

Importance of Nonvolatile Compounds to the Flavor of Coffee

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The chemical composition of green and roasted coffee was reviewed in an attempt to correlate chemical changes which occur during roasting with the formation of nonvolatile flavors. Examination of free and combined amino acids indicate that arginine, cysteine, lysine, and serine are markedly destroyed during roasting. The carbohydrates also undergo marked changes—i.e., sucrose and arabinogalactan are destroyed in proportion to degree of roast. Even though the soluble mannan increases with roast, the mannose content of the

holocellulose fraction decreases under the same conditions. The acids in green and roasted were examined in greater detail and methods for their quantitative analysis were described. Volatile, nonvolatile, and phenolic acids all decreased with increased roasting time. Chlorogenic acid, a major component of the acids, can be used to measure degree of roast. The current analytical methods for separation and analysis of nonvolatile flavors in coffee were reviewed.

Coffee has been a widely accepted beverage for many centuries. It has grown steadily in commercial importance for the last 150 years so that today it is a major food item and one of the most important articles of international trade.

The flavor of a cup of coffee varies widely and it is well known by people in the field that this flavor is dependent on the green bean variety, the region from which it comes, the altitude at which it is grown, the soil, and method of handling. Thus, for the past hundred years, chemists have been analyzing various green coffees to obtain a chemical rationale for the production of the desired flavors and to explain the differences in flavor which are obtained by the roasting of different beans. Along with this effort, many have analyzed coffee before and after roasting—again to point up the reactants and reactions in the green bean which are important to the development of flavor.

The objective of this paper is to review briefly the existing data on chemical composition of green coffee beans, the changes in composition which occur as the beans are roasted, and the nonvolatile flavors which are produced during processing.

CHEMICAL COMPOSITION OF GREEN COFFEE

Moore and Stefanucci (1964) in Table I depicted the average composition of green and roasted coffee. A similar average composition of green and roasted coffee was reported by Sivetz and Foote (1963). Although this is an overly simplified composition of coffee, it clearly indicates that the sucrose, chlorogenic acid, protein, and trigonelline undergo the greatest change during roasting.

Inherent in the averages given in Table I are values in chemical composition which show that between various samples analyzed, the protein, hemicellulose, lignin, and ash contents of green coffees lie in a narrow range. The fat varies from 10 to 17%, with the observation that Robusta coffees are lower than Arabica; sucrose varies between 5 and 10%, reducing sugars 0 to 0.7%, and chlorogenic acid 5 to 8%. Caffeine varies between 1 and 2%, again higher in Robusta. Trigonelline can vary between 1 and 3% with the trend Colombian > Brazilian > Robusta.

Table I. Average Composition of Green and Roasted Coffee

Constituent	Green, %	Roasted, %
	D.B. ^a	D.B. ^b
Hemicelluloses	23.0	24.0
Cellulose	12.7	13.2
Lignin	5.6	5.8
Fat	11.4	11.9
Caffeine	1.2	1.3
Sucrose	7.3	0.3
Chlorogenic acid	7.6	3.5
Protein (based on nonalkaloid N)	11.6	3.1
Trigonelline	1.1	0.7
Reducing sugars	0.7	0.5
Unknown	14.0	31.7
Total	100.0	100.0

^a Dry basis.

^b Not corrected for dry weight roasting loss, which varies from 2 to 5%.

EFFECT OF ROASTING ON MAJOR CHEMICAL COMPONENTS

Green coffee has no desirable taste or aroma of its own. The desired flavor is developed in the roasting of the beans. The obvious changes which occur during this process are a change in color from green to brown and a concomitant increase in size of the bean. During the roast, as the temperature of the bean reaches about 425° F. (220° C.), a strong exothermic reaction ensues which is accompanied by sudden expansion or puffing of the bean.

In brief, the major water soluble constituents of coffee, e.g., proteins, sucrose, chlorogenic acids, trigonelline, and ash, which account for 70 to 80% of water soluble solids, as well as the water insoluble fractions, react to give new high molecular weight solids, both soluble and insoluble, carbon dioxide, and volatile and nonvolatile flavors which are important to quality of the beverage. It is possible, as shown later, that green coffee insolubles also enter into the over-all reaction, but due to limitations of analysis, it is not apparent in the gross compositional data. Thus, it might be informative to look at compounds in green coffee in greater detail and to follow their interaction during roasting to explain their contribution to coffee flavor.

It is the purpose of this paper to review and bring up-to-date the information on the chemical composition of coffee. Proteins and carbohydrates are discussed and particular attention is given to recent unpublished results obtained in this

Table II. Effect of Roasting on Amino (Protein) Nitrogen in Coffee (% calc. on dry basis)

	Amino Nitrogen			Total Protein		
	Green	Roasted		Green	Roasted	
		1	2		1	2
Haiti	1.63	1.25	1.16	10.19	9.04	8.76
Colombia	1.55	1.27	1.15	9.69	9.24	8.70
Angola Robusta	1.61	1.15	0.94	10.07	7.16	5.86

laboratory dealing with phenolic and nonphenolic acids. Some of the techniques used to analyze nonvolatiles in coffee and their inherent limitations will be mentioned to emphasize the complexity of the analytical problems involved and the need for the imaginative use of newer skills and techniques in the determination of coffee composition.

PROTEINS

The importance of the reaction of proteins (and amino acids) with carbohydrates to produce characteristic flavors has been well-documented by El-Ode *et al.*, (1966), Hodge (1967), Ryder (1966), Wiseblatt (1963), and many others. Volatile carbonyl, dicarbonyl, sulfur, furanoid, and other flavor compounds reported by Stoll *et al.* (1967) and Gian-turco (1967) in coffee could very well come from these reactions. However, in contrast to volatile compounds of coffee, there appears to be relatively little information on proteins, polypeptides, and free amino acids as they exist in green coffee, as well as on their fate after roasting.

The first intensive investigation in coffee proteins was made by Underwood and Deatherage (1952). They developed conditions for hydrolysis of coffee protein, the separation of 14 amino acids and quantitative analysis of nine amino acids. All of the amino acids in the green coffee were found in comparable amounts in the roasted coffee. They also found an appreciable amount of amino acids in the water extract of roasted coffee. Barbera *et al.* (1956) identified cystein, methionine, and proline in green and roasted coffee.

Thaler and Gaigl (1962, 1963a, 1963b, 1964) published a series of articles on the proteins in green and roasted coffee. No significant difference was observed in protein nitrogen of Arabica, Robusta, and Liberica coffees. In their treatment of roasted coffee, two varieties of Arabica coffee (Haiti and

Table III. Relationship Between Degree of Roast and Per Cent Roasting Loss

	1	2
Haiti	13.8	17.0
Colombia	14.0	17.6
Angola Robusta	14.5	22.6

Colombia) and a Robusta, were roasted to two different degrees of roast (Table III), acid hydrolyzed, and the resultant amino acids analyzed by the Moore *et al.* method (1958).

Table II illustrates the loss of amino nitrogen (free and combined) with degree of roast of Haiti, Colombia, and Angola Robusta coffees.

The degree of roast, Table III, is expressed as roasting loss.

Thus, in Table II the lighter roasted Haitian sample lost 35% of amino nitrogen, the Colombian 29%, and Angola Robusta 39%. In the darker roast, Haiti lost 29%, Colombian 39%, and Angola Robusta 53% of amino nitrogen (calculated on green bean basis).

The composition of the amino acids in the hydrolyzed green and roasted Haiti, Colombia, and Robusta coffees is detailed in Table IV.

The data in Table IV show that arginine, cysteine, lysine, serine, and threonine are markedly destroyed during the roast. Glutamic acid, on the other hand, increased to 50%; leucine, phenylalanine, and proline also increased. Valine in dark roast Robusta increased by 36%. Thaler did not indicate the amount of free amino acids or peptides in the various green and roasted coffees. Free amino acids would be expected to react more rapidly with carbohydrates than combined amino acids. Thaler recognized that his method of analysis (acid hydrolysis) included amino acids derived from Maillard reaction products and that these were only partly reversible.

CARBOHYDRATES IN GREEN AND ROASTED COFFEE

The carbohydrates comprise 50 to 60% of the weight of the green bean. This is made up of 6 to 10% sucrose, 5 to 12% cellulose, 3% pectic substances, and the remainder as high molecular weight polysaccharides. The carbohydrates in coffee have been widely explored by Barbaroli (1965), Courtois and LeDizet (1962), Courtois *et al.* (1963), Natarajan *et al.* (1966, 1967), Thaler (1959, 1965), Thaler and Arneth (1967),

Table IV. Composition of Amino Acids (%) in Green and Roasted Haiti, Colombian, and Angola Robusta Coffees (after Acid Hydrolysis)

Amino Acid	Haiti			Colombia			Angola Robusta		
	Green	Roast 1	Roast 2	Green	Roast 1	Roast 2	Green	Roast 1	Roast 2
Alanine	4.91	5.97	5.48	4.75	4.76	5.52	4.87	6.84	7.85
Arginine	4.72	0.00	0.00	3.61	0.00	0.00	2.28	0.00	0.00
Asparagine	10.50	9.07	9.02	10.63	9.53	7.13	9.44	8.94	8.19
Cysteine	3.44	0.38	0.34	2.89	0.76	0.69	3.87	0.14	0.14
Glutamic acid	18.86	20.86	23.29	19.88	21.11	23.22	17.88	24.01	29.34
Glycine	5.99	6.86	7.08	6.40	6.71	6.78	6.26	7.68	8.87
Hystidine	2.85	1.99	2.17	2.79	2.27	1.61	1.79	2.23	0.85
Isoleucine	4.42	4.75	4.91	4.64	4.76	4.60	4.11	5.03	5.46
Leucine	8.74	9.95	11.19	8.77	10.18	10.34	9.04	9.65	14.12
Lysine	6.19	2.54	2.74	6.81	3.46	2.76	5.36	2.23	2.56
Methionine	2.06	2.32	1.48	1.44	1.08	1.26	1.29	1.68	1.71
Phenylalanine	5.79	6.75	6.05	5.78	5.95	6.32	4.67	7.26	6.82
Proline	6.58	6.52	6.96	6.60	6.82	7.01	6.46	9.35	10.22
Serine	5.60	1.77	1.26	5.88	2.60	0.80	4.97	0.14	0.00
Threonine	3.73	2.43	1.83	3.82	2.71	1.38	3.48	2.37	1.02
Tyrosine	3.54	4.31	3.54	3.61	4.11	4.35	7.45	9.49	8.87
Valine	5.50	6.86	3.31	8.05	6.93	8.05	6.95	10.47	9.49

Table V. Sucrose in Green and Roasted Coffee (Per Cent on Dry Bean Basis)

	Green	Light Roast	Medium Roast	Dark Roast
Colombian	4.59	0.45	0.17	0.06
Santos	5.47	0.68	0.27	0.10

Wolfrom (1960 and 1961), and Wolfrom and Patin (1964 and 1965).

Sucrose is a major component in green coffee. In roasting of coffee, the sucrose is destroyed quickly as shown in Table V.

These analyses were made in this laboratory using gas chromatography of trimethylsilyl derivatives.

Along with sucrose, Courtois and LeDizet (1962) and Courtois *et al.* (1963) isolated and identified raffinose (galactoglucoside) and stachyose (galactogalactoglucoside) in small yield in green Robusta coffee. The fate of these compounds during roasting is not known.

Courtois *et al.* (1963), Wolfrom and Patin (1965), and Thaler and Arneith (1967) all found that the major water soluble polysaccharide in coffee was an arabinogalactan (2 to 5). Courtois isolated glucogalactomannan by cold water extraction. Wolfrom *et al.* (1961) and Thaler (1957, 1959) isolated mannan from roasted coffee. It was postulated that mannan originally existed in green coffee cell wall as galactomannan.

The polysaccharide content of green and roasted coffee is graphically summarized in Figure 1, using Thaler's data (1967) (without indication of individual component—i.e., whether it is arabinogalactan or a mixture of arabinan, galactan, etc.).

In the graphs comprising Figure 1 the following abbreviations are used: Ga = galactose, Ma = mannose, Ar = arabinose, Gl = glucose.

The polysaccharides of green and roasted coffee were separated by Thaler and Arneith (1967) into hot water solubles, chlorine dioxide degradable polysaccharides, and holocellulose. Thus, the major component of water soluble polysaccharide in green coffee is arabinogalactan, the ClO₂ degradable is arabinogalactan and holocellulose is galactomannan (60 to 70% mannose) and some cellulose. On roasting the water soluble fraction, the arabinose practically disappears, the galactose decreases markedly, and the mannose increases from 10 to 70%. Also during roasting, the arabinose and galactose of degradable polysaccharides decrease and mannan increases proportionately.

In the holocellulose fractions, the mannan decreases slightly with roast, the galactan is almost constant, and the cellulose (as indicated by glucose value in Figure 1) increases proportionately with degree of roast. Thus, the cellulose is unaffected by roasting.

The data thus far would account for the formation of complex flavor of coffee—both volatile and nonvolatile. Thus, reactive amino acids and sugars enumerated could react in a variety of ways to yield a large variety of flavors. Conditions exist in the bean that produce a clearly distinguishable coffee flavor regardless of variety of coffee used. The analyses are still inadequate since they fail to explain the differences in reactants which are responsible for the characteristic flavors of roasted Colombian, Brazil, and Robusta coffees.

ACIDS

The acids are important to the flavor of coffee and have received considerable attention by Marbrouk and Deatherage

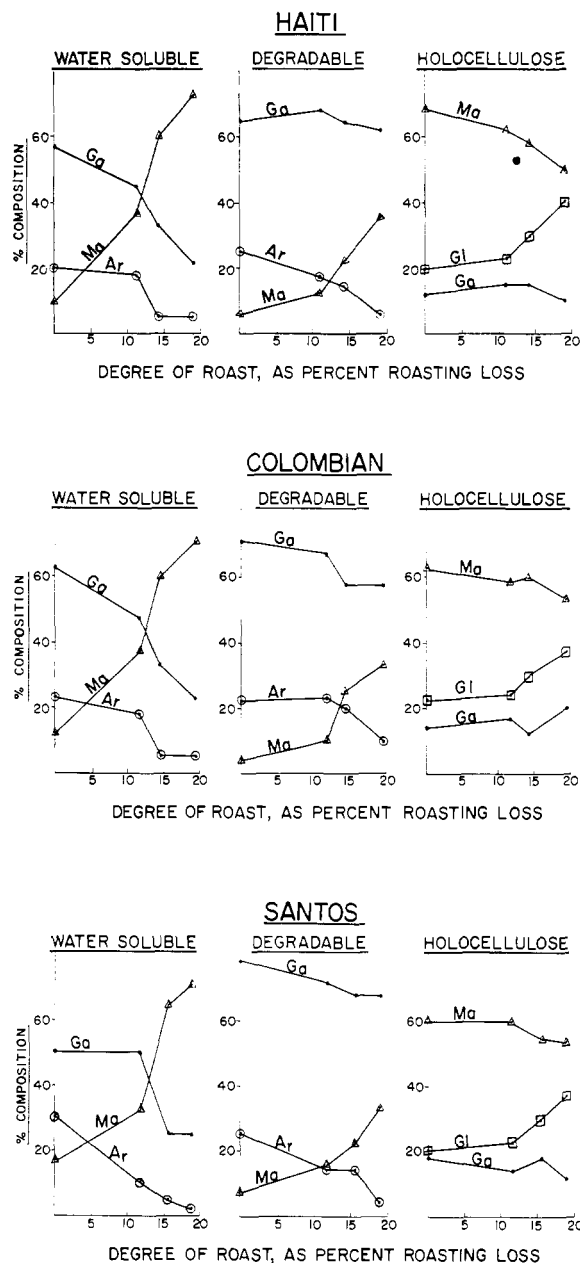


Figure 1. Polysaccharides in green and roasted Haiti, Colombian, and Santos coffees (Thaler, 1967)

(1956), Kung *et al.* (1967), and Woodman *et al.* (1967). This "acid" flavor in the coffee beverage varies with the variety of beans used and the extent to which they are roasted. In this laboratory volatile acids in roasted coffee were determined by Kung *et al.* (1966), and the nonvolatile, nonphenolic acids and the phenolic acids by Kung *et al.* (1967). Gas chromatographic analysis was utilized because of its inherent sensitivity, resolving power, speed, and accuracy.

Volatile Acids. With the exception of formic acid, the volatile acids were isolated from coffee by solvent extraction of acidified extract and analyzed directly by gas chromatography. Formic acid was determined by NMR (Kung *et al.*, 1966).

The volatile acid composition of three varieties of coffee at two degrees of roast was determined as shown in Table VI. The Robusta samples were significantly higher in formic acid but slightly lower in acetic acid than the Colombian and Santos samples. Santos had the lowest formic acid content.

Table VI. Volatile Acid Composition (%)^a in the Beverages of Colombian, Santos, and Robusta Coffee

Volatile Acids	Colombian		Santos		Robusta	
	Dark roast	Medium roast	Dark roast	Medium roast	Dark roast	Medium roast
C ₁	0.072	0.092	0.066	0.079	0.12	0.14
C ₂	0.31	0.31	0.30	0.33	0.25	0.29
C ₃	0.011	0.0092	0.0063	0.0056	0.0055	0.0050
C ₄	0.0076	0.0052	0.0069	0.0068	0.0053	0.0057
C ₅	Trace	Trace	Trace	Trace	Trace	Trace
C ₆	0.0010	0.00071	0.0019	0.0019	0.0020	0.0029
C _{7x}	0.00074	0.0014	0.0012	0.0015	0.0016	0.0018
C ₇	0.0061	0.0063	0.010	0.0094	0.0031	0.0040
C _{8x}	0.0013	0.00083	0.00074	0.00023	Trace	Trace
C ₈	0.00028	0.00022	0.0016	0.0032	0.0006	0.0008
C ₉	0.00025	0.00083	0.0013	0.0013	0.0014	0.0021
C _{10x}	0.0020	0.0017	0.0028	0.0015	Trace	Trace
C ₁₀	0.00051	0.0010	0.0028	0.0015	0.0015	0.0020
C ₁ to C ₁₀ (Total)	0.41	0.43	0.40	0.44	0.39	0.45

^a Calculated on dry coffee bean basis.

Table VII. Distribution on Acids in Milliequivalents^a in Beverages of Roasted Colombian, Santos, and Robusta Coffee

	Colombian		Santos		Robusta	
	Dark roast	Medium roast	Dark roast	Medium roast	Dark roast	Medium roast
C ₁ to C ₁₀	7.34	7.41	6.72	7.68	7.10	8.33
Nonvolatile						
nonphenolic acids	4.20	4.55	4.50	4.13	6.57	7.67
Phenolic acids	19.25	16.18	15.50	20.82	16.24	16.71
Total by G. C. analysis	30.79	28.14	26.72	32.63	29.91	32.71
pH of beverages	4.62	4.82	5.35	5.18	5.44	5.32

^a Milliequivalent values calculated on 100-gram dry roasted bean basis.

Table VIII. Nonvolatile (Nonphenolic) Acids in Coffee Brew (Per Cent on Dry Coffee Basis)

Acids	Colombian		Santos		Robusta	
	Dark roast	Medium roast	Dark roast	Medium roast	Dark roast	Medium roast
Lactic	0.077	0.078	0.058	0.055	0.066	0.088
Pyruvic	0.051	0.058	0.028	0.050	0.058	0.085
Oxalic	0.035	0.047	0.024	0.029	0.044	0.049
Malonic	0.0062	0.0071	0.0055	0.0063	0.012	0.0061
Succinic	0.0064	0.0055	0.016	0.00076	0.011	0.0067
Glutaric	0.013	0.012	0.013	0.0082	0.037	0.018
Maleic	0.0085	0.0038	0.038	0.020	0.0085	0.054
Fumaric	0.014	0.0072	0.012	0.0048	0.028	0.023
Malic	0.018	0.015	0.039	0.0025	0.030	0.026
Tartaric	0.035	0.046	0.041	0.061	0.079	0.093
Citric	0.018	0.035	0.027	0.042	0.069	0.075
Total (%)	0.28	0.31	0.30	0.28	0.44	0.52

Woodman *et al.* (1967) found that formic and acetic acids decreased during roasting. Table VI shows that the darker the roast, the lower the volatile acid content. The decrease of volatile acids by prolonged roasting may be due to volatilization. Natural variation of coffee beans prevents drawing firm conclusions based on a limited number of samples regarding the relationship of brew acidity and roast color. The present data suggest, however, that the volatile acids of coffee are not a major factor in the total acidity. The nonvolatile (nonphenolic) acids and the phenolic acids are probably responsible to a greater degree for the variations observed in beverage acidity—i.e., pH and titratable acidity—as shown in Table VII.

Nonvolatile (Nonphenolic) Acids. The nonvolatile (nonphenolic) acids were isolated from coffee brews either by solvent extraction or by separation with DEAE Sephadex, converted to methyl esters, and analyzed by gas chromatography.

As shown in Table VII and Table VIII, the concentrations of

these acids (like the volatile acids) in coffee brews are low and the differences among varieties are slight. Evidently, they are not the major contributors of the acidity in the coffee brew. In general, they are lower in dark roasts than in medium roasts. The loss of the nonvolatile acids with roast is believed to be due to thermal decomposition.

Recently, four additional acids were identified: 2-furoic, itaconic, citraconic, and mesaconic acids in roasted coffee, thus corroborating the results reported by Woodman *et al.* (1967). The concentrations of these acids in coffee are also low. 2-Furoic acid is believed to be formed from polysaccharides during roasting. Itaconic, citraconic, and mesaconic acids are derived from the degradation of citric acid.

Phenolic Acids. Chlorogenic acid was isolated from coffee by Gorter in 1908 and its structure established as 3-caffeoylquinic acid by Fischer and Dangschat in 1932. Interest in this and related compounds, however, continues to the present time, stimulated by identification of several

Table IX. Mono- and Dicafeoylquinic Acids in Green and Roasted Santos Coffee

Acid	% on Dry Coffee Beans Basis		Ratio, Roasted/Green
	Green	Roasted	
3-Caffeoylquinic (Chlorogenic)	5.56	1.96	0.35
4-Caffeoylquinic (Cryptochlorogenic)	0.41	0.24	0.58
5-Caffeoylquinic (Neochlorogenic)	0.88	1.02	1.16
Isochlorogenic acids:			
3,4-Dicafeoylquinic	0.28	0.010	0.036
3,5-Dicafeoylquinic	0.21	0.092	0.45
4,5-Dicafeoylquinic	0.11	0.010	0.09

isomers of chlorogenic acid and their widespread occurrence as pointed out by Sondheimer and Griffin (1960) and Sondheimer *et al.* (1961). Also, these compounds are closely related to shikimic acid, an intermediate in the biosynthesis of many aromatics, and whose depsides are also known to occur widely in nature (Maier *et al.*, 1965). Chlorogenic acid is also of biochemical significance in view of its inhibitory action toward indoleacetic acid oxidase and potato phosphorylase (Gortner and Kent, 1958).

Chlorogenic acid, a major constituent of green coffee, was isolated in 1908 as a crystalline solid from its potassium caffeine complex by Gorter. Moores *et al.* (1948) developed an analytical method for the determination of this compound in coffee. Two years later, Barnes *et al.* (1950) published a method for isolating isochlorogenic acid from green coffee as a noncrystalline solid. This method, still in use today, has led to the development of methods for the isolation of isochlorogenic, chlorogenic, neochlorogenic acids, and another isomer designated as Band 510 from green coffee. Subsequent investigators (Carangal *et al.*, 1956; Corse *et al.*, Scarpati and Esposito, 1964; Sondheimer *et al.*, 1961) using more sophisticated analytical techniques and NMR for structure elucidation have established that isochlorogenic acid exists as three isomers which are 4,5-, 3,4-, and 3,5-dicafeoylquinic acids and that chlorogenic acid and its isomers, neochlorogenic acid and Band 510 or cryptochlorogenic acid, are monocaffeoylquinic

acids, respectively, or the 3-, 5-, and 4-positional isomers, respectively, of caffeoylquinic acids.

DETERMINATION OF PHENOLIC ACIDS IN GREEN AND ROASTED COFFEE

Pictet and Brandenberger (1960) identified ferulic, caffeic, isochlorogenic, chlorogenic, neochlorogenic, and three feruloylquinic acids in green and roasted coffee by paper chromatography. They found *p*-coumaric acid in Robusta coffee but not in Arabica. With the availability of *bis*-(trimethyl-silyl) acetamide (BSA) reagent for the formation of TMS derivatives of nonvolatile compounds, the gas chromatographic method was extended to the determination of phenolic acids in green and roasted coffee. To date, six compounds have been identified in which quinic acid is combined with caffeic acid. Results are presented in Tables IX, X, XI. Thus far, the feruloylquinic acids identified by Pictet and Brandenberger (1960) have not been firmly established by the gas chromatography method.

As shown in Tables VII and IX, the major acids in coffee are the phenolic acids. These account for 84 to 87% of the total acid found, of which 67 to 76% are chlorogenic and neochlorogenic acids. As expected, the dark roasts are also lower in phenolic acids than are the medium roasts.

As shown in Table VII, the total milliequivalents of acid calculated from gas chromatographic analytical data ranges from 26 to 33 meq. per 100 grams, depending on the degree of roast, and accounts for about 75% of total acid as determined by titration of de-ashed coffee extracts.

DETERMINATION OF CHLOROGENIC AND ISOCHLOROGENIC ACIDS IN GREEN AND ROASTED SANTOS COFFEE

Since the chlorogenic acids are the major acid components in coffee and the isochlorogenic acids are chemically closely related to them, the determination of these acids could serve as an index for the characterization of green and roasted coffee, and perhaps contribute to the better understanding of the chemical changes produced during roasting.

On account of their low concentrations in coffee, the isochlorogenic acids were determined in the coffee extracts from which the high molecular weight materials had been removed by precipitation with 2-propanol. The results are given in Table IX. All chlorogenic and isochlorogenic acids, with the exception of neochlorogenic acid, decreased on roasting with

Table X. Phenolic Acids in Coffee Brew (Per Cent on Dry Coffee Basis)

Acid	Colombian		Santos		Robusta	
	Dark roast	Medium roast	Dark roast	Medium roast	Dark roast	Medium roast
Quinic	0.35	0.27	0.49	0.29	0.65	0.71
Ferulic	0.056	0.064	0.073	0.052	0.19	0.29
Caffeic	0.0068	0.0041	0.0050	0.0036	0.0078	0.0095
Cryptochlorogenic	0.46	0.26	0.27	0.18	0.18	0.023
Chlorogenic	2.55	2.02	2.12	2.88	1.53	1.74
Neochlorogenic	3.05	2.82	2.05	3.67	2.48	2.29
Total	6.47	5.44	5.01	7.08	5.04	5.06

Table XI. Changes in Phenolic Acids During Roasting

Acid	% of Dry Coffee Bean Basis							
	Santos				Colombian			
	Green	Light roast	Medium roast	Dark roast	Green	Light roast	Medium roast	Dark roast
Chlorogenic	5.56	2.90	1.96	1.11	3.77	2.74	2.16	0.93
Neochlorogenic	0.88	1.59	1.02	0.63	0.60	1.53	1.16	0.49
Caffeic	0.24	0.24	0.27	0.28	0.17	0.17	0.18	0.21
Ferulic	—	0.02	0.03	0.03	—	0.01	0.02	0.02

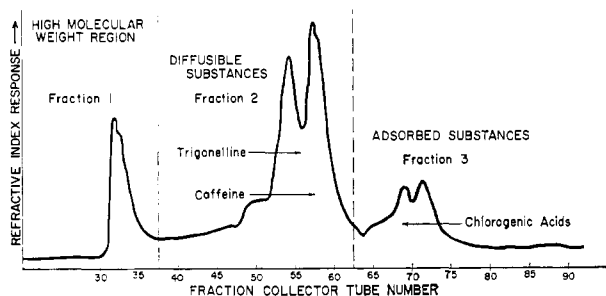


Figure 2. Chromatographic separation of roasted Robusta coffee water extract on Sephadex G-25 column

respect to their concentrations in the green extract. The neochlorogenic acid increased slightly in the roasted sample. Later investigation has shown that neochlorogenic acid decreased in darker roasted samples. The increase of neochlorogenic acid is believed to occur at the expense of chlorogenic acid. This increase of neochlorogenic acid was also found by Kröplien (1963), who reported that coffee treated with superheated steam had a higher neochlorogenic acid content than untreated coffee.

As indicated in the last column of Table IX, cryptochlorogenic acid appears to be more thermally stable than chlorogenic acid, and 3,5-dicaffeoylquinic acid is more stable than its two isomers.

CHANGES IN ACIDS DURING ROASTING

Lentner and Deatherage (1959) stated that during roasting of coffee beans, the amounts of formic and acetic acids increased. Between 32 and 52% of the chlorogenic acid was destroyed during roasting, whereas the loss in citric acid was 33 to 56% and for malic acid the loss was 16 to 40%, depending on the particular coffee used and the final roast temperature.

Analyses performed in this laboratory show that the chlorogenic acid content of green coffees averages about 7.5%, whereas roasted coffees fall within the range of 3.1 to 3.8% for normal roast. Similar results have been reported by Natarajan *et al.* (1967).

Data for the changes in phenolic acids during roasting of Santos and Colombian coffees are given in Table XI. In both varieties, chlorogenic acid decreased gradually during roasting, while neochlorogenic acid increased on the initial stage of roasting but decreased on further roasting. Caffeic acid contents in green coffees were low and increased slightly on roasting. Ferulic acid was not detected in green coffees of either variety but was present in trace amounts in roasted samples. Although the increase in neochlorogenic acid in the roasting could occur from the degradation of isochlorogenic acids, the magnitude of this increase exceeds the amount of isochlorogenic acids available and is, therefore, assumed to occur through the rearrangement of chlorogenic acid.

Other methods of separation of nonvolatile compounds of coffee appear to have promise, one of which is column chromatography using Sephadex.

SEPARATION OF COFFEE SOLUBLE SOLIDS BY GEL FILTRATION

Streuli (1962) separated an extract of roasted coffee into three principal fractions on a chromatographic column containing Sephadex G-25 using ultraviolet absorption as a monitor.

The first fraction contained high molecular weight substances estimated to be in excess of 4000. Melanoidin, caffeine, and trigonelline were found in the second fraction and chlorogenic acid in the third fraction.

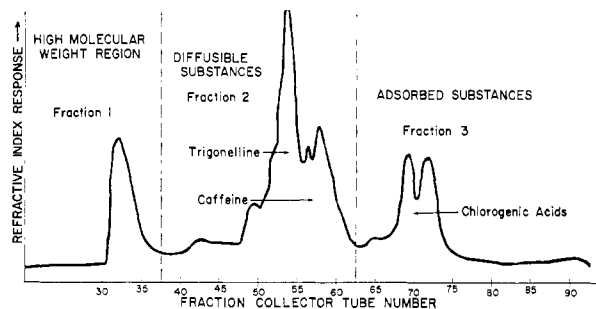


Figure 3. Chromatographic separation of roasted Santos coffee water extract on Sephadex G-25 column

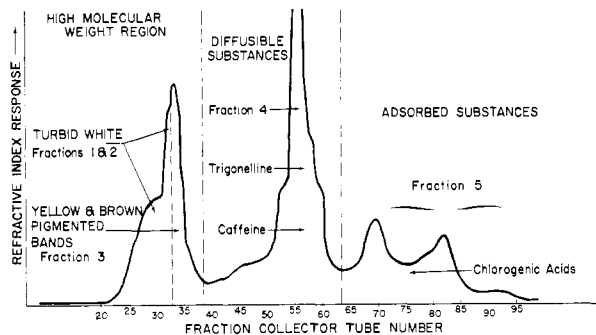


Figure 4. Chromatographic separation of green Santos coffee water extract on Sephadex G-25 column

The ratio of the three Sephadex fractions was reported to change with degree of roast. It was also implied that the color, taste, and chemical composition of fractions from Colombian, Santos, and African coffees were clearly different. These points were not discussed in further detail.

The characteristic taste of cold coffee was found in the second fraction. The presence of flavor, however, could not be correlated with any spectral characteristic.

At General Foods, Sephadex G-25 has been used to fractionate solutions of coffee soluble solids from green or roasted coffees. Elution patterns obtained, using refractive index monitor show three major peak areas as suggested by Streuli.

With roasted coffee (Figures 2 and 3), the first fraction contains high molecular weight substances and is highly pigmented, being very dark brown to almost black in color on the Sephadex column. The second fraction represents smaller molecular weight substances which diffuse into and out of the gel matrix. This is a very complex fraction containing many coffee components and which appears in the refractive index pattern as at least two major peaks which are only partially separated. Chemical analyses have located sugars, organic acids, amino acids, simple peptides, caffeine, and trigonelline in this fraction. The third Sephadex fraction contains substances which are retarded in their elution due to adsorption forces. This fraction, which often appears as a doublet, includes the chlorogenic and isochlorogenic acids.

Green coffee (Figure 4) provides a somewhat different refractive index pattern than roasted coffee. The high molecular portion of green coffee extract contains a lightly pigmented fraction which appears at the same elution volume as the heavily pigmented fraction of roasted coffee. Ahead of this fraction, however, two fractions occur which are invisible on the column and which elute as greyish-white turbid solutions. The differences in elution volume of these high molecular weight fractions presumably is due to solubility and possibly polarity differences.

The fourth major fraction in green coffee corresponds to the

second fraction in roasted coffee. The composition of this fraction is similar to that of roasted coffee with respect to caffeine, trigonelline, and the classes of compounds present. However, in terms of total composition, the green coffee fraction is much simpler due to the absence of products of roasting. This is reflected in the shape in the refractive index curve, being more or less a single large peak with two shoulders.

The fifth major fraction of green coffee in this scheme corresponds to the third and strongly adsorbed fraction in roasted coffee. This green coffee fraction often appears as three partially resolved peaks. Chlorogenic and isochlorogenic acids and other strongly adsorbed materials—i.e., small, highly polar compounds—occur in this fraction.

Flavor notes are found only in the diffusible and absorbed coffee fractions. The basic tastes and a distinctive raw green vegetable or herbaceous flavor occur in green coffee. In roasted coffee, a complex array of flavor notes are found: sour, astringent, caramel, bitter, medicinal, phenolic, as well as a number of aromatic notes.

CONCLUSION

Green coffee has no desirable taste or aroma of its own. The chemical components of green coffee include numerous complex, relatively insoluble substances, which in the main are nonflavorful and nonvolatile. The intact coffee bean may be considered as a tiny autoclave wherein the roasting process causes these chemical substances to react and interact under rather controlled conditions producing a desirably flavored end-product.

The nature of the reactants and analyses performed before and after roasting indicate the occurrence of Maillard reactions, Strecker degradations, base catalyzed sugar reactions, etc., perhaps regulated along uncommon pathways by the low moisture environment, localized buffer systems and a fluctuating balance of reaction products.

Gradually, certain aspects of the so-called browning reactions involving sugars and amino acids and the development of flavorful reaction products is being defined. To date, about 300 compounds have been identified in coffee, most of which are so-called volatile substances which appear to contribute to the aroma of freshly ground coffee and to the bouquet of freshly brewed coffee. Yet, as shown by the work with Sephadex gel-filtration, there is a base or nucleus of flavorful substances which survives dehydration treatments and even solvent extractions, appears to be nonvolatile in nature, and which contributes to the sour, bitter, astringent tastes and to the characteristic heavy aromatic flavor of coffee. It is this category of flavor materials which constitutes the current challenge to flavor research—substances whose needed identification will yield only to diligent work and the application of modern instrumentation.

REFERENCES

- Anet, E. F. L. J., *Australian J. Chem.* **12**, 280 (1959).
 Barbaroli, G., *Res. Chim.* **17**, 261 (1965).
 Barbera, C. E., Barbera, D., Messina, S., *Coffee Tea Ind.* **79**, 12 (1956).
 Barnes, H. M., Feldman, J. R., White, W. V., *J. Am. Chem. Soc.* **72**, 4178 (1950).
 Bondarovich, H. A., Friedel, P., Krampl, V., Renner, J. A., Shephard, F. W., Gianturco, M. A., *J. Agr. Food Chem.* **15**, 1093 (1967).
 Carangal, A. R., Jr., Arnaldo, S. R., Tucay, E. A., *Philippine Agriculturist* **40**, 99 (1956).
 Corse, J., Lundin, R. E., Waiss, A. C., Jr., *Phytochemistry* **4**, 527 (1965).
 Corse, J., Lundin, R. E., Sondheimer, E., Waiss, A. C., Jr., *Phytochemistry* **5**, 767 (1966).
 Courtois, J. E., LeDizet, P., *Ann. Pharm. Franc.* **20**, 588 (1962).
 Courtois, J. E., Percheron, R., Glomoud, J. Cl., *Cafe, Cacao, The* **7**, 231 (1963).
 El'Ode, K. E., Dornseifer, T. P., Keith, E. S., Powers, J. J., *J. Food Sci.* **31**, 351 (1966).
 Fischer, H. O. L., Dangschat, G., *Chem. Ber.* **65**, 1037 (1932).
 Gianturco, M. A., "Chemistry and Physiology of Flavors," p. 431, Avi, Westport, Conn., 1967.
 Glomoud, J. Cl., Percheron, F., Courtois, J. E., *Colloq. Int. Chim. Café-Paris* **1965**, p. 39.
 Gorter, K., *Ann.* **358**, 327 (1908).
 Gortner, W. A., Kent, M. J., *J. Biol. Chem.* **233**, 731 (1958).
 Herz, W. J., Shallenberger, R. S., *Food Res.* **25**, 491 (1960).
 Hodge, J. E., *J. Agr. Food Chem.* **1**, 928 (1953).
 Hodge, J. E., "Chemistry and Physiology of Flavors," p. 465, Avi, Westport, Conn., 1967.
 Kaufman, C. W., *Food Technol.* **5**, 154 (1951).
 Kröplien, U., Green and Roasted Coffee Tests, Gordian, Hamburg, 1963.
 Kung, J. T., McNaught, R. P., Yeransian, J. A., *J. Food Sci.* **32**, 455 (1966).
 Kung, J. T., Ryder, W. S., Feldman, J. R., A.S.I.C. (Trieste) **1967**, p. 223-30.
 Langner, E. A., Tobias, J., *J. Food Sci.* **32**, 495 (1967).
 Lentner, C., Deatherage, F. E., *Food Res.* **24**, 483 (1959).
 Maier, V. P., Metzler, D. M., *J. Food Sci.* **30**, 747 (1965).
 Marbrouk, A., Deatherage, F. E., *Food Technol.* **10**, 194 (1956).
 Merritt, M. C., Proctor, B. E., *Food Res.* **24**, 672 (1959).
 Moore, S., Spackman, D. H., Stein, W. H., *Anal. Chem.* **30**, 1185 (1958).
 Moores, R. G., McDermott, D. L., Wood, T. R., *Anal. Chem.* **20**, 620 (1948).
 Moores, R. G., Stefanucci, A., "Encyclopedia of Chemical Technology," V, Wiley, New York, 1964.
 Natarajan, C. P., Balachandran, A., Shivashankar, S., Ramamani, S., Bhatia, D. S., *J. Food Sci. Technol.* **2**, 7 (1966).
 Natarajan, C. P., Iyengar, J. R., Bhatia, D. S., *J. Sci. Ind. Res. (India)* **16C** **2**, 42 (1967).
 Pictet, G., Brandenberger, H., *J. Chromatog.* **4**, 396-409 (1960).
 Rabin, R. S., Klein, R. M., *Ann. Biol. Animale Biochim. Biophys.* **70**, 11 (1957).
 Reynolds, T. M., *Advan. Food Res.* **12**, 1 (1963).
 Reynolds, T. M., *Advan. Food Res.* **14**, 167 (1965).
 Ryder, W. S., *Advan. Chem. Ser.* **56**, 70 (1966).
 Scarpati, M. L., Guiso, M., *Ann. Chim. (Rome)* **53**, 1315 (1963).
 Scarpati, M. L., Esposito, P., *Ann. Chim. (Rome)* **54**, 35 (1964).
 Schwimmer, S., *Nature* **180**, 149 (1957).
 Sivetz, M., Foote, H. E., "Coffee Processing Technology" Vol. II, pp. 165-7, Tables 88 and 89, Avi, Westport, Conn., 1963.
 Sondheimer, E., *Arch. Biochem. Biophys.* **75**, 131 (1958).
 Sondheimer, E., Griffin, D. H., *Science* **131**, 672 (1960).
 Sondheimer, E., Szymanski, C. D., Corse, J., *J. Agr. Food Chem.* **9**, 146 (1961).
 Stoll, M., Winter, M., Gautschi, F., Flament, I., Willhalm, B., *Helv. Chim. Acta* **50**, 628 (1967).
 Streuli, H., *Chimia (Aarau)* **16**, 371 (1962).
 Talley, E. A., Porter, W. L., *J. Agr. Food Chem.* **16**, 212 (1968).
 Thaler, H., *Z. Lebensm.-Untersuch.-Forsch.* **106**, 125 (1957).
 Thaler, H., *Z. Lebensm.-Untersuch.-Forsch.* **110**, 442 (1959).
 Thaler, H., *Z. Lebensm.-Untersuch.-Forsch.* **125**, 369 (1965).
 Thaler, H., Arneth, W., 3rd Int. Collog. on Coffee, Trieste, **1967**, p. 127.
 Thaler, H., Gaigl, R., *VI Z. Lebensm.-Untersuch.-Forsch.* **118**, 22-32 (1962).
 Thaler, H., Gaigl, R., *VII Z. Lebensm.-Untersuch.-Forsch.* **119**, 10-25 (1962), C. A. **58**, 11897 (1963a).
 Thaler, H., Gaigl, R., *VIII Z. Lebensm.-Untersuch.-Forsch.* **120**, (5) 357-63 (1963), C. A. **59**, 14865 (1963b).
 Thaler, H., Gaigl, R., *IX Z. Lebensm.-Untersuch.-Forsch.* **120**, (6) 449-54 (1963), C. A. **60**, 11292 (1964).
 Underwood, D. E., Deatherage, F. E., *Food Res.* **17**, 425 (1952).
 Waiss, A. C., Jr., Lundin, R. E., Corse, J., *Chem. Ind.* **1964**, p. 1984.
 Wiseblatt, L., Zoumat, H., *Cereal Chem.* **40**, 162 (1963).
 Woodman, J. S., Giddey, A., Egli, R. H., "The Carboxylic Acids of Brewed Coffee," A.S.I.C. (Trieste) 1967, pp. 137-43.
 Wolfrom, M. L., Plunkett, R. A., Laver, M. L., *J. Agr. Food Chem.* **8**, 58 (1960).
 Wolfrom, M. L., Laver, M. L., Patin, D. L., *J. Org. Chem.* **26**, 4533 (1961).
 Wolfrom, M. L., Patin, D. L., *J. Agr. Food Chem.* **12**, 376 (1964).
 Wolfrom, M. L., Patin, D. L., *J. Org. Chem.* **30**, 4060 (1965).

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