SPICES HERBS &
EDIBLE FUNGI

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PAPRIKA CHEMISTRY AND ITS RELATIONSHIP TO SPICE QUALITY

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1. INTRODUCTION

Paprika spice is the ground,dried fruit of selected cultivars of Capsicum annuum var.annuum[1a].High quality paprika has a deep red color(due to the ketocarotenoids) and a minimum pungency(burning sensation).Low pungency fruit is the result of breeding programs to select cultivars containing very low concentrations of capsaicinoids,the constituents causing pungency[1c].Since paprika is primarily utilized for its red color,quality standards for the spice are based on its carotenoid pigment content usually estimated on the basis of an absorbance measurement of the acetone extract[1d].The preparation of the spice from fresh fruits differs slightly in the various countries.The most important producers are Hungary,Spain,U.S.A.,Bulgaria, Yugoslavia and Morocco[1b].

Since the highest concentrations of carotenoid pigments occurs in the pericarp of the fruit,spice which contain only pericarp will have the highest pigment content and thus be evaluated as the highest quality.Grades which contain added dissipiment and seeds are lower quality and usually are more pungent since these parts of the fruit contain higher concentrations of capsaicinoids.A small amount of seed is said to be required for grinding[2].

2. CAPSICUM PIGMENTS

Due to the complexity of native pigment extracts,which contain primarily mono- and difatty acid esters of carotenoid alcohols and diols,hydrolyzed mixtures were used for the early pigment investigations.Figure 1 illustrates the unesterified structures of some major Capsicum carotenoids.Thirty one carotenoids have been separated by countercurrent distribution and adsorption column chromatography[3]. Recently the natural occurance of a cis isomer of capsanthin,capsanthin-3,6-epoxide and an antheraxanthin diol derivative has been established[4,5].These compounds may indicate interesting new pathways in carotenoid biosynthesis.HPLC separations of hydrolyzed pigments have been described using both normal phase and reversed phase modes[6-8].

The first report of separation of native Capsicum pigments is

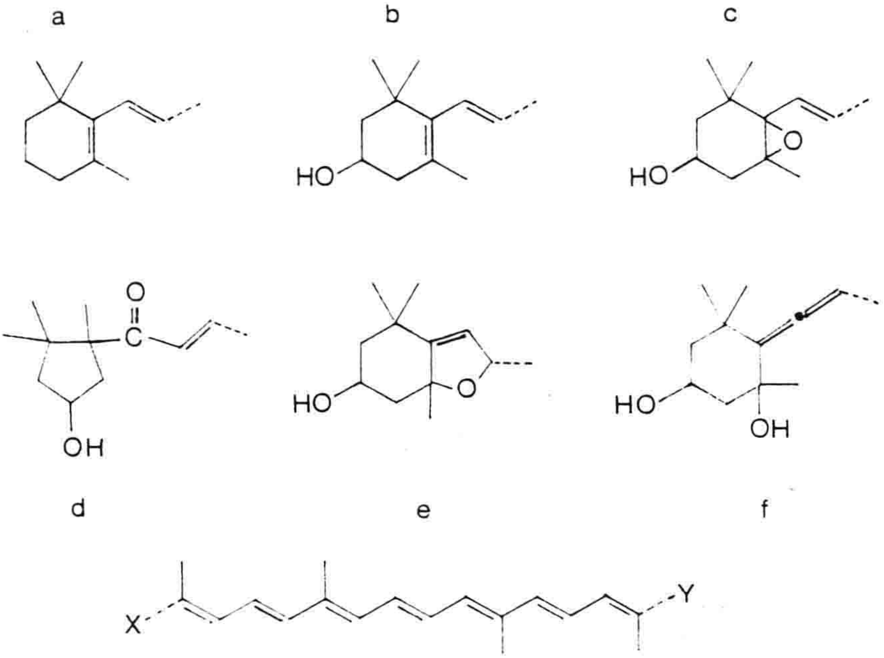

Figure 1 Structures of selected Capsicum pigments

	X	Y		X	Y
capsanthin	b	d	zeaxanthin	b	b
ß-carotene	a	a	antheraxanthin	b	c
violxanthin	c	c	capsanthin epoxide	c	d
cryptoxanthin	a	b	mutatoxanthin	b	e
capsorubin	d	d	neoxanthin	c	f
cryptocapsin	a	d			

the generally overlooked work of Egger which describes the separation of 66 carotenoid esters,probably from red bell peppers,using a combination of silica gel adsorption columns and reversed phase thin layer chromatography[9].All combinations of capsanthin diesters of lauric,myristic and palmitic acids(except the laurate-palmitate) were separated and the fatty acids identified after hydrolysis.The

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*Unsymmetrical diols like capsanthin, antheraxanthin,and capsanthin epoxide can form two isomeric monoesters and mixed diesters. Synthetic lutein monomyristate has been resolved chromatographically with semipreparative HPLC with a C-18 column and reversed phase TLC. Only one of the isomers was found naturally in squash[10]. The unsymmetrical diester isomers have not been resolved yet.

three synthetic diesters of single fatty acids co-chromatographed with naturally occurring compounds. Similar TLC separations revealed six diesters of zeaxanthin, antheraxanthin, capsorubin, violaxanthin and capsorubin epoxide. Three monoesters of cryptocapsin, cryptoxanthin, capsolutein, zeaxanthin, antheraxanthin, capsanthin, violaxanthin, capsanthin epoxide, capsorubin and four unidentified carotenoids were also isolated. In addition, three capsanthin mixed diesters of a single unidentified polar fatty acid and the three dominant saturated fatty acids were isolated.

The chemistry of the Capsicum carotenoid esters has not been investigated, probably because of the difficulty of their isolation. The application of high performance preparative separations will undoubtedly stimulate interest in the chemistry of these compounds.

Recently HPLC of bell pepper and paprika spice pigments using C-18 reversed phase columns has given chromatographic profiles similar to the partition TLC chromatograms of Egger[8,11-13]. Isocratic separations are inferior to those utilizing gradient elution[14-16]. Figures 2a-f are gradient HPLC traces of extracts from paprika spice samples of different quality. Although about 55 peaks can be found in a typical chromatogram, considerable peak overlap is apparent. The Hungarian paprika grades examined in order of decreasing quality were delicatesse, Edelsüss and rose. The corresponding Spanish grades are dulce, agridulce and picante[1]. Unesterified carotenoids elute during the first seven minutes followed by monoesters of primarily capsanthin and capsorubin. ß-Carotene elutes at about 28 min. and is followed by diesters of the two dominant carotenoids. Since the Hungarian delicatesse sample (fig. 2a) has the highest content of fruit pericarp, the total peak area of the HPLC trace is greater than those for Edelsüss and rose samples (figs. 2b&2c). The total pigment content (ASTA value) shows a high correlation with the logarithmic values of major peak areas (table 1). The HPLC trace of a Spanish agridulce extract (fig. 2d) is similar to that of the Hungarian rose sample but contains less ß-carotene. Figure 2e is a trace from a Hungarian Edelsüss sample after 4.5 months exposure to UV light while figure 2f is the trace of a sample purchased as Hungarian Edelsüss paprika. This profile most resembles that of the Spanish agridulce sample. These results indicate that pigment profiles can be used directly for quality evaluation.

Different carotenoid and ester ratios have been found for different cultivars of Capsicum annuum var. annuum. For example, in early work on paprika spice, capsanthin dilaurate was found to be

Table 1 Linear Correlation of Total Pigment Content(ASTA) with log(HPLC peak area) and TLC scanner Peak Height[13,36] #

HPLC peak	R	Std.error	HPLC peak	R	Std.error
B	0.932	11.2	F+H	0.964	8.5
C	0.935	11.0	G+H	0.965	8.5
F	0.933	11.2	G+J	0.975	7.2
G	0.958	8.8			
H	0.960	8.6	TLC spot	R	Std.error
J	0.927	11.6			
A+H	0.966	8.3	C	0.926	12.2
B+H	0.965	8.4	J	0.941	10.9
B+G	0.966	8.3	K	0.949	10.2
			L	0.945	10.6

HPLC n=16 TLC n=12

Peak identities indicated in figures 2 and 8

the dominant derivative,while in other studies of paprika spice and bell peppers the laurate-myristate, the dimyristate or the myristate-palmitate dominates[9,11,12,14,17]. The occurance of lutein also seems to be variable[3,6,7,9,11,14,17-19]. Lutein has been found to be present in high concentrations in the unripe fruit of some cultivars,but its concentration decreased sharply during ripening[19]. Characteristic differences can be seen in the HPLC profiles of hydrolyzed carotenoids from Hungarian and Spanish Capsicum cultivars[6,7].Especially striking are the relative concentrations of ß-carotene and cryptocapsin in the reported cultivars.The variation in carotenoid composition in Capsicum annuum cultivars is consistent with the extreme variation in fruit morpholgy[1a].

A recent article on the application of overpressured TLC shows a native paprika spice pigment separation[20].The separation illustrated is similar to that obtained utilizing conventional equipment[9].An extreme degree of spot overlap is evident.

The two positional isomers of lutein monomyristate have been separated by reversed phase TLC[10a.]

Silica gel adsorption TLC has been examined in our laboratory as a rapid method to generate pigment profiles in combination with scanning densitometry[13].In spite of extensive chromatographic overlap,pigment profiles were obtained which could be related to sample quality.See figures 3a-f.Scanner peak areas and total scanner trace area was highly correlated to total pigment content.See table 1.

2.1 Pigment Profile Evaluation of Paprika Quality[13]

When the areas of 11 prominent HPLC peaks shown in figure 2 are subjected to principle component analysis(PCA) different quality groups emerged.The PC projection (figure 4) reveals that the samples are separated on the abscissa(PC1) according to total pigment content(ASTA).Four groups are evident,high ASTA(80-120) Hungarian delicatesse or Edelsüss,moderate ASTA(60-80) Hungarian Edelsüss, low ASTA(ca.50) Hungarian rose and low ASTA(ca.50) Spanish agridulce samples.The low ASTA Hungarian and Spanish samples are separated on the ordinate(PC2).Although many of the peaks in the HPLC trace represent more than one compound,HPLC profile data can be used to recognize quality types.Table 2 shows the results of PC classification of the samples using the high ASTA group as a single quality type.The distance from the class model is an indication of how close the chromatographic pattern fits that of the high ASTA group.Sample number 7(HPLC shown in fig.2f) appears on the PC trace far from the high quality group and near the Spanish samples although it was purchased as a Hungarian Edelsüss paprika.The low total pigment value supports this classification of sample 7.Seven year old Hungarian Edelsüss paprika(sample 8) is located farthest from the high quality group.A Hungarian Edelsüss sample irradiated with UV light(sample 9) is located near the Hungarian rose samples implying that the consequences of pigment degradation,although initiated by quite different mechanisms,may be similar.

Although there is much less separation indicated in the TLC scanner traces than in HPLC traces,if peak heights of 12 prominent spots were used for PC analysis results similar to those obtained from HPLC data were obtained.See figure 5 and table 2.As expected, due to the extensive chromatographic overlap,the quality groups are more diffuse in the PC projection and the classification is less effective.The poor quality Hungarian Edelsüss sample(here no.8) again fails to fit the high quality group.

Examination of the importance of the various HPLC peaks and TLC spots for classification(modeling power) does not show the dominance of a few chromatographic entities.Since many of the pigments have similar chemical structures,this is not surprising.HPLC and TLC data give more refined quality classification than total pigment

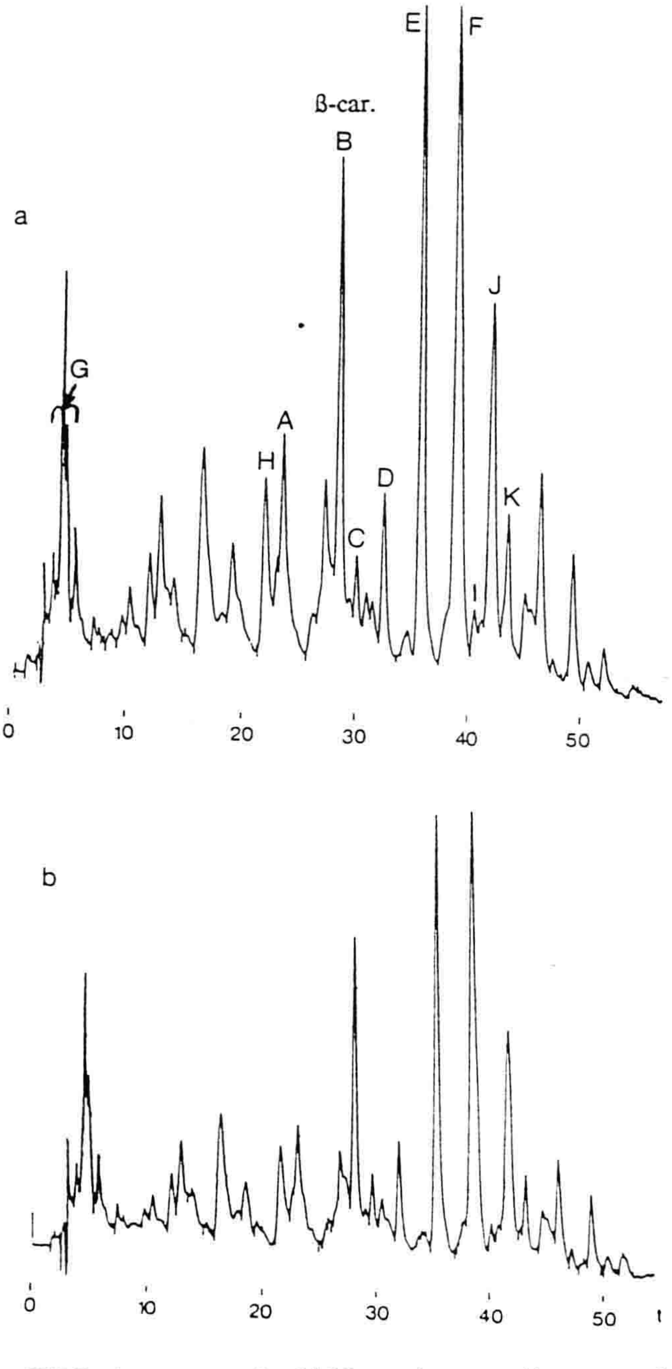
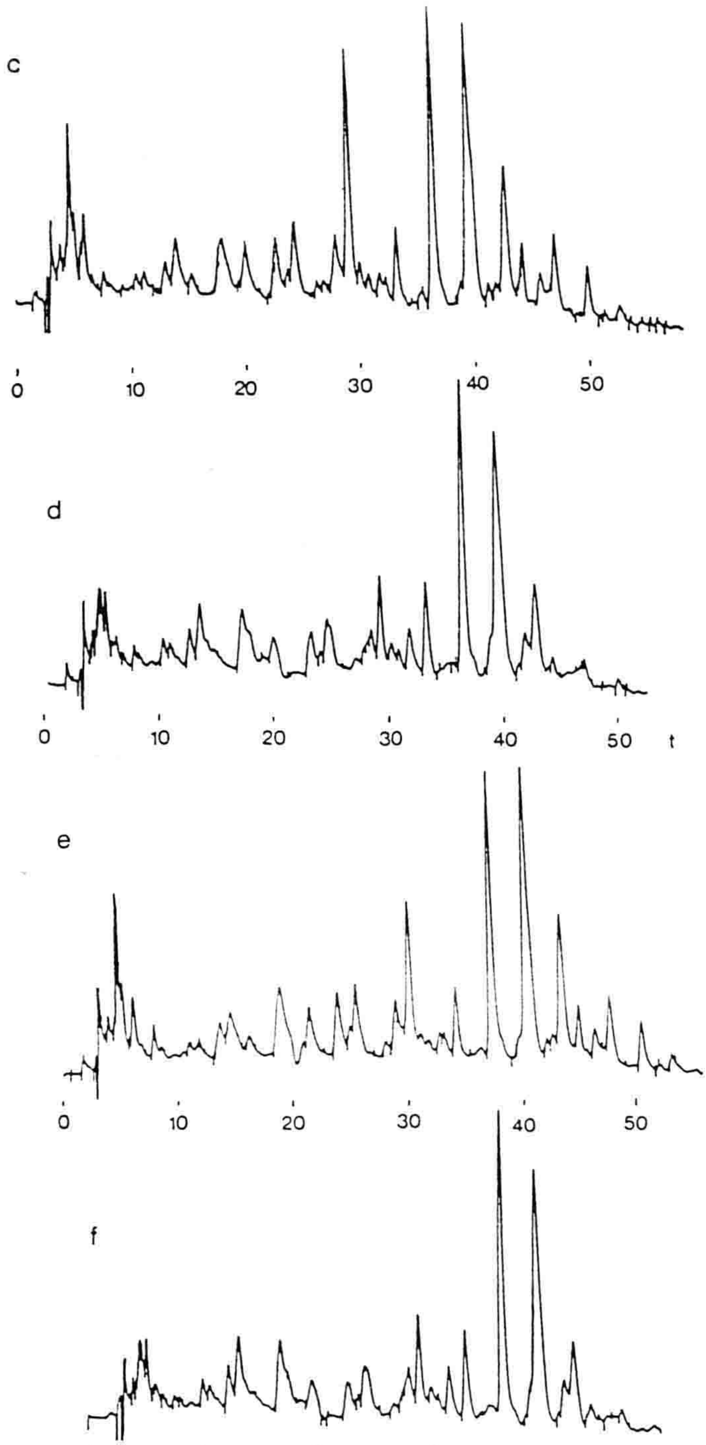


Figure 2 HPLC traces of different paprika samples:a.Hungarian delicatesse,b.Hungarian Edelsüss paprika c.Hungarian rose,d.Spanish agridulce,e.Hungarian Edelsüss after 4.5 months exposure to UV radiation,f.supposed Hungarian Edelsüss [13.36]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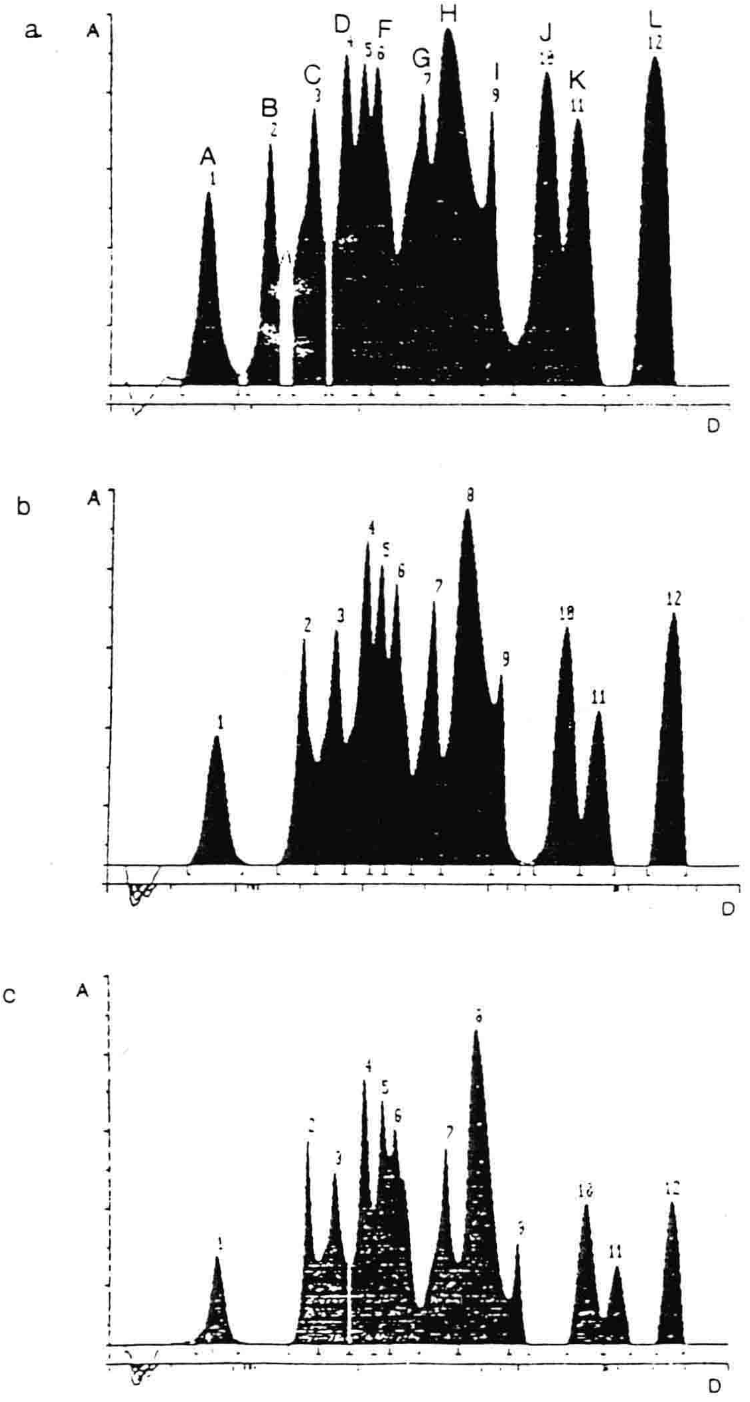
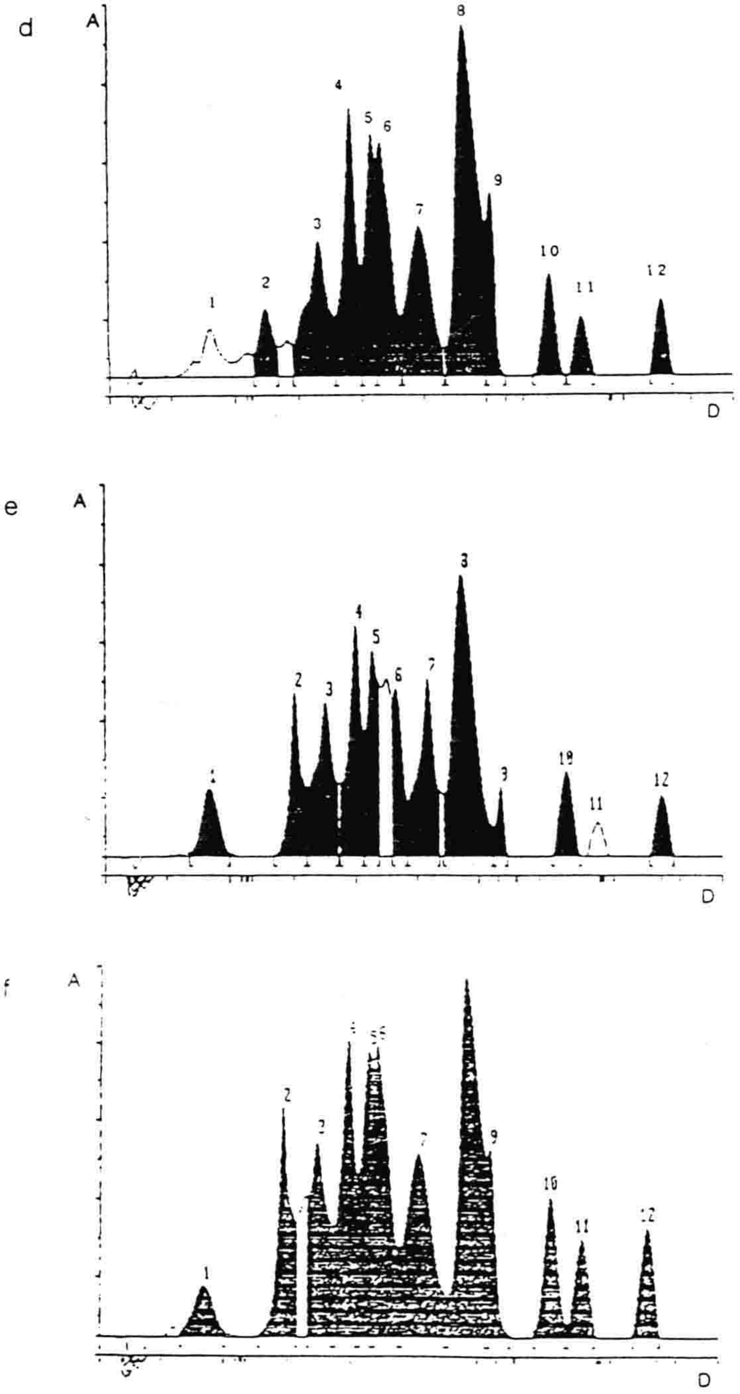


Figure 3.TLC scanner profiles of paprikas:a.Hung.delicatesse,b. Hung.Edelsüss,c.Hung.rose,d.Span.agridulce,e.Hung.Edelsüss sam-



ple after 4.5 months UV exposure to UV radiation,f.supposed Hung. Edelsüss(as table 2) [13,36]

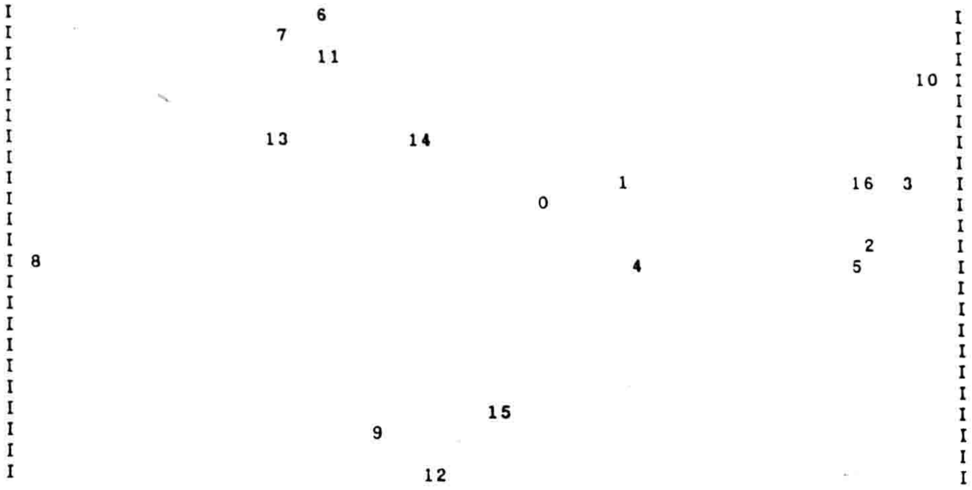


Figure 4. PC projection from HPLC peak area data

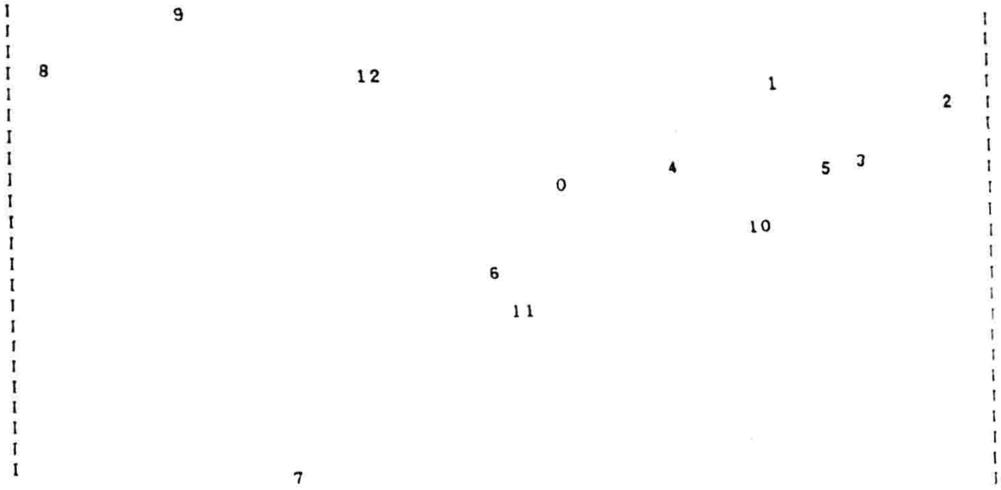


Figure 5. PC projection from TLC scanner data

Table 2. PC analysis results from HPLC and TLC data

	HPLC data-11 peaks		TLC data-12 spots	
	PC 1	PC 2	PC 1	
CSV/SD	0.882	0.675	0.702	
% variance explained	41.0	40.0	62.0	
residual Sd	0.768	0.436	0.617	
2xSd		0.872	1.23	
distance from the class models:				ASTA
1.Hungarian Edelsüss 1		3.60	0.890•	85
2.Hung.Edelsüss 2		0.410•	0.439•	117
3.Hung.Edelsüss 3		0.678•	0.529•	116
4.Hung.Edelsüss 4		3.77	1.19	75
5.Hung.delicatesse 1		0.427•	0.605•	105
6.Spanish agridulce 1		8.35	2.03	52
7.Hung.Edelsüss 5		9.40	2.70	50
8.Hung.Edelsüss 6(7 years old)		13.1	4.61	32
9.Hung.Edelsüss 1 exposed to UV		6.67	3.40	44
10.Hung.Edelsüss 6		0.191•	0.403•	106
11.Span.agridulce 2		8.43	1.95	49
12.Hung.rose 1		6.28	1.88	54
13.Span.picante 1		9.74	-	41
14.Span.picante 2		7.53	-	54
15.Hung.rose 2		4.49	-	62
16.Hung.delicatesse 2		0.323•	-	95

• class members

content

PC classification of different Hungarian paprika types has been carried out using total ash content and the concentrations of Fe,Ca, K,P,SI.Ti,Cu,Sr and Ni[21].Although sweet(delicatesse) paprikas were well separated from semisweet(Edelsüss) and hot(rose) samples, semi-sweet and hot samples were not separated.In our PC analyses Edelsüss and delicatesse samples were not separated but total pigment content of our samples could not distinguish these groups either.

The potential of pattern recognition for spice and herb quality classification is illustrated in the case of paprika which had a priori established criteria for quality evaluation.Similar analyses of condiments have appeared for ginger,black pepper and oregano[22,23].

3. Paprika Volatiles

Although there have been several studies reporting the identification of fresh Capsicum volatiles[24-27],the recent report is the first on paprika spice aroma to our knowledge[28].Paprika aroma is rather weak and indeed some authors suggest that paprika contains no essential oil[29,30].Dynamic headspace sampling of nitrogen purged paprika at 50°C allowed the concentration of enough volatile material to identify 37 compounds using GC/MS and GC/FTIR.A typical chromatogram is shown as figure 6.Six of these,pentanal,N-methylpyrrole,2-methyltetrahydrofuran-3-one,α-terpinene,safranal and estragole,had not been previously identified in Capsicum.The major constituents, monoterpene hydrocarbons,which are commonly found in plant material,are probably present in the fresh fruits in low concentrations.Compounds which may be remnants of terpenoid biosynthesis or degradation include 2- and 3-methylbutanal and safranal. Safranal has been identified as a product of the thermal degradation of ß-carotene[31].1-Pentanol, pentanal,1-hexanol and hexanal are probably products of fatty acid oxidation.N-Methylpyrrole,2-acetylfuran and 2-methyltetrahydro-furan-3-one are often found in heated products like bread and cacao and appear to be derived from carbohydrates[32].

It should be mentioned that pyrazine derivatives were not detected in paprika.This is in essential agreement with an unpublished GC/MS study of paprika which was air dried at 80°C,in which tetramethylpyrazine,the only derivative reported,could only be detected by selective ion monitoring[33].

The presence of seed in paprika markedly increases the concentration of lipids and lipoxygenase which have been shown to be involved in the oxidation of the carotenoid pigments[14,34-35].Enzymic activity has been demonsrated in paprika spice and it is likely that paprika aroma arises in part from oxidative processes during drying and storage.

Since our sample group exhibited extreme variation in composition(grade),genetic origin,age and drying and storage conditions, no clear patterns were apparent in the GC profiles[36].On the other hand,examination of GC profiles of the samples in our Hungarian Edelsüss storage series revealed consistent patterns for some constituents.Specifically the ratio of the peak areas of myrcene to limonene clearly decreased under storage conditions which favored oxidation(table 3).These observations undoubtedly reflect the relative reactivities of the two compounds.

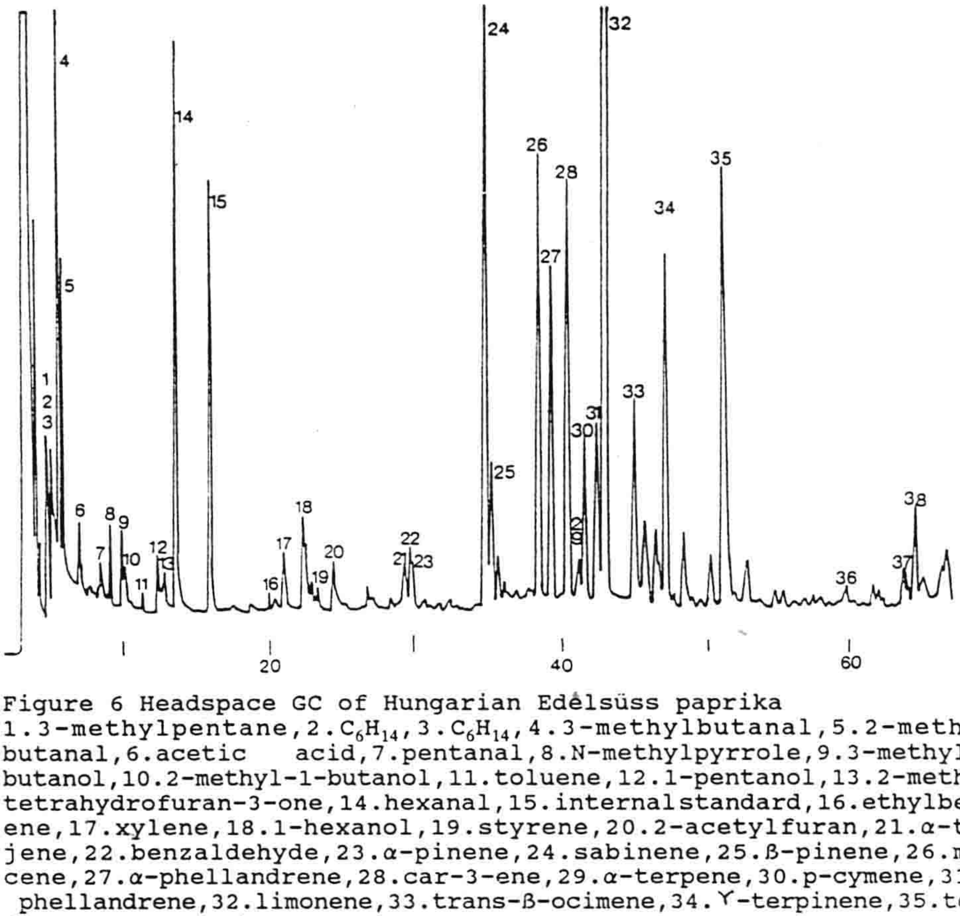


Figure 6 Headspace GC of Hungarian Edelsüss paprika
1.3-methylpentane, 2.C_6H_{14}, 3.C_6H_{14}, 4.3-methylbutanal, 5.2-methylbutanal, 6.acetic acid, 7.pentanal, 8.N-methylpyrrole, 9.3-methyl-1-butanol, 10.2-methyl-1-butanol, 11.toluene, 12.1-pentanol, 13.2-methyltetrahydrofuran-3-one, 14.hexanal, 15.internal standard, 16.ethylbenzene, 17.xylene, 18.1-hexanol, 19.styrene, 20.2-acetylfuran, 21.α-thujene, 22.benzaldehyde, 23.α-pinene, 24.sabinene, 25.ß-pinene, 26.myrcene, 27.α-phellandrene, 28.car-3-ene, 29.α-terpene, 30.p-cymene, 31.ß-phellandrene, 32.limonene, 33.trans-ß-ocimene, 34.γ-terpinene, 35.terpinolene, 36.terpinen-4-ol, 37.safranal, 38.estragole

Table 3.Paprika Headspace GC Peak Area Ratios After Six Months Storage.Limonene/Myrcene[36]

Ambient,glass container	4.62
Ambient,plastic bag	5.98
2°C in nitrogen	5.57
40°C in nitrogen	6.61
40°C in air+oxygen	15.6
UV exposure in nitrogen	7.78
UV exposure in air	13.7

3.1 GC profiles and Paprika Quality[13,36]

Principal component analysis of 14 GC peak areas resulted in the separation of paprika samples into two groups, those with high (ASTA 75-120) and low (ASTA 40-75) pigment content(table 4).Only one of the commercial samples(no.1-8,11-16) deviated from this pattern.The delicatesse sample 14 is not included in the high quality class as is shown by the large distance from the class model,a result which reflects the sample variance.It is possible that delicatesse samples require a separate group since the other delicatesse sample examined gave the largest distance to the class model in the high quality class(no.5).In this group,no.7(same as HPLC analysis) as well as no.15,which was also purchased as a Hungarian Edelsüss paprika,are excluded from the class.

Table 4. PC analysis of paprika GC data (one PC)

CSV/SD 0.545 % variance explained 74.2 residual Sd 0.508

2xSd 1.16

	Distance from the class model	ASTA
1.Hungarian Edelsüss A	0.404•	85
2.Hung.Edelsüss B	0.348•	117
3.Hung.Edelsüss C	0.434•	116
4.Hung.Edelsüss D	0.635•	75
5.Hung.delicatesse A	0.635•	105
6.Spanish agri ulce A	2.05	52
7.Hung.Edelsüss E	2.34	50
8.Hung.Edelsüss 7 years old	1.64	32
9.Hung.Edel.A,3 mo. exposure to UV	5.52	44
10.Hung.Edel.A,3 mo.,40°C,air+O_2	6.48	2
11.Span.agridulce B	3.95	49
12.Hung.rose	4.31	54
13.Span.picante	2.81	41
14.Hung.delicatesse B	1.59	93
15.Hung.Edelsüss F	7.63	27
16.Hung.Edelsüss G	0.631	66
17.Hung.Edel.A,6 mo,ambient,glass	0.253	68
18.Hung.Edel.A,6 mo.,ambient plastic	0.812	61
19.Hung.Edel.A,6 mo.,2°C,N_2	2.42	81
20.Hung.Edel.A,6 mo.,UV,N_2	2.70	75
21.Hung.Edel.A,6 mo.,UV,air	5.12	35
22.Hung.Edel.A,3 mo.,40°C,N_2	1.60	68

• class members

3.2 Sensory Studies of Paprika Aroma[13]

A systematic GC splitter sniffing study of three fresh Capsicum annuum cultivar extracts resulted in 18 sensory descriptions of the eluate components[27].Benzaldehyde was the only paprika spice component identified in that study.Of the compounds found in fresh green bell peppers,hexanal and limonene were also identified in Hungarian Edelsüss paprika[24].Threshold values for some of the compounds identified in paprika are listed in table 5.The aldehydes,methylbutanol isomers and unsaturated monoterpenes have the lowest threshold values of the compounds listed.

Table 5 Olafactory Detection Threshold Values(in water)[37]

Benzaldehyde	4.29 ppb
2-Methyl-1-butanol	1.25
3-Methyl-1-butanol	1.5
Cumene	4.7
Hexanal	4.5
1-Hexanol	4870.
Limonene	1.0
Myrcene	13.
Pentanal	1.2
2-Pinene	6.
2(10)-Pinene	140.
Sabinene	730.
Terpinolene	200.

Descriptors for paprika spice aroma were developed and using a standard Hungarian Edelsüss sample with agreed intensity levels, aroma intensities for some commercial samples were determined.Some of the data is listed in table 6 and aroma profiles for some samples are shown in figure 7.There was considerable variation for the Hungarian Edelsüss samples,profiles a-d,which correspond to samples 1-4 in table 4.Profiles f-i correspond to samples 5,6,11 and 13. There are striking differences between the profiles of the Spanish and Hungarian samples.It is interesting to note that the profile of the Hungarian rose sample(g) resembles that of some Hungarian Edelsüss samples(a,d,e) and that the Hungarian delicatesse profile resembles those of the other Hungarian Edelsüss paprikas(b,c).The later observation is consistent with our inability to separate these quality groups using GC and HPLC profiles.The aroma profile of sample 7,

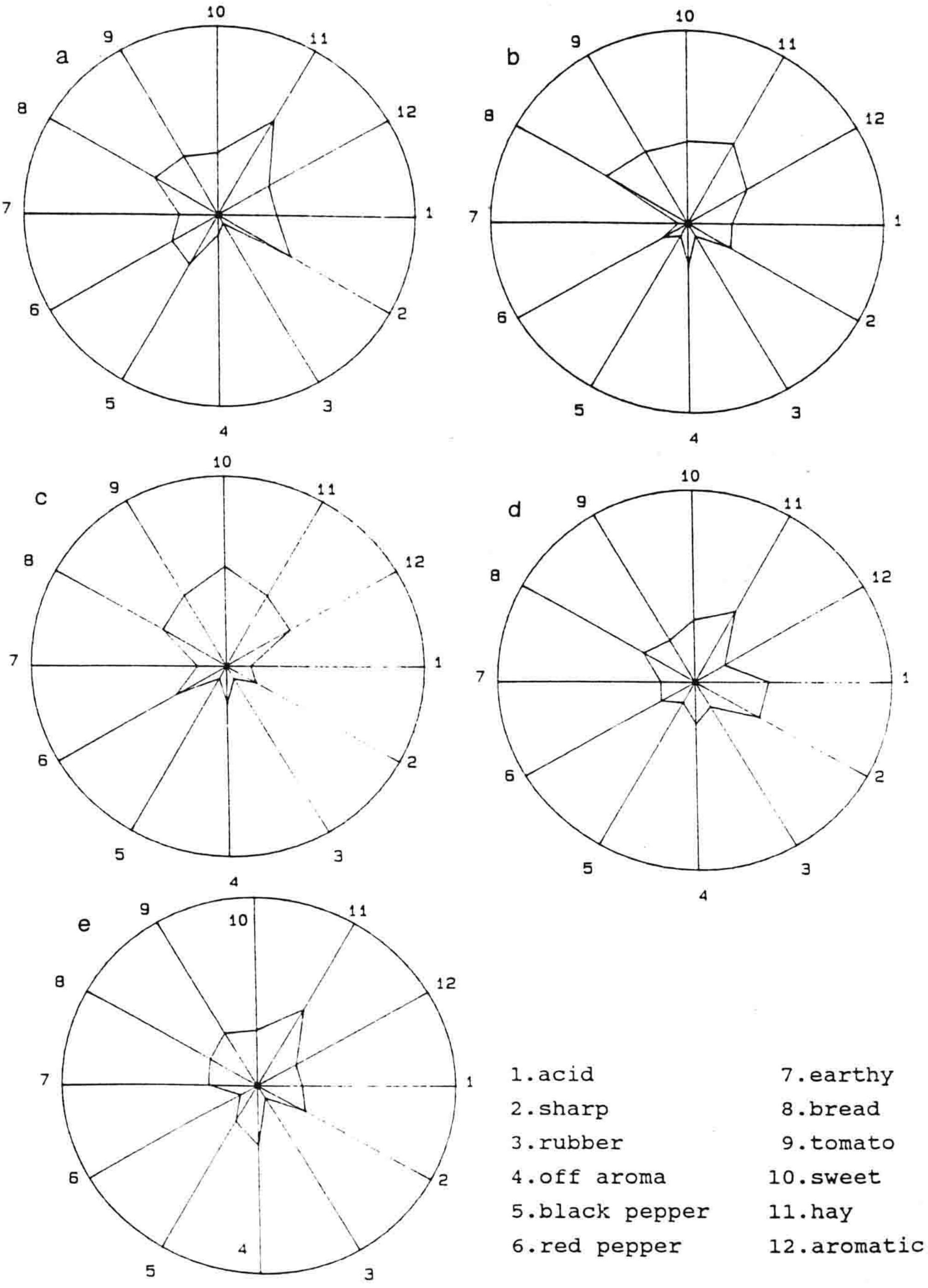


Figure 7.Aroma profiles of paprica samples. a.Hungarian Edelsüss A, b.Hung.Edel.B,c.Hung.Edel.C,d.Hung.Edel.D,e.Hung.Edel.E,f.Hung.delicatesse A,g.Spanish agridulce A,h.Span.agri.B,i.Hung.rose,j.Hung.