Carob Bean Aroma Dependence on Roasting Conditions

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The carob bean, commonly known as locust bean and St. John's Bread, is the dried fruit of Ceratonia siliqua, a leguminous tree native to eastern Mediterranean countries and commercially cultivated throughout most of the Mediterranean region in countries such as Spain, Greece, Cyprus, Turkey, Italy and Portugal, and in the Arabian peninsula.[1] As harvested, the mature bean is a dark brown pod averaging 4-12 inches long and 0.25-0.75 inches thick; it contains 5-16 brown bony seeds. Since ancient times, the carob tree has seen a number of versatile uses, including nourishing beverages for human consumption, a liquor and coffee substitute (carob coffee), and medicines such as laxatives and diuretics.[2] However, its major use was and still is as animal feed, because the deseeded pulp of the carob bean (up to 90% of fruit dry weight) has limited nutritional use due to its low protein content and the presence of highly condensed polyphenols.[3,4]

Sophisticated technologies in the food/flavor industry have spawned recent discoveries and new uses for carob in the modern market. The dried and powdered endosperm of the seeds is used for the manufacture of locust bean gum[5] and is employed, primarily in the food industry, as an emulsifier/stabilizer in products such as ice cream, cheese, salad dressings and doughs.

The carob pulp composition is well documented,[6-11] although variations have been observed according to varieties and cultivars. The pulp is rich in sugar (40-50% of the dry matter), low in protein (2.7-3% of the dry matter) and lipids (0.4-0.8% of the dry matter), and high in phenolic compounds (mostly, highly condensed tannins) that account for nearly 20% of the dry matter. The high, easily extractable sugar content of the pulp makes this material suitable for syrup production,[12,13] with "carob sugar" manufactured at the industrial level in Portugal.[14] Fermentation studies have also investigated its use as a suitable substrate for manufacture of ethanol and single cell protein.[15-17] The majority of the carob pulp is milled for the production of carob powder, which is roasted in order to enhance its cocoa-like flavor for its use as a cocoa/chocolate substitute and extender in the food industry. Carob's extremely low fat content (<1%), combined with high levels of natural fruit sugars and the absence of caffeine and theobromine, give carob powder great potential as a component in the coloring, flavoring and sweetening of a wide range of confectionery products, beverages, biscuits and cakes sold in health food stores worldwide.

The significance of carob as a flavoring and food additive is clear, and its many useful applications within the food industry are continually expanding. However, research and reporting on its volatile/aromatic composition is scarce. Data of this type have been mainly proprietary in the food/flavor industry and scarcely reported in the academic journals and literature. No meaningful literature is presently available pertaining to carob's flavor generation during the roasting process. Therefore, the necessity for characterization of the flavor constituents of carob and its roasted powders is apparent. The objective of the work described in this article is a) to analyze and compare the flavor components of carob and its different degrees of roast, and b) to describe the chemistry of carob flavors generated during the roasting process. Our samples are a typical commercial unroasted carob and four roasted carob powders presently being used in the food industry.

Experimental

Materials: Commercial unroasted carob kibbles and four of its roasted powders were purchased under the trademark "Virginia Roast Carob Powder" from Famarco Ltd, Inc. (Virginia Beach, Virginia). The roasted carob powders are referred to as FM-20, FM-40, FM-60 and FM-80, with each larger number designating a higher roasting temperature used during processing.

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Preparation: The unroasted kibbled carob sample was ground in the presence of dry ice in a Bell Art bench-top grinder from Micromill (Cleveland, Ohio) and passed through a 200-mesh sieve to ensure particle consistent with the size of the roasted carob powder samples. The sample holders, $\frac{1}{4}$-inch x $13\frac{3}{8}$-inch glass tubes, were washed, then dried at 100°C for 30 minutes. The prepared samples (100 mg) were then weighed into the sample holders and positioned between two plugs of silanized glass wool. A mixture of 1.012 mg each of δ-8-naphthalene and δ-8-toluene internal standards (that is, eight deuterium atom substituted naphthalene and toluene) per ml of diethyl ether was prepared, and 1 µl of this mixture was administered to the plug at the side where the carrier gas passed through the sample.

Purge and trap: The volatiles were purged in a Scientific Instrument Services solid matrix sampling oven at 80°C using nitrogen at a flow rate of 40 ml/min and a duration of 30 minutes. Volatiles were trapped using silanized glass-lined stainless-steel desorption tubes (30 mm i.d. x 10 cm) with a 4 cm total bed volume containing an equal mixture of Tenax TA[a] and Carbo Trap[b] adsorbents. Following isolation of the flavor volatiles, this adsorbent trap was further purged with dry nitrogen gas for 30 minutes to remove traces of accumulated water vapor.

Short path thermal desorption—GC/MS: The short path thermal desorption technique permits analysis of solid samples without prior solvent extraction or elaborate sample preparation. A concise and detailed description of this method and its different applications has been published.[20,21]

Desorption of the entrapped volatiles from each sample was performed using the Short Path Thermal Desorption System from Scientific Instrument Services, Inc. (Ringoes, New Jersey). The system consists of an electronics control unit and a thermal desorption unit (Model TD-2) mounted on a Varian 3400 gas chromatograph. The short path of sample flow eliminates transfer lines that, in other desorption systems, are easily contaminated by samples. Each sample was desorbed at a temperature of 220°C for a five-minute period, utilizing helium as the carrier gas with a linear flow rate of 1 ml/min and an initial purge time of three minutes.

The sample was analyzed by GC/MS. This was accomplished by utilizing a Varian 3400 gas chromatograph equipped with an FID coupled to a Finnigan MAT 8230 high resolution mass spectrometer. The volatiles were separated using a nonpolar fused silica capillary column (60 m x .32 mm i.d., 25 µm thickness, DB-1, from J&W Scientific, Folsom, California). The GC was operated with an injection temperature of -20°C, and a temperature increase of 4°C/min from -20°C to 280°C with a 20-minute isothermal hold at the final program temperature.

The mass spectrometer ionization was set at 70 eV, and the source temperature was 250°C with a filament emission current of 1mA and a mass range of 35-350. Spectra obtained were identified by utilizing an on-line computer library (NFST/EPA/MSDC, version 3.0, June 1990), a computerized software program (Wiley Registry of Mass Spectral Data, 5th Edition, 1989) and the Eight Peak Index (MSDC, 1984).

Results

Applied to the five samples, GC/MS positively identified 79 volatiles, of which 28 are reported for the first time here as carob bean components. We labeled ten classes: 8 aliphatic organic acids, 2 aliphatic aldehydes, 8 aliphatic ketones, 10 aliphatic esters and 5 aliphatic alcohols, as well as 4 sulfur-containing compounds, 13 terpenoids, 7 furanoids/pyranoids, 10 aromatics and 1 pyrrole. Eleven other compounds were identified and labeled miscellaneous. A summary of these volatiles, identified with their respective quantification data, is presented in Table I. Unidentified components were present in such low amounts that either no mass spectrum could be recorded or the mass spectrum was too poor for interpretation.

As seen in the table, the unroasted carob powder contains the highest volatile yield of any of the powders with 509.9 ppm, while the least roasted powder, FM-20, contains the lowest concentration at 68.1 ppm. The remaining three roasts, FM-40, 60 and 80, displayed total volatile yields of 124.4, 109.3 and 115.7 ppm, respectively. The dramatic reduction in the concentrations of volatiles in the roasted powders, as compared to the unroasted powder, is a direct result of the high temperatures used in the roasting process; the volatiles vaporize when subjected to these temperatures. Subsequent roasting at higher temperatures results in darker roasted carob powders with total volatile yields nearly double that of the FM-20 roast. Interpretation of this data clearly indicates that most of the volatiles responsible for the characteristic "cocoa-like" aroma attributed to these roasted carob powders are generated during the roasting period between the "light" and "medium" stage, while further roasting brings about insignificant changes in the total volatile yield of the two remaining "darker" roasts. However, this is not to suggest that there aren't significant sensory differences between the medium and darker roasts; this data does not reflect the aromatic volatile profile of each individual sample and its overall impact upon the sample's organoleptic properties. That data will be presented later in this paper.

Volatiles Identified and Yield Comparisons

The various classes of identified volatile aroma compounds originate from the carob plant's basic nutrients, such as the carbohydrates (particularly the mono- and disaccharides), the proteins (particularly the free amino acids) and—to a lesser extent—the fats and triglycerides or their derivatives. In the following discussion we'll

[a] Tenax TA is a registered trade name of the Enka Research Institute, Arnhem, The Netherlands

[b] Carbo Trap is a registered trade name of Supelco Inc., Bellefonte, Pennsylvania

Table I. Volatiles identified and quantified in unroasted carob and its roasted powders

Compounds identified	Identified first time	R.I.*	Concentration (ppm) Unroasted	FM-20 "light"	FM-40 "medium"	FM-60 "darker"	FM-80 "darkest"
Organic acids							
acetic acid		597.0	12.7	nd	3.1	12.9	13.5
isobutyric acid		844.0	290.6	8.3	19.3	5.4	18.6
butanoic acid		849.0	14.4	2.8	3.1	4.1	2.7
2-methylbutanoic acid		896.0	12.5	5.3	4.7	4.0	1.0
isovaleric acid	x	919.0	3.1	nd	nd	nd	nd
hexanoic acid		1008.0	nd	3.4	5.1	5.4	1.3
pentanoic acid	x	1028.0	7.0	nd	nd	nd	nd
octanoic acid		1190.0	nd	1.0	1.6	1.6	0.5
Total/class	**2**		**340.3**	**20.8**	**36.9**	**33.4**	**37.6**
Aliphatic esters							
methyl isobutanoate		650.0	16.0	nd	nd	nd	nd
methyl butanoate		684.0	5.2	nd	nd	nd	nd
ethyl isobutanoate		728.0	0.5	nd	nd	nd	nd
methyl 2-methylbutanoate	x	750.0	0.6	nd	nd	nd	nd
methyl n-pentanoate	x	799.0	0.2	nd	nd	nd	nd
methyl hexanoate		914.0	30.7	nd	nd	nd	nd
isopentyl isobutanoate		1003.0	2.0	nd	nd	nd	nd
methyl octanoate	x	1108.0	0.9	nd	nd	nd	nd
ethyl 3-octenoate	x	1267.0	1.3	nd	nd	nd	nd
octyl butanoate	x	1332.0	0.9	nd	nd	nd	nd
Total/class	**5**		**58.3**	**nd**	**nd**	**nd**	**nd**
Furanoids/Pyranoids							
furfural		783	nd	nd	1.1	0.8	2.6
furfuryl alcohol		845	nd		nd	6.2	6.2
5-methylfurfural		923	nd	nd	nd	nd	trace
cyclotene		990	nd	nd	nd	nd	0.1
3-methyl-2,5-furandione		1012	nd	nd	2.0	3.8	1.4
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	x	1114	nd	trace	15.3	17.5	8.8
5-hydroxymethyl-2-furfural		1197	nd	nd	nd	2.3	26.1
Total/class	**1**		**nd**	**trace**	**18.4**	**30.6**	**45.2**
Terpenoids							
δ-limonene		1011	5.4	1.2	nd	nd	nd
1,4-terpineol		1163	nd	nd	0.9	0.5	0.3
anethole		1171	nd	nd	1.1	0.7	0.5
δ-carvone		1219	nd	nd	1.8	0.8	1.2
isothymol		1273	nd	0.6	0.9	0.8	0.6
thymol		1276	nd	0.9	1.1	0.7	0.6
eugenol		1328	nd	nd	3.1	2.0	1.0
a sesquiterpene		1375	nd	nd	1.2	0.9	0.6
β-caryophyllene		1410	nd	nd	5.5	2.7	1.9
α-humulene		1443	nd	nd	1.5	1.3	0.5
α-curcumene		1468	nd	nd	1.3	1.0	0.4
a sesquiterpene		1474	nd	nd	0.9	0.5	0.3
a sesquiterpene		1506	nd	nd	1.0	0.5	0.3
Total/class	**0**		**5.4**	**2.7**	**20.3**	**12.4**	**8.2**
Aliphatic ketones							
diacetyl		527	1.0	nd	nd	nd	nd
2-heptanone		851	39.1	nd	0.6	0.7	0.3
2-nonanone		1076	2.0	0.4	0.5	0.4	0.3
2-undecanone		1278	1.4	nd	nd	nd	nd
7-decen-2-one	x	1464	1.1	nd	nd	nd	nd
2-tridecanone		1472	2.2	0.7	0.9	0.7	0.3
2-pentadecanone	x	1675	7.7	4.1	4.9	3.3	1.9
2-heptadecanone	x	1887	1.9	2.4	2.1	1.3	0.9
Total/class	**3**		**56.4**	**7.6**	**9.0**	**6.4**	**3.7**

Table I. Continued

Compounds identified	Identified first time	R.I.*	Concentration (ppm) Unroasted	FM-20 "light"	FM-40 "medium"	FM-60 "darker"	FM-80 "darkest"
Aliphatic aldehydes							
isovaleraldehyde		596	nd	nd	0.1	0.6	0.1
2-methylbutanal		600	nd	nd	nd	0.1	0.8
Total/class	**0**		**nd**	**nd**	**0.1**	**0.7**	**0.9**
Aliphatic alcohols							
1-butanol		657	nd	nd	0.9	nd	nd
2-nonanol		1087	1.7	nd	nd	nd	nd
2-undecanol	x	1297	0.8	nd	nd	nd	nd
2-tridecanol	x	1495	1.2	nd	nd	nd	nd
2-pentadecanol	x	1695	1.4	1.6	1.7	1.1	0.7
Total/class	**3**		**5.1**	**1.6**	**2.6**	**1.1**	**0.7**
Sulfur-containing							
allyl methyl sulfide	x	640	0.6	nd	nd	nd	nd
dimethyl disulfide		687	0.5	nd	nd	nd	nd
dimethylthiophene	x	846	14.8	0.7	nd	nd	nd
cyclohexyl isothiocyanate		1196	nd	1.3	2.4	1.2	nd
Total/class	**2**		**15.9**	**2.0**	**2.4**	**1.2**	**nd**
Pyrroles							
2-acetylpyrrole		1032	nd	1.2	2.3	1.8	1.0
Total/class	**0**		**nd**	**1.2**	**2.3**	**1.8**	**1.0**
Aromatics							
benzene	x	597	0.1	2.3	0.4	0.2	0.7
benzaldehyde		922	nd	trace	0.9	trace	trace
benzoic acid		1172	nd	nd	0.3	1.1	1.3
4-isopropylbenzaldehyde		1217	nd	nd	2.9	1.7	2.1
cinnamic aldehyde		1240	nd	nd	nd	1.1	0.8
2,6-di-tert-butyl-p-benzoquinone		1442	nd	nd	0.9	trace	nd
BHT		1493	nd	1.2	1.5	1.0	0.5
2,4-di-tert-butylphenol		1499	nd	0.9	nd	nd	nd
6,6-dimethylcyclooct-4-en-1-one	x	1526	1.2	nd	nd	nd	nd
2,5-di-tert-amylquinone	x	1636	1.6	3.1	1.0	1.0	0.3
Total/class	**3**		**2.9**	**7.5**	**9.1**	**6.1**	**5.7**
Miscellaneous							
ethyl isopropyl ether	x	690	2.1	nd	nd	nd	nd
n-decane	x	991	1.0	0.4	0.9	0.5	0.2
5-undecen-3-yne		1254	nd	nd	0.9	0.6	0.3
3-methyl-5-propylnonane		1348	nd	nd	0.4	0.4	0.8
hexadecane	x	1597	0.6	0.5	0.6	0.5	0.2
12-methylpentadecane	x		0.9	0.7	0.8	0.6	0.3
hexahydrofarnesyl acetone	x	1738	1.8	2.0	2.1	1.5	1.0
1-octadecane	x	1819	15.0	15.6	14.8	9.2	8.5
methyl 14-methylpentadecanoate	x	1903	2.1	2.1	1.4	1.2	0.8
ethyl palmitate	x	1970	0.6	1.4	0.6	0.4	0.2
methyl octadec-10-enoate	x	2086	1.5	2.0	0.8	0.7	0.4
Total/class	**9**		**25.6**	**24.7**	**23.3**	**15.6**	**12.7**
Total/sample	**28**		**509.9**	**68.1**	**124.4**	**109.3**	**115.7**

* R.I. is the retention index, based on the dry weight of the carob bean
nd = none detected
trace = < 0.1 ppm

consider the role of these basic nutrients in the chemistry of the important volatiles in each class.

Organic acids: Observation of Table I immediately shows the presence of an extraordinarily high level of aliphatic acids in the unroasted carob. They originate largely from the high sugar content of the carob pulp, with a smaller contribution from the lipids present.[1] At a concentration of 340.3 ppm, these aliphatic acids represent as much as 66.8% of the total volatiles isolated. That is a very high proportion for any given class of volatile components in comparison with most fruits and vegetables.

The major contributor is isobutyric acid, which represents 56.7% of the volatiles identified in the unroasted carob and as much as 85.4% of the acidic fraction alone. Although no reference or publications are available detailing its formation in the carob fruit, its biosynthesis may be the result of the intracellular metabolism of certain fermentative organisms present in the pulp of the carob and their catabolic action upon both the amino acids and carbohydrates present.[23] Other methods of formation are via the Strickland reaction[24] and/or the isomerization of butanoic acids via butyric acid-forming microorganisms. Smaller contributions were made by butanoic, acetic, 2-methylbutanoic, isovaleric and pentanoic acids. This supports the data published by Stubbs et al.[18] and MacLeod and Forcen[19] with the exception of pentanoic acid, which was not identified by either party.

All the roasted powders exhibited far less organic acids relative to those reported for the unroasted carob (approximately 10%), but these acids still represented approximately 30% of the total volatiles identified for each roast. Again, isobutyric acid was the major contributor, with acetic acid following.

Aliphatic esters: Ten aliphatic esters were identified in the unroasted carob. They constitute the largest class of volatiles of the non-acid fraction. With a concentration of 58.3 ppm, they represent 11.4% of the total volatile yield. They probably occurred as a result of the esterification of those acids formed by the metabolism of the carbohydrates, lipids and amino acids present in the carob fruit with methyl, ethyl, isopentyl and octyl alcohols. No ester components were identified in any of the roasted carob powders.

Aliphatic aldehydes: No aliphatic aldehydes were isolated in the unroasted carob. Two were identified in the roasted powders at levels of less than 1% for the darker and darkest roasts. These aldehydes—isovaleraldehyde and 2-methylbutanal—are the Strecker aldehydes resulting from the Strecker degradation of leucine and isoleucine, respectively, and are products of the Maillard reaction. Their extremely low concentration may be a result of the small amount of protein existing in the carob fruit in an unbound available form.[9]

Aliphatic ketones: The eight aliphatic ketones identified in the unroasted carob constitute the second largest class of volatiles of the non-acid fraction, with 2-heptanone contributing the most at 70%. With the exception of diacetyl, they are primary products of the oxidation of the lipids present in the carob fruit. Diacetyl is a well-known sugar degradation product and is produced via the fermentation of citrate. All the roasted powders yielded far less of the aliphatic ketones than did the unroasted carob; the darkest roast contained approximately 3%.

Sulfur-containing compounds: The three sulfur-containing compounds—allyl methyl sulfide, dimethyl sulfide and dimethylthiophene—identified in the unroasted carob at 15.9 ppm, represent 3.1% of the total volatile yield and may result from enzymatic reactions involving the hydrolysis of the non-volatile glucosinolates by enzymes, such as thioglucosidases, present within the cellular structure of carob.[25] This may occur as a result of the disruption of the cellular structure during the kibbling process in which an industrial commutator coarsely breaks up the carob pods into deseeded pieces ranging from $\frac{1}{4}$-inch to $\frac{1}{2}$-inch in length. The sulfur volatiles could also have been formed non-enzymatically by the Strickland degradation of methionines.[26]

Three of the roasted powders, FM-20, 40 and 60, displayed a substantially lower concentration of sulfur volatiles than that reported for the unroasted carob. A fourth sulfur compound, cyclohexyl isothiocyanate, was identified in all three of these roasts and may result from thermal degradation of the glucosinolates present in the carob. No sulfur volatiles were identified in the darkest roast, FM-80.

Terpenoids: δ-Limonene, the only terpenoid identified in the unroasted carob, represents 1% of the total volatile yield. It is a product of the biochemical reactions directly associated with the plant's metabolism of carbohydrates and is of no surprise since carbohydrates, in the form of sugars, exist in an extremely large amount in the carob fruit.

Twelve other terpenes or their oxygenated derivatives were identified only in the roasted powders and result from the thermal processing. The medium roast, FM-40, had the highest yield at 20.3 ppm, representing 15.5% of its total volatile concentration. β-Caryophyllene and eugenol were the main contributors at 28% and 16%, respectively. δ-Carvone, a well-known oxidation product of δ-limonene, was also identified.[27]

Of these twelve terpenoids, six are oxygenated monoterpene derivatives. Four of the six are monoterpene alcohols, which are of some interest because these compounds are the major contributors to the distinctive odors and flavors of many plants. Their formation has been the subject of a number of studies,[28-30] and their absence in the unroasted carob is probably due to their existence as the aglycon moiety of a glycoside. It has become well known through studies of fruits such as pineapple, ginger, passion fruit, grapes and tomatoes,[31-35] that significant quantities of

glycosidically bound flavor constituents are contained within the glycoside, and that some of the terpenoid alcohols originate from nonvolatile terpenoid glycosides through the action of enzymes, acid and/or heat. These glycosides may include the monosaccharides (i.e., glucose, fructose) or the disaccharides (i.e., sucrose) identified in the carob and its roasted powders in this research, but not reported here because the saccharides are not volatiles. The effect of the roasting process on the volatile yield of terpenoids can be seen in Table I. Interpretation of these results clearly establishes that all of the oxygenated monoterpenes exist entirely as the aglycon moiety of glycosides present in the unroasted carob, and those monoterpenes can be transformed into important aroma components by chemical, enzymatic and/or heat catalyzed reactions. This is the first time that the existence of glycosidically bound terpenes in the carob fruit has been established and reported in the literature.

Furanoids/pyranoids: No furan/pyran compounds were identified in the unroasted carob. This is to be expected, since these volatiles are the product of sugar degradation brought about by dehydration reactions at high temperatures.

Six furans and one pyranoid compound were isolated in the roasted powders, with only the pyranoid 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (hydroxydihydromaltol) common to all the roasts. While only trace amounts of furanoids and pyranoids were quantified in the least roasted carob powder, 18.4, 30.6 and 45.2 ppm were quantified for the increasingly darker roasts. This represented 14.8%, 28% and 39% of their respective total volatile yields. Hydroxydihydromaltol was the major component in both the FM-40 and FM-60 powders, representing 83.1% and 57.2% of this fraction, while methyl-4H-pyran-4-one 5-hydroxy methyl-2-furfural (HMF) at 58% was the major contributor in the darkest roast. Thus, the general trend observed throughout the entire roasting process is the absence of furans and pyrans in the unroasted sample and their generation in the roasted samples, with their concentration increasing as the processing temperature of the roast increases.

All the furan/pyran compounds result directly or indirectly from the thermal degradation of the high sugar content present in the carob. The thermal degradation of these sugars produces both acids that contribute to their degradation and an ionic environment that results in the dehydration of the sugar molecules, favoring the formation of those furanoids and pyranoids identified in this study. Thus, furfural, HMF and furfuryl alcohol result from the dehydration and subsequent rearrangement/cyclization of the principal sugars present in the carob and its roasted powders. 5-Methylfurfural and cyclotene may result from the thermal degradation of HMF and hydroxydihydromaltol, respectively.[36]

The only pyranoid compound identified, hydroxydihydromaltol, is found in all of the roasted powders and is generated in increasing yields as the roasting temperatures

increase. The largest amount of hydroxydihydromaltol is generated in the FM-60 roast with a comparable yield observed in the intermediate roast. This suggests that the temperatures used to produce those roasts favor the pyranoid's production in the carob fruit system. Its decrease in the darkest roast, FM-80, indicates it may be thermally labile at those higher roasting temperatures. Hydroxydihydromaltol's generation results from the thermal degradation of the high amount of fructose present in the carob fruit (fructose is one of the sugars identified in carob and its roasted powders in this study), and its formation is of great interest since it is present in all the roasted powders and contributes greatly to the "cocoa-like" aroma associated with roasted carob powders. Whether or not hydroxydihydomaltol is the intermediate in the formation of maltol cannot be definitively answered based on the data obtained. However, the hypothesis that hydroxydihydromaltol is not a precursor of maltol[37] is supported by the results of this study since no maltol was identified in any of the roasted powders. As previously discussed, the decrease in the concentration of hydroxydihydromaltol in the roast receiving the severest roasting process is attributed to the compound's instability at those temperatures, rather than to dehydration leading to the formation of maltol. Therefore, the absence of maltol in these roasted powders supports the mechanism of hydroxydihydromaltol formation proposed by Yaylayan and Mandeville,[37] rather than the mechanism of hydroxydihydromaltol as an intermediate of maltol.

Pyrroles: Only one pyrrole compound, 2-acetylpyrrole, was identified and found to be common to all roasted samples in low concentrations. The medium roast, FM-40, contained the highest level, representing 1.8% of the total volume yield for that sample. As seen in Table I, the trend of 2-acetylpyrrole's formation is directly related to the roasting process and is a result of the thermal degradation of the sugars and amino acids via the Maillard reaction pathways.

Aromatics: Three aromatic compounds were identified in the unroasted carob sample. They represented a low level of the total volatile yield at 0.6%. Seven other aromatics were identified in the roasted powders with the FM-40 powder having the highest level at 9.1 ppm and representing 39% of the total volatile yield for that roast. Of the ten aromatic compounds identified, only one, 2,5-di-tert-amylquinone, was common to all five samples analyzed. These aromatics are based on benzene and result from biochemical reactions directly associated with the plant's metabolism.

The seven aromatics seen in the roasted powders result from the thermal processing of the carob. The three aldehydes of this class—benzaldehyde, 4-isopropylbenzalde-hyde and cinnamic aldehyde—may be glycosidically bound to the sugars present in the carob fruit and liberated during the roasting process. Glycosidic benzaldehyde has been reported in bitter almond, ginger,[32] peaches and apricots.[38]

Miscellaneous: Eleven other compounds were identified, including one ether, five hydrocarbons, two ketones and three esters. Although the unroasted carob displayed the highest individual yield at 25.6 ppm, this concentration represented the lowest total-volume yield of all the samples (5%) while the highest total-volume yield (36.2%) was observed in the least roasted carob powder. Most of the miscellaneous compounds are long chain, saturated/unsaturated, high molecular weight molecules having little or no effect upon aroma and flavor characteristics of carob and its roasted powders, and they are either products of the plant's metabolism or result from the numerous reactions occurring during the roasting process.

Sensory Observations

We evaluated and compared the aroma and the flavor of carob in relation to the volatiles identified, and as a function of the roasting process. The results are shown in Table II.

An immediate observation was the powerful, diffusive sour/acrid odor present in the unroasted carob. This is due to the extremely high level of isobutyric acid present, and to the presence of other organic acids such as butanoic and acetic which contribute, respectively, to the rancid and pungent odors. The strong fruity aroma is characteristic of the large amount of aliphatic esters present. A slight cheesy note is perceptible and probably due to the methyl ketones, such as 2-heptanone. The sulfurous notes are due to the sulfur compounds identified in the carob. The overall flavor of the unroasted carob is somewhat creamy-fruity because the powerful

Table II. Effect of roasting upon the flavor of carob

Sample	Aroma	Taste
unroasted	fruity sour acrid/pungent slightly rancid/cheesy/ sulfurous	creamy-fruity sweet
FM-20	little "cocoa-like" faintly acrid/pungent	creamy-fruity sweet little "cocoa-like"
FM-40	"cocoa-like" spicy	sweet "cocoa-like" faintly spicy
FM-60	"cocoa-like" less spicy	less sweet caramellic rich "cocoa-like"
FM-80	weak "cocoa-like" slightly burnt	bitter-caramel "cocoa-like" burnt

sour, buttery-cheesy notes attributed to the high level of isobutyric acid are adequately diluted by the high levels of sugars also present.[39]

The initial roasting process drastically decreases the level of all the volatiles responsible for carob's characteristic odor, while generating trace amounts of sugar degradation products which result in a low level of "cocoa-like" aroma and flavor. The fruity aroma attributed to the aliphatic esters is absent due to their complete vaporization, while a similar decrease in the level of organic acids, notably isobutyric, also occurs, resulting in a roasted carob powder having a faintly acrid aroma. The initial roasting process also significantly decreases the aliphatic ketones and sulfur volatiles, so they do not effect the aroma to any extent. A perceptible "cocoa-like" odor results from the trace amount of hydroxydihydromaltol generated. The generation of 2-acetylpyrrole contributes a pleasant caramel aroma that complements that of hydroxydihydromaltol. The taste of the FM-20 roast is still creamy-fruity and probably due to the dilution of the isobutyric acid and concomitant decrease of the other volatiles via the roasting process. The powder is also very sweet due to the large amount of sugars present, as well as the inversion of the sucrose to glucose and fructose.

The aroma of the FM-40 roast is "cocoa-like" without the presence of the sour/acrid note described in the previous roast, due mainly to the dramatic increase of hydroxydihydromaltol. Interestingly, a distinct spicy note is also discernible; a result of the thermal acid-catalyzed hydrolysis of glycosides liberating oxygenated monoterpene compounds as well as sesquiterpenes. The absence of the spicy note in the previous samples tested is due to these highly odoriferous volatiles existing in "bound" form as the aglycons of glycosides present in the carob. The taste of the medium roast is sweet and "cocoa-like" for the same reasons stated for the FM-20 roast. The terpenoids are responsible for a warm, faintly spicy taste complementing the sweet taste from the high sugar content.

The aroma of the FM-60 roast is similar to that of the FM-40 roast, but more "cocoa-like" since the spicy note is fainter due to the thermal degradation of the terpenoids and the simultaneous increase of hydroxydihydromaltol at these higher roasting temperatures. As a result, the FM-60 roast has a rich "cocoa-like" taste that is less sweet and caramellic due to the further degradation of the high sugar content and subsequent formation of caramel.

The most roasted carob powder, FM-80, displays a weak "cocoa-like" aroma due to the thermal degradation of hydroxydihydromaltol. The slight burnt note detected is due to advanced sugar and volatile degradation at extremely high temperatures leading to the formation of caramel and polycarbonyl compounds. The taste is somewhat bitter due to the presence of caramellic compounds, with an accompanying "cocoa-like" after-taste due to a lower level of hydroxydihydromaltol in relation to that of the previous roast. Due to the high temperatures used, there is little if any sweetness, leading to a dark, roasted powder with a distinct burnt taste.

Overall Trend of Roasting

The overall trend of roasting and its effect upon the aroma and flavor of carob is evident from Tables I and II. The unroasted carob displays a characteristic sour/acrid aroma due to the presence of an unusually large amount of organic acids. It also displays odoriferous notes originating from a variety of other volatiles that result from the carob bean's biochemical process during maturation. The initial roasting process drastically decreases the level of all the volatiles responsible for the characteristic aroma of carob, while generating trace amounts of sugar degradation products, such as hydroxydihydromaltol, that give the roasted carob powder a low level of "cocoa-like" aroma and flavor. Continued roasting decreases sugar content and generates thermal degradation products (i.e., furans/pyrroles) in amounts that produce a "cocoa-like" flavor in subsequent roasts. It liberates bound volatiles (monoterpene alcohols and sesquiterpenes), existing as the aglycons of glycosides present in the carob bean. The roasting parameters used in the production of the medium and dark carob powders

(i.e., FM-40 and FM-60, respectively) result in powders having aromas and tastes characteristic of cocoa with some spicy notes. Further roasting at extremely high temperatures results in a carob powder having a weaker cocoa flavor with a slightly burnt note and bitter taste characteristic of caramel.

Conclusions

From this study, it is clear no single or small group of compounds is entirely responsible for the characteristic aroma and taste of carob and its roasted powders. It was hypothesized for the first time that the terpenoid fraction identified in the roasted carob powders exists as aglycons of glycosides present in the carob bean before it is subjected to the roasting process. Interpretation of the GC/MS data, sugar analysis (not presented) and sensory observations attributed the "cocoa-like" and caramellic aromas perceived as due mainly to the thermal degradation of the high sugar content, and secondarily to subsequent formation of hydroxydihydromaltol, furanoids and 2-acetylpyrrole (a Maillard reaction product). Finally, it was concluded that the overall aroma and flavor profile of the roasted carob powders is almost entirely due to the thermal degradation of the high sugar content and its related reaction pathways, rather than to any Maillard reaction. This conclusion is supported by the fact that those heterocyclic volatiles normally resulting from the Maillard reaction (volatiles such as thiophenes, pyrazines and pyridines) were not identified in any of the roasted carob powders.

Future work should be focused in several areas.

- We need more comprehensive studies viewing sugar degradation/Maillard reactions as a myriad of simultaneous reactions that determine the aroma/flavor profile of foods such as carob. Control of the processing conditions will dictate the direction and extent of each reaction, leading to a collective understanding of the reactions and, eventually, to a means of controlling their outcome.
- We should examine the differences between findings reported here and findings reported elsewhere in the literature, particularly with regard to a) aroma constituents of the unroasted carob, b) identification of sugar degradation products (i.e., the furans, a pyrrole and pyrazine), and c) identification of terpenes. Our research experiment did not identify any sugar degradation products in the unroasted carob and found only one terpene there. We confidently claimed that these products (with the exception of the lone terpene) were generated by the roasting process. Perhaps the different findings by other researchers (such as MacLeod and Forcen[19]) resulted from their use of sample preparation and isolation techniques that did not incorporate heat.
- We should focus on the terpene fraction existing in the carob bean and on the generation of terpenes during the roasting process, since knowledge of the reaction pathways involved would lead to a better understanding of the flavor potential existing in the carob fruit.

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