is 80–90% water, the concentration of vitamin C on day 2 of the experiment would range from 1.2×10^{-3} to 1.4×10^{-3} M. As shown in the cell-free experiments, this is definitely within the range of maximum activation at pH 4.45.

The results of this study indicate that food processing may have a significant effect upon the enzymatic release of natural flavorants and toxicants.

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Volatile Flavor Components of Licorice

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Sixty-three compounds never found before in heated licorice essential oil have been identified by use of GLC, GLC–MS coupling, and IR spectrometry. In addition, a few preliminary results on some components identified in unheated licorice juice are reported. Many heated licorice compounds are furan derivatives; this fact may be due to pyrolysis and condensation reactions which may occur during heating between sugars of which licorice roots are very rich. The most abundant components are: acetol, propionic acid, 2-acetylpyrrole, 2-acetylfuran, and furfuryl alcohol. None of the identified compounds is alone responsible for licorice flavor, while on the other hand, total extract shows a typical licorice aroma, indicating that this may be due to an integrated response to the proper mixture of the proper volatiles, rather than to the odor of one or two components.

Continuing our past studies on volatile drug components (Frattini et al., 1976), we have obtained some results in a few preliminary experiments carried out on samples of unheated licorice (Frattini et al., 1976). We now report our data on heated licorice flavoring components compared with those of unheated licorice.

Licorice (*Glycyrrhiza glabra* L.) is a very well-known plant since antiquity, known in ancient Egypt and in India as well as in Greek–Roman medicine. Since early times it has been employed as an emollient and expectorant; it is still under discussion whether it has therapeutic value for the treatment of gastric ulcer or not. Generally, licorice is employed in confectionery and beverages industries; the commercial product comes from the roots heated at 130–150 °C: juice is collected and solidified in black pats with typical flavor and taste.

Glycyrrhiza glabra L. var. *typica* grows in Italy, while the variety *glandulifera*, also named Russian licorice, grows in Russia, Turkey, and Asia Minor.

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Owing to its great pharmacological and commercial interest, components of licorice have been widely studied; we know many of its active principles and, particularly, substances which give it taste: glycyrrhetic acid, liquiritigenin, liquiritin, and glycyrrhizin (the latter is responsible for its sweet taste, with a sweetening power 50 times greater than sugar). Very little, however, is known of its volatile flavor components, although its essence oils have been mentioned (Haensel, 1899; Isaev, 1934).

In our analyses we use GLC, GLC–MS coupling, and infrared spectrometry. Each approach is discussed below. Sixty-three compounds (Table I), never found before in heated licorice, have been identified by comparison of their retention times and mass and/or infrared spectra with those of authentic samples or on the basis of literature spectral data.

EXPERIMENTAL SECTION

Licorice pats (8 kg) (manufactured in Calabria by handicraftsmen or obtained from foreign sources) were crushed and suspended in water (6 L). This slurry was extracted in Soxhlet with 6 L of dichloromethane for 1 week. The extract, concentrated to 250 mL, was again water-suspended and steam distilled in a two-phase continuous distiller (1 L of water, 0.3 L of dichloromethane). Distillation was controlled at regular intervals; the qualitative composition of the extract was constant

Table I. Licorice Essential Oil Components

Identified Compounds	Mol wt	GC-MS	t_R	IR
Acids, anhydrides, and esters				
Propionic acid	74	+	+	
Hexanoic acid	116	+	+	
Octanoic acid	144	+	+	
Nonanoic acid	158	+	+	
Benzoic acid	122	+	+	
Phenylpropionic acid	150	+	+	
Butyric anhydride (tentative)[a]	158	+		
Ethyl palmitate	284	+	+	
Ethyl linoleate	308	+	+	
Ethyl linolenate	306	+	+	
Ethyl phenylacetate	164	+	+	
Butyl phthalate	278	+	+	
Butyrolactone	86	+	+	+
γ-Heptalactone	128	+	+	+
γ-Nonalactone	156	+	+	
Aldehydes, ketones, and hydroxy ketones				
Tiglaldehyde (tentative)	84	+		
Benzaldehyde	106	+	+	
Methyl ethyl ketone	72	+	+	
Acetol	74	+	+	+
1-Butanol-2-one (tentative)	88	+		
1-Butanol-3-one	88	+	+	
Acetoin (tentative)	88	+		
2-Hydroxy-3-methyl-2-cyclopenten-1-one	112	+	+	+
4-Hydroxy-4-methyl-2-pentanone	116	+	+	+
Alcohols and glycols				
α-Terpineol	154	+	+	+
2-Phenylethanol	122	+	+	
2,3-Butanediol	90	+	+	
1,2-Heptanediol	132	+	+	
Phenols				
Phenol	94	+	+	+
o-Methoxyphenol	124	+	+	
p-Methoxyphenol	124	+	+	
Carvacrol	150	+	+	
2,4-Dihydroxyacetophenone	152	+	+	
Furan derivatives				
Furfural	96	+	+	
Furfuryl alcohol	98	+	+	+
5-Methyl-3-hydrofuran-2-one	98	+	+	
2-Methyl-2-tetrahydrofuran-3-one	100	+	+	+
2-Acetylfuran	110	+	+	
5-Methylfurfural	110	+	+	+
Dihydrobenzofuran	120	+	+	
2-Acetyl-5-methylfuran	124	+	+	
1-(2-Furyl)-2-propanone (tentative)	124	+		
Furyl ethyl ketone	138	+	+	
1-(5-Methyl-2-furyl)1,2-propanedione	152	+	+	+
Furfuryl formate	126	+	+	
Furfuryl acetate	140	+	+	
Furfuryl propionate	154	+	+	
Furfuryl butyrate	168	+	+	
2,2-Difurylmethane	148	+	+	
2,2-Difurylethane (tentative)	162	+		
2,2-Difurylethylene (tentative)	160	+		
Difurfuryl ether	178	+	+	
1-Furfuryl-2-formylpyrrole	175	+	+	
1-Furfuryl-2-acetylpyrrole	189	+	+	
2,4-Difurfurylfuran (tentative)	220	+		
Heterocycle compounds				
2-Acetylpyrrole	109	+	+	+
1-Methyl-2-formylpyrrole	109	+	+	
2-Formyl-5-methylpyrrole	109	+	+	+
Pyrazole (tentative)	68	+		
2,6-Dimethylpyrazine	108	+	+	
2-Ethyl-6-methylpyrazine	122	+	+	
Trimethylpyrazine	122	+	+	
Maltol	126	+	+	+

[a] "Tentative" refers to compounds which were identified only on the basis of a comparison of their mass spectra with those recorded in the literature. Positive identification is given for compounds which were identified, at least, by comparison of their retention times and mass spectra to those obtained from authentic samples.

after 18 and 30 h: distillation was stopped after 30 h because no more significant amounts of essential oil were collected. After careful concentration with a long Widmer column under reduced pressure, we obtained 4.185 g of essential oil, d^{20}_4 1.136. On standing, the oil partly crystallized to give 1.9 g of maltol in white needles (mmp,

Table II. Components Identified in Licorice Juice (Unheated)

Acids and esters	Hydrocarbons
γ-Hexalactone	Tetradecane
γ-Octalactone	*p*-Cymene
γ-Nonalactone[a]	4-Propenyltoluene
p-Ethoxybenzoic acid[b]	Ketones
Propyl *p*-hydroxybenzoate[b]	Thujone
Alcohols and ethers	Fenchone
Hexanol	Phenols
Linalool	Guaiacol (*o*-methoxyphenol)[a]
α-Terpineol[a]	Cresol
Lavandulol	Ethylphenol
Cymenol	Thymol
Benzyl alcohol	Heterocycle compounds
Phenylethyl alcohol[a]	Furylmethylketone[a]
Dimethylphenethyl alcohol	2-Methyltetrahydrofuran-3-one[a]
Linalyl oxide	Indole
4-Propenylanisol	2-Acetylpyrrole[a]

[a] Common to heated licorice (see Table I). [b] Probably additives from manufacturers.

IR, MS). Residual oil (2.272 g) (see Figure 1) was chromatographed on a silica gel 60 (70–230 mesh ASTM) Merck column (60 g; length, 20 cm; i.d., 1.2 cm) with solvents of increasing polarity. The elution sequence was pentane, pentane–dichloromethane, dichloromethane, dichloromethane–ethyl ether, dichloromethane–acetone. Forty-five fractions were eluted and analyzed by GLC, GLC–MS, or collected for infrared analyses by GLC.

GLC analyses of eluted fractions were performed with a Perkin-Elmer Model 800 and a P.E. Model 3920 gas chromatograph equipped with a FID A 2 m × 0.125 in. NPGS 5% stainless steel column, a 3 m × 0.125 in. 20% Carbowax 20M TPA, and a 2 m × 0.125 in. FFAP 5% stainless steel columns were used. Column flow rate was 25 mL of nitrogen/min. Temperatures were chosen to obtain a satisfactory separation in a reasonable time of analysis (e.g., for most fractions, temperatures were programmed from 80 to 200 °C at 6 °C/min). Total oil GLC analysis was carried out by use of a P.E. Model 900 gas chromatograph equipped with a FID: a Scot FFAP 200 ft × 0.020 in. column was employed, flow rate 6.5 mL/min, column programmed from 80 to 185 °C at 4 °C/min.

Gas chromatographic–mass spectral analyses were made by use of a modified Varian Aerograph 1200 interfaced by means of a Biemann separator with a Varian CH7A spectrometer. A 2 m × 0.125 in. stainless steel column packed with 5% NPGS on Chromosorb W, 80–100 mesh, was used. Helium flow rate was 9 mL/min in output of fore-vacuum pumps. Temperatures were again chosen to obtain a satisfactory separation in a reasonable time of analysis (e.g., for most fractions, temperatures were programmed from 80 to 200 °C at 6 °C/min). Mass spectra were recorded at 70 eV: line-off-sight inlet system temperature, 200 °C; ion source temperature, 220 °C; ion source pressure, 2×10^{-6} Torr. Infrared analyses were made with a Perkin-Elmer Model 257 infrared spectrophotometer equipped, when necessary, with a SPECAC Beam condenser 9000 in a 2-μL cell.

GENERAL COMMENTS

The data in Table I indicate that many of the licorice compounds are furan derivatives. This fact appears rather consequential if we consider that licorice roots are rich of sugars (up to 12% of glucose and saccharose) and are manufactured at 130–150 °C: during heating, pyrolysis and condensation reactions may occur, which can lead to the formation of furan or pyran derivatives. Many of these components, of course, are also present in the flavor of other natural roasted products, like coffee, cocoa, and peanuts (Van Straten and De Vrijer, 1973; Van Straten et al., 1976), but in different percentages; on the other hand, in licorice extracts we only find traces of nitrogenous compounds, such as 2-formyl-5-methylpyrrole, 1-methyl-2-formylpyrrole, and a few pyrazines. Only 2-acetylpyrrole is present in considerable amount. At the present time, since the samples of heated and unheated licorice do not have the same commercial origin, we are not able to state with sufficient certainty whether the quantity of 2-acetylpyrrole increases remarkably on heating or not. The lack of nitrogenous compounds in comparison to furan derivatives can be explained, if we consider that sugars are much more abundant than amino acids or proteins in roots.

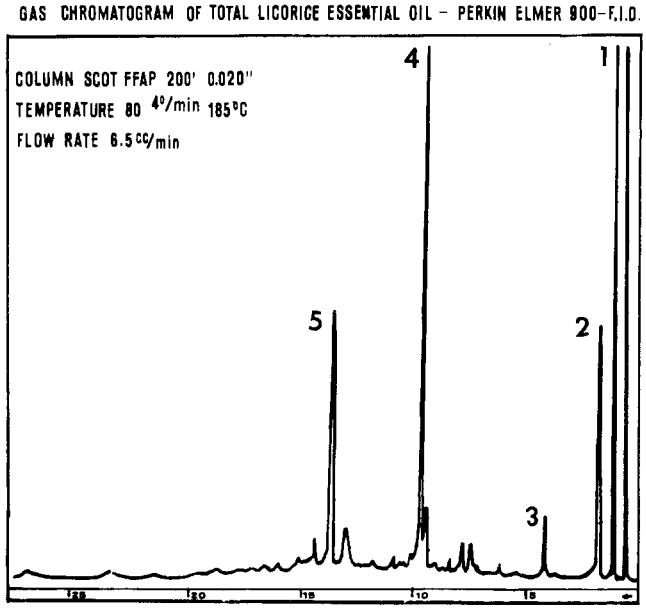

Figure 1. Numbered peaks are mainly: (1) acetol, (2) propionic acid, (3) 2-acetylfuran, (4) furfuryl alcohol, (5) 2-acetylpyrrole.

At the present time we have not identified any sulfur-containing compounds, but, obviously, we cannot exclude their presence completely. In a few experiments, previously reported (Frattini et al., 1976), carried out on samples of unheated licorice (these samples, of commercial origin, were obtained by maceration of licorice roots in water and concentration of the extracts at reduced pressure, at room temperature, and were treated in the same way described for heated licorice), we have identified some components rather different from those reported in Table I, with a remarkable decrease of furan derivatives and a large increase of terpenic constituents. This situation may be explained on the basis of the expounded theory

of sugar pyrolysis and condensation and taking into account the fact that protracted heating can remove terpenes. Twenty-eight compounds identified in unheated licorice are reported in Table II for comparison.

In conclusion, we can say that none of the identified compounds is alone responsible for licorice flavor, although many taste panels have been made on fractions, e.g., at the gas chromatographic column exit. Total extract, instead, shows a typical licorice aroma, indicating that this may be due to an integrated response to the proper mixture of the proper volatiles, rather than to the odor of one or two components.

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Quantitative Determination of 5α-Androst-16-en-3-one by Gas Chromatography–Mass Spectrometry and Its Relationship to Sex Odor Intensity of Pork

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Chemical analysis for determining the level of 5α-androst-16-en-3-one by an isotope dilution/carrier technique utilizing selected ion monitoring (SIM) mass spectrometry is described. Levels of 5α-androst-16-en-3-one were correlated with sex odor scores from a meat packing house panel and a selected laboratory panel, which resulted in "r" values of 0.27 and 0.46, respectively. Only the latter value was statistically significant ($P < 0.05$). The low relationships between odor scores and actual levels of 5α-androst-16-en-3-one lend support to the theory that other C_{19}-Δ^{16} steroids may contribute to sex odor in pork.

Meat from sexually mature boar (uncastrated male) pigs frequently gives off an offensive odor upon heating, which has been described as "urine-like" or "perspiration-like" (Craig et al., 1962). This odor not only occurs in the meat from boars and cryptorchids but has been noted to a lesser extent in the meat from sows, barrows, and gilts (Williams et al., 1963). Bishop (1969) has suggested that the low incidence of the undesirable odor in the meat from females (sows and gilts) and in castrated males (barrows) may be due to the presence of intersexes and cryptorchids, whereas, Sink (1967) has discussed other possibilities including the stage of estrus in sows and gilts, castration, and adrenal hypertrophy.

Patterson (1968) isolated 5α-androst-16-en-3-one from boar fat and concluded that it is responsible for the offensive odor, which confirmed the suggestion of Sink (1967) that the C_{19}-Δ^{16} steroids may be responsible for sex odor in pork. Beery et al. (1971) and Thompson et al. (1972) also verified the contribution of 5α-androst-16-en-3-one to sex odor in pork and presented evidence for the involvement of other C_{19}-Δ^{16} steroids. Although the relative importance of 5α-androst-16-en-3-one and the other C_{19}-Δ^{16} steroids has not been resolved, Canadian governmental meat inspection regulations require condemnation of all boar and stag carcasses, whereas, USDA (1973) regulations specify that carcasses with "slight odor" can be used in comminuted sausages and those with "strong odor" must be condemned. Obviously, the problem of meat inspection and packing house personnel properly identifying the level of sex odor in the carcasses and their ability to relate it to the amount of 5α-androst-16-en-3-one in the fatty tissues is an important one from the regulatory standpoint. Kloek (1961) has shown that there is wide variation in the ability of human subjects to smell different steroid hormones. More recently, Griffiths and Patterson (1970) have demonstrated some people are unable to smell 5α-androst-16-en-3-one, whereas, others vary widely in their olfactory reaction to this compound, with some finding it pleasant and others extremely nauseating.

Since cryptorchid pigs may be frequently encountered in the normal population of slaughter hogs, it seemed desirable to see if meat inspectors and packing house personnel could relate sex odor scores of cryptorchid pigs with the levels of 5α-androst-16-en-3-one present in the fatty tissues. Thus, the present study involved a comparison of the results from packing house olfactory tests

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