to the experimental parameters. The most critical factor was the O_2 content of the water used for extraction, and careful control of O_2 exposure at the extraction stage could be a crucial factor for generating products with resistance to oxidative processes during storage.

KEYWORDS: *coffee whole bean extract, EPR spectroscopy, O_2, free radical, manganese(II), iron(III)*

INTRODUCTION

Oxidative processes are responsible in a major way for the flavor characteristics of coffee, but they also contribute to the instability of flavor during storage.[1−4] The electron transfer reactions, which are characteristic of oxidative processes, often involve the generation of free radicals, and free radical species and paramagnetic metal ions are believed to be involved in the oxidative degradation of coffee solutions.[5,6] For example, the oxidation of caffeine to 1,3,7-trimethyluric acid (oxocaffeine) proceeds via a free radical intermediate,[7] and the 1,3,7-trimethyluric acid molecule is considered to represent a good chemical marker for the oxidative status of coffee brews.[8]

Many free radicals have very short half-lives at room temperature, especially in the fluid environment, but some of the products of their reaction are "stable" radicals that can be investigated directly by electron paramagnetic resonance (EPR) spectroscopy, a technique that selectively detects free radicals and other paramagnetic species. However, the study of such radical products is complicated by the fact that there is a strong free radical signal in the EPR spectra of roasted and ground (R&G) and soluble coffee[9−13] and in coffee solutions.[14,15] These free radicals, which are found in the melanoidin fraction of the roasted coffee, are formed by either Maillard or caramelization reactons during roasting and have been studied extensively by EPR spectroscopy.[16−19] However, changes in the free radical contents of coffee are observed during storage,[12,20,21] and Pascual et al.[15] established that radical formation and decay occur in aerobic soluble coffee solutions.

Research to produce high-quality and stable liquid coffee products is motivated by numerous business benefits, the most cited being (i) convenient dosing and adaptation to vending machines, (ii) short brewing time (fast preparation), (iii) high consistency, and (iv) easily adjustable product strength. Various approaches have been utilized to attempt to increase the stability of liquid coffees,[22,23] but these products appear to be intrinsically unstable, and attempts to control degradation are often frustrated by the complexity of the chemical mechanisms that are responsible for it. This is likely because of the fundamental role of the hydroxyl radical in initiating the various degradation reactions, because coffee solutions contain the components required for Fenton reaction chemistry.[24]

Hydroxyl radicals react more or less indiscriminately, and in a complex matrix they can be responsible for multiple reactions as reported by Blank et al.[25] for the degradation of the coffee aroma compound furfuryl mercaptan; such reactions are still poorly understood at the molecular level. However, these hydroxyl radical mediated reactions could be responsible for decreasing the concentration of specific aroma impact

Received: July 9, 2012
Revised: March 6, 2013
Accepted: March 9, 2013
Published: March 9, 2013

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molecules,[3] revealing intrinsic off-flavors or inducing physical or chemical changes in nonvolatile molecules in the coffee matrix. Thus, the changes in stable free radical contents and various sensory molecules in coffee probably have a common origin.[3]

One approach that is being considered for extending the shelf lives of liquid coffees is supplementing the beverage with a very slow continuous extraction from intact whole roasted beans that are held within the container. The objective is to selectively extract small, flavor active compounds while leaving behind many of the larger, less soluble macromolecules, which are thought to contribute to the instability of the coffee extract.[26] We report here investigations of the influence of different amounts of O_2 at the extraction stage on the intensities of the free radical and paramagnetic metal ion EPR signals during anaerobic storage conditions at elevated temperatures. These measurements thus provide information on the stability of the liquid coffee preparations and also provide further insight into the use of EPR spectroscopy as a probe to monitor free radical processes and the stability of liquid coffee.

MATERIALS AND METHODS

Samples. The coffee beans used in these measurements were a blend of 30% Colombian, 30% Brazilian, and 40% Guatemalan, and these were roasted to 84 Color Test Neuhaus medium-roast degree.

Coffee Solution Extraction and Storage. A series of coffee solutions, in which different O_2 contents were present during extraction of the whole roasted coffee beans, were prepared at the Nestlé Research Center as described in Table 1. Beans were filled into

Table 1. Extraction Conditions for Samples from Whole Bean Extraction

sample	O_2 content of water used during extraction (ppm)	headspace composition	
		extraction	storage
O_2 free	~0.01	N_2	N_2
2 ppm	2	N_2	N_2
4 ppm	4	N_2	N_2
air	8	air	N_2

330 mL aluminum cans, and water of various O_2 contents was added for extraction of the beans. The cans were filled to the top, so as to leave only minimal headspace, and then sealed with a manual can seamer, leaving the beans inside during subsequent retorting and storage. All of these operations were done inside a glovebox under N_2. These samples were then retorted at 121 °C for 6 min and stored for periods of up to 8 weeks at either 30 or 50 °C (Table 2). EPR measurements were made at both room temperature and 77 K without any further treatment of the samples, except that methanol was added for the low-temperature measurements (to give a 80:20 water/methanol ratio). For quantitative comparisons, identical volumes of samples were used in either a quartz flat cell or a 4 mm o.d. quartz tube for measurements at room temperature and 77 K, respectively. Spectra were recorded from three replicates of each sample, and the results are presented as the mean of these measurements.

EPR Spectroscopic Measurements. EPR measurements were made at room temperature or 77 K at X-band frequencies (~9.5 GHz) using a Bruker ESP300E computer-controlled spectrometer incorporating an ER4103TM cylindrical microwave cavity; low-temperature measurements were made by immersing the sample in liquid nitrogen in a quartz "finger dewar", which was inserted into the microwave cavity. The reasons for making measurements at low temperature as well as room temperature were (a) to improve the free radical signal-to-noise ratios by avoiding microwave absorption by liquid water and

Table 2. Storage Conditions for Samples from Whole Bean Extraction

storage time (weeks)	treatment[a]	storage temp (°C)	sample code
0	none		0N
	retorted		0R
2	retorted	30	2R30
	retorted	50	2R50
6	retorted	30	6R30
	retorted	50	6R50
8	retorted	30	8R30
	retorted	50	8R50

[a]Retorted samples were retorted for 6 minutes at 121 °C after extraction.

(b) to observe the Fe(III) signal, the relaxation properties for which are such that low temperatures are required for it to be observed.

All spectra were collected in 1024 data points using a modulation frequency of 100 kHz. Sweep widths of 5, 200, and 500 mT were used to optimize the spectra of the free radical, Mn(II), and Fe(III) components, respectively. Microwave powers of 1, 0.016, 5, and 10 mW were used for measurement of the signal from the free radical at room temperature, free radical at 77 K, Mn(II) at room temperature, and Fe(III) at 77 K, respectively. The modulation amplitude for Mn(II) and Fe(III) measurements was set to 1 mT (10 G = 1 mT), which for free radical measurements was 0.5 mT. Other parameters such as receiver gain were adjusted as necessary to obtain good signal-to-noise ratios. As is conventional in EPR spectroscopy, all spectra were recorded as first derivatives of the microwave absorption and displayed as functions of absorption versus magnetic field at a constant microwave frequency.

Analysis of EPR Data. Because all samples contained the same sets of signals, the peak-to-peak distances between inflection points of the first-derivative spectra were used as measures of EPR absorption intensity; in the absence of saturation, the heights of individual components are proportional to their concentrations.[27] This approach was preferred to the double-integration method, because of difficulties in separating the individual contributions from overlapping components with greatly different linewidths. However, it must also be appreciated that the relative intensities of different types of EPR signal can be compared only after double integration of the spectra, and the results presented in this paper thus represent trends in spectral intensities and not absolute concentrations. To minimize the contribution of instrumental noise to the intensity measurements, all spectra were smoothed by applying a second-order polynomial function after cubic background correction. Finally, because there was little if any variation in the Mn signal between samples, the results for the free radical and Fe(III) signals are expressed as their ratio to the Mn signal in each sample.

RESULTS AND DISCUSSION

Compared to the corresponding Fe and Mn signals, the free radical signal was much weaker than that observed previously with solutions of soluble coffee,[15] and the solutions were also much paler in color. Thus, this result suggests relatively low levels of melanoidins in these extracts. Variations in the free radical spectral intensity with time and storage conditions are shown in Figure 1. There was little difference between the free radical signals from the freshly extracted samples, although that in the sample extracted with aerated water was somewhat lower than the others. The effects of retorting were also small, although, with the exception of the sample extracted with 2 ppm O_2, there was a small increase in the free radical signal

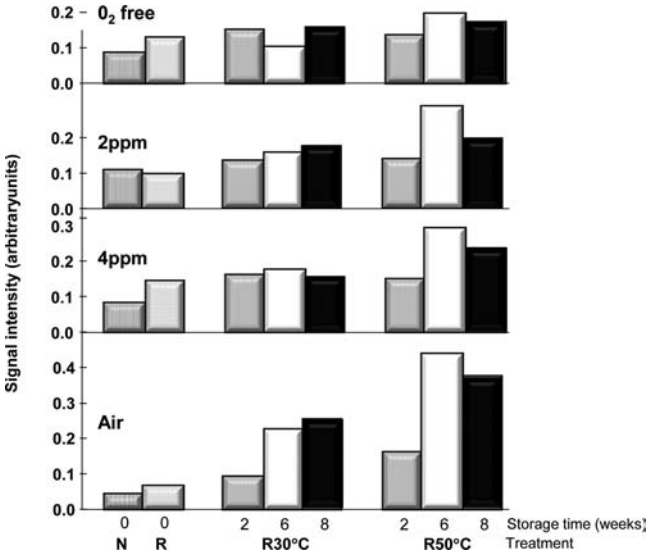

Figure 1. Variation with time and storage conditions of the intensity of the free radical signal observed at ambient temperature (~21 °C) in aqueous extracts of whole roasted coffee beans prepared as described in Tables 1 and 2.

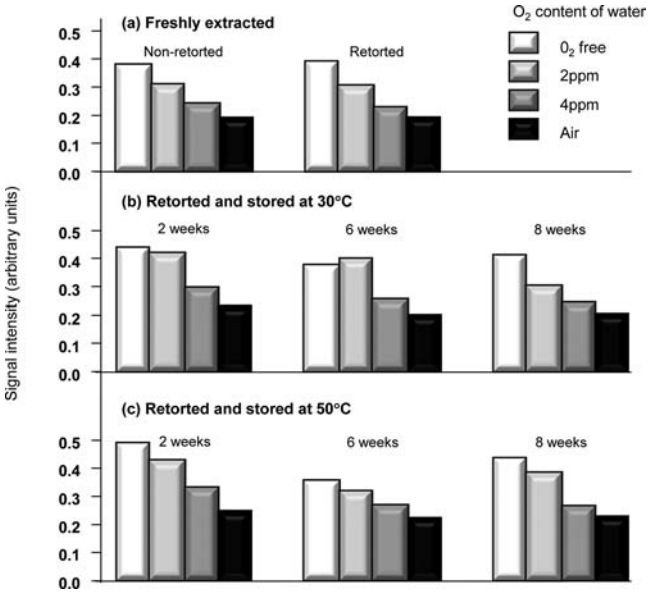

Figure 2. Variation with time and storage conditions of the intensity of the Fe(III) g = 4.3 signal observed at 77 K for aqueous extracts of whole roasted coffee beans prepared as described in Tables 1 and 2.

intensity. For the sample extracted under air, there was a minor increase in intensity of the free radical signal after storage at 50 °C for 2 weeks, but major increases after 6 and 8 weeks; storage at 30 °C showed smaller increases than observed with the equivalent samples stored at 50 °C. Some increases in intensity of the free radical signal were also observed with samples extracted using water with 2 and 4 ppm O_2 when stored at 50 °C, but not at 30 °C. However, the free radical signal from the sample extracted under O_2-free conditions showed only minor changes in intensity over 8 weeks of storage at both temperatures.

The low-temperature EPR spectra showed the presence of free radical, Fe(III), and Mn(II) signals that were qualitatively similar to those seen with measurements on soluble coffee.[15] Also, although there was a significant increase in the signal-to-noise ratio of the free radical signal compared to fluid solution measurements, the variations of spectral intensity with time and storage conditions were similar to those observed with the room temperature spectra.

The intensity of the Fe(III) (g = 4.3) signal decreased with increasing O_2 content of water used during extraction, although similar results were obtained with both nonretorted and retorted solutions, and only small changes were observed as a function of either storage time or temperature (Figure 2). At first sight this result is surprising, because increasing the O_2 content of the extraction water might be expected to result in increased Fe(III) contents of the coffee solution as a result of oxidation of Fe(II). However, these results can be explained by changes in Fe(III) speciation from momonuclear organic complexes to polymeric species. Thus, the g = 4.3 signal corresponds to mononuclear complexes, but polymeric iron-(III) oxide or oxyhydroxide species give a much broader (and hence lower amplitude) signal with a g value around 2.0.[28]

In these measurements, the free radicals detected by EPR correspond to the paramagnetic components in the coffee solutions with g values close to that of an unpaired electron. They represent the (relatively) stable components in the solutions at the time the measurements were made, and no information is provided on highly unstable radicals, such as hydroxyl radicals, which are likely precursors of those measured here via the following type of reaction:

$$R - H + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O$$

However, because the only compositional variable in these measurements was the O_2 content of the extraction water, the signals observed here are considered to be proportional to the oxidative challenge to which the coffee solutions were subjected.

The Mn(II) and Fe(III) signals showed qualitatively different dependencies on the O_2 content of the extraction water, storage time, and temperature. There were no significant changes in the Mn(II) EPR signal with respect to extraction conditions, whereas the Fe(III) (g = 4.3) signal intensity was inversely related to the O_2 content of the water used during extraction, but was insensititve to subsequent storage conditions. This latter result suggests that there is probably O_2-mediated degradation of the original ligand(s) to which Fe(III) was coordinated in roasted coffee. The fact that no further changes were observed during storage under N_2 (at either 30 or 50 °C) suggests that the intensity of the g = 4.3 Fe(III) signal could be an indicator of O_2 load during extraction.

The decreased stability (with respect to free radical content) of coffee samples that had been exposed to O_2 during the extraction process could be related to the iron speciation. Because hydrogen peroxide is produced during the coffee roasting process by the pyrolysis of chlorogenic acids and other polyphenols,[29,30] the chemical forms of iron may be important for initiating hydroxyl radical production via Fenton reaction chemistry.[24] Thus, the present results suggest that the presence of O_2 in the water used for extraction results in the conversion of some of the Fe(III) from mononuclear complexes to polymeric oxide or oxyhydroxide species, which have been reported to be able to catalyze the Fenton reaction.[31,32]

In conclusion, these measurements show that exposure to O_2 during extraction of whole coffee beans results in products that have increased sensitivity to change during storage under anaerobic conditions. No critical level of O_2 could be discerned,

dx.doi.org/10.1021/jf305028s | J. Agric. Food Chem. 2013, 61, 3301−3305

which corresponded to a discontinuity in the O_2 impact on EPR intensity (at the actual sensitivity and resolution of the current EPR experiments). The O_2 concentration in the extracting water also had a major effect on the iron speciation, a result which suggests that the chemical forms of this metal may influence the stability of coffee solutions during storage under inert atmospheres. However, the much higher relative stability to storage at elevated temperatures (50 °C) of whole bean coffee extracts from which O_2 was excluded during the extraction phase indicates that products with considerable "shelf life" could be produced if there is careful control of the extraction conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preliminary measurements of the impact of O_2 in the headspace during extraction of whole roasted coffee beans. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

Funding for C.Y. and E.C.P. was provided by Nestlé.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

R. Fumeaux of Nestlé Research Center (NRC) is acknowledged for the preparation of the liquid coffee specimens. In addition, the EPR facilities were provided by the Scottish Office Agriculture Environment and Fisheries Department (SOAEFD) (now Agriculture Environment and Fisheries Department Scottish Executive).

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