Chemistry of Brazilian green coffee and the quality of the beverage. VII. Total carbonyls, activity of polyphenol oxidase, and hydroperoxides*[1/]

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R E S U M O

Carbonilos totais e hidroperóxidos foram determinados no óleo de café verde classificados quanto a qualidade da bebida em Mole e Rio. Os carbonilos totais decrescem com o armazenamento e é maior no café Mole. Não foram observadas diferenças para hidroperóxidos entre cafés Mole e Rio armazenados por dois anos. A atividade da polifenol oxidase (DOPA como substrato) é maior no café Mole e decresce com o tempo de armazenagem. Estes resultados são discutidos com os já existentes na literatura — Os autores

Introduction

THE roles of hydroperoxides (9, 17) and carbonyls (6) in flavor of foods are well documented in the literature. In coffee, however, most of the references deal with research on roasted coffee (19). Calle (4, 5) was the first to call attention to the importance of aldehydes in green coffee and in quality of the beverage. His qualitative tests showed that the best coffees gave a more positive reaction for aldehydes than did the poor. Also by qualitative tests, Harigopal (7) confirmed these results. The probable importance of phenolics on the protection of aldehydes was illustrated by Amorim and Silva (1), who showed that spoiled coffee had lower polyphenol oxidase activity in comparison to good coffee. Several authors also found that polyphenol oxidase activity is higher in the best coffees (1, 15, 16, 11, 8).

The purpose of this research was to recheck the reports of Calle (5) and Harigopal (7) pertaining to the carbonyls, along with concurrent of polyphenol oxidase activity and the estimation of hydroperoxides by the conjugated diene hydroperoxide method (17).

Materials and methods

Commercial coffee samples, classified for quality of the beverage by professional cup tasters from the Brazilian Institute of Coffee as Soft (Mole) and Rio, were examined.

The beans were frozen in liquid nitrogen and ground in a Wiley mill under liquid nitrogen. The powder (<40 mesh) was used immediately for oil and enzyme extractions.

Hydroperoxides were estimated by the method of St. Angelo *et al.* (17). The oil was extracted in a mortar and pestle with spectroscopic grade hexane (2 g/30 ml) and allowed to stand for 1.5 hr in stoppered contrifuge tubes with occasional mixing. After centrifugation (20,000 g, 2-4°C), 0.2 ml of the supernatant was added into a 3 ml spectrophotometric cuvette containing 2.8 ml of hexane, mixed, and read at 234 nm against a hexane blank. The concentration of CDHP (conjugated diene hydroperoxide) was calculated from an e-max of 24,500. Values are given in μmoles of CDHP/g of coffee powder. Two extractions and four determinations were made for each sample. Total carbonyls were extracted four times from 3 g of coffee powder with 10 ml carbonyl-free benzene. After each extraction, the homogenate was centrifuged at 37,000 g for 10 min at —4°C. The supernatants were combined and diluted

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to 50 ml with benzene. Two aliquots of 10 ml were removed and evaporated under nitrogen at 50°C to measure oil content. Two additional aliquots were immediately removed and total carbonyls were estimated by the procedure of Brown *et al.* (3), which used the 2,4-dinitrophenylhydrazone complex to analyze volatiles from peanuts and peanut products.

Polyphenol oxidase was extracted from 1 g of the powder with 20 ml of 0.05 M phosphate buffer, pH 6.5, for 10 min (1). The homogenate was centrifuged at 27,000 g for 20 min at 0-2°C. The supernatant was used for enzymatic assay. Aliquots of 0.5 ml of the supernatant were made to 3 ml with either DL-Dopa (DL-dihydroxyphenylanine, 8 mg/10 ml) in 0.1 M phosphate buffer pH 7.0, or with the buffer only as a blank. The increase in absorbance at 410 nm was measured continuously in a Gilford spectrophotometer and the absorbance at 10 min was used for the calculations. Duplicate readings were made for each sample.

Table 1.—Total carbonyls in the coffee oil and activity of polyphenol oxidase (PPO) of Arabica green coffee beans.

Sample		Total carbonyl μmol/g oil	Activity of PPO Abs. 10 min/g powder
Soft (Stored 1 yr)	1	125.8	1.78
	2	119.7	1.46
	3	114.9	1.66
	4	94.5	1.96
	(mean)	113.7 a	1.72 a
Rio (Stored 1 yr)	5	80.5	0.96
	6	102.2	0.52
	7	67.6	0.60
	8	104.8	0.78
	(mean)	88.8 b	0.72 b
Soft (Stored 2 yr)	9	97.5	0.84
	10	98.8	1.14
	11	78.4	0.88
	12	97.5	1.02
	(mean)	93.0 b	0.97 c
Rio (Stored 2 yr)	13	102.6	0.00
	14	70.4	0.46
	15	95.9	0.66
	16	81.7	0.92
	(mean)	87.6 b	0.51 d

Different simbols (columns) mean significancy at 5 per cent level

Results and discussion

Table 1 shows the results of total carbonyl and polyphenol oxidase (PPO) activities on samples classified as Soft and Rio, which had been stored for 1 and 2 yr.

Table 2 shows the hydroperoxide values found for the same coffees stored for 2 yr.

The activity of PPO was higher in Soft coffee and decreased on storage. These data agree with results obtained by Amorim and Silva (1), Pereira (13), Oliveira (11), Valencia (18) and Rotemberg and Iachan (15). The total carbonyls followed the same pattern as the enzyme activities; they were higher in the Soft coffee and decreased with storage. However, Rio coffee stored for one and two years were not statistically different. These results agree with the qualitative work of Calle (5) and Harigopal (7) on beverage quality. The higher activity of PPO and total carbonyls, which decreases with age, in the good (Soft) coffee, may be due to the protection which phenolics exert on aldehydes. The explanation for the lower PPO activity found in spoiled and old coffee may be the inactivation of the enzyme by the oxidized phenolics produced by the PPO system (2) which lowers the protection of the aldehydes.

The results of our current study apparently substantiate the hypothesis that chlorogenic acid, a phenolic compound, may act as a protective agent for aldehydes in coffee (1), although acetaldehyde was found to be associated with spoiled coffee after long fermentation (14). Rodríguez *et al.* (14), however, did not estimate total carbonyls or total aldehydes. A more detailed analysis of the carbonyls, identifying the ketones and aldehydes, could shed more light on this matter and should determine if there really is a correlation between certain types of carbonyls in the green bean and the quality of the beverage.

Table 2.—Total conjugated diene hydroperoxide in coffee oil extracted from green coffee beans stored for two years (Average of 2 extractions and 4 estimations).

Rio	μm CDHP/g powder	Soft	μm CDHP/g powder
1	6.69	1	4.99
2	5.13	2	5.09
3	5.45	3	4.25
4	3.98	4	4.55
5	4.01	5	3.99
6	4.39	6	5.59
—	—	7	5.23
—	—	8	2.97
mean	4.94 ± 0.42	mean	4.58 ± 0.29

No statistically significant

Unsaturated fats can undergo oxidative reactions catalyzed by metals or by lipoxygenase, and the hydroperoxides formed may contribute to off flavors in foods (12). The same peroxide values found for Soft and Rio coffee stored 2 yr do not mean that hydroperoxide formation is not important in coffee flavor, because hydroperoxides can undergo further degradation to form aldehydes, ketones, and alcohols that generally have unpleasant flavors. However, some of the degradation products do contribute to desirable flavors. Jordão *et al.* (10) estimated peroxide values by the A.O.C.S. method (Cd 8-53) in coffees stored in different conditions and they did not find peroxide up to 21 months of storage. The quality of the coffee at the start of the experiment was one of the intermediate type (Hard) and it did not change flavor even after peroxide was detected; however it is not known if hydroperoxide can affect the flavor of a good coffee (Soft).

Although we have found more total carbonyls in the good and young coffee beans, these findings do not invalidate the hypothesis that off flavors may be caused by production of hydroperoxide and further reactions, since the samples analysed were two years old and numerous transformations could have taken place.

Summary

Soft and Rio green coffee beans were analyzed for total carbonyls, polyphenol oxidase activity, and hydroperoxides. Total carbonyls and polyphenol oxidase activity were higher in Soft than in Rio coffees, decreasing with length of storage, although the difference on carbonyls between Rio coffees stored for one and two years were not different statistically. After 2 years storage, total hydroperoxides did not differ between Rio and Soft coffees.

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