Chemical Significance of the Volatile Components of Spices in the Food Preservative Viewpiont III*

Antibacterial Activity of Volatile Components of Nutmeg

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(Tables 1-4)

INTRODUCTION

The present study was undertaken in order to clarify the volatile constituents and antibacterial properties of the condiments which are used to season fish ham and fish sausage. The chief volatile components of nutmeg[1] known to date are α-pinene, α-terpineol, linalool, geraniol, safrol and myristein. Nutmeg was subjected to steam distillation and the distillate was extracted with ether. This ether extract was separated into fatty acid, phenol, carbonyl and terpene fractions by the method that was used for the separation of volatile constituents of seaweeds[2),3),4)]. Terpene fraction was further fractionated by fractional distillation. Antibacterial properties of each fraction were tested with *Bacillus subtilis*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus morganii* and *Escherichia coli*. The fractions showed antibacterial activity at dilutions lower than 20～200 times.

EXPERIMENTAL

I. Fractionation of the Volatile Components of Nutmeg

About 2 kg of nutmeg was submitted to steam distillation, the distillate was saturated with sodium chloride and extracted with ether. Ether was distilled off after drying and the crude oil thereby obtained was fractionated into lower fatty acids, higher fatty acids, phenols, carbonyls and terpenes[2),3),4)]. The yield of each fraction of volatile components from 2 kg of nutmeg is given in Table 1.

Table 1. Yield of volatile compounds from 2 kg of nutmeg

Fraction	Yield (g)
1. Lower fatty acid fraction	0.3
2. Higher fatty acid fraction	0.7
3. Phenol fraction	3.2
4. Carbonyl fraction	0.1
5. Terpene fraction	7.2

* The previous report—II. This journal, **2**: 349 (1959).

The terpene fraction was further fractionated with WIDMER microfractionation apparatus[5] with the result shown in Table 2.

Table 2. Result of fractional distillation of terpene fraction

No.	Pressure (mm)	b. p. (°C)	Yield (g)	N_D^{25}	Color
I	10	~45	1.0	1.4756	Light yellow
II	〃	46~50	0.5	1.4812	〃
III (a)	〃	51~60	0.7	1.4820	〃
(b)	〃	〃	0.7	1.4834	〃
(c)	〃	〃	0.7	1.4822	〃
(d)	〃	〃	0.2	1.4810	〃
(e)	〃	〃	0.2	1.4825	〃
IV	〃	61~70	1.0	1.4840	〃
V (a)	〃	71~80	1.0	1.4829	〃
(b)	〃	〃	0.2	1.4838	〃
VI	5	~70	0.3	1.4830	〃
VII (a)	〃	71~80	0.8	1.4844	〃
(b)	〃	〃	0.7	1.4934	〃
VIII	3	45~60	0.2	1.5000	〃
IX	〃	61~70	0.4	1.5164	〃
X (a)	〃	71~80	0.8	1.5290	〃
(b)	〃	〃	0.5	1.5240	〃
XI	〃	81~90	0.4	1.5192	Light brown
XII	〃	91~100	0.7	1.5317	〃
XIII (a)	〃	101~110	1.0	1.5370	〃
(b)	〃	〃	1.0	1.5423	〃
(c)	〃	〃	0.6	1.5379	〃
XIV	〃	111~120	1.0	1.5338	〃
XV	〃	121~130	0.4	1.4859	〃

Each fraction was subjected to chromatostripping[6),7),8),9),10),11),12)]. From Rf value, coloration and pressure-boiling point curve, the presence of α-pinene, α-terpineol, linalool and geraniol was presumed. These results are shown in Table 3.

Table 3. Fractionation of neutral compounds by chromatostripping

Fraction	Pressure (mm)	b. p. (°C)	Rf values												
I	10	~45								0.74					0.93
II	〃	46~50								0.74					0.92
III (a)	〃	51~60								0.73					0.92
(b)	〃	〃								0.74					0.92
(c)	〃	〃								0.74				α-pi	nene
(e)	〃	〃					linal	ool		0.74					
IV	〃	61~70					0.34		0.55						
V	〃	71~80					0.33		0.54				0.83		
VI	5	~70		α-te	rpine	ol	0.34		0.54						

VII (a)	5	71～80			0.19		0.34		0.56		0.75			0.85
(b)	〃	〃			0.20		0.34		0.57		0.74			0.85
VIII	3	～60			0.19				0.58		0.74			0.85
IX	〃	61～70			0.19	0.26					0.75			0.85
X	〃	71～80				0.26					0.75			0.86
XI	〃	81～90				0.27		0.45			0.75			
XII	〃	91～100				geraniol		0.45		0.67				
XIII	〃	101～110						0.45		0.66				
XIV	〃	111～120	0.05	0.13				0.45		0.67				
XV	〃	121～130	0.05	0.13				0.46		0.67		0.71		

II. Assay of Antibacterial Activity

Antibacterial activity of the phenol, terpene and distilled terpene fractions was tested with *Bacillus subtilis*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus morganii* and *Escherichia coli* by the streak method described in the preceding report[13]. The results are shown in Table 4.

Table 4. The antibacterial activity of volatile compounds

Fraction	b. p. (°C)	Maximum dilution at which bacterial growth was inhibited					
		Bac. subtilis	*Esch. coli*	*Salm. enteritidis*	*Staph. aureus*	*Prot. morganii*	*Pseud. aeruginosa*
Phenol		20	10	10	10	10	10
Terpene		200	20	100	20	100	20
Terpene I	～45(10mm)	—	—	—	—	—	—
Terpene II+III	46～60(〃)	10	—	—	—	10	—
Terpene III(b)	51～60(〃)	20	—	—	20	20	—
Terpene IV	61～70(〃)	20	20	20	20	20	100
Terpene V	71～80(〃)	20	20	20	100	20	10
Terpene VI+VII	～80(5mm)	100	100	100	100	100	20
Terpene VIII+IX	～70(3mm)	20	20	20	20	20	20
Terpene X+XI	71～90(〃)	20	10	20	20	100	—
Terpene XII+XIII	91～110(〃)	20	10	20	20	20	10
Terpene XIV	111～120(〃)	100	—	—	—	100	—
Terpene XV	121～130(〃)	200	—	—	100	100	—

All the fractions inhibited bacterial growth at dilutions lower than 20～200 times.

SUMMARY

1. Nutmeg, which is used as a condiment in manufacturing fish ham and fish sausage,

was subjected to steam distillation and the distillate was extracted with ether. The volatile compounds were separated into fatty acids, phenols and terpenes.

2. The terpene fraction was refractionated, each fraction was submitted to chromatostripping, and the presence of α-pinene, α-terpineol, linalool and geraniol was presumed.

3. Antibacterial activity of each fraction was tested with *Bacillus subtilis*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus morganii* and *Escherichia coli*. All the fractions inhibited bacterial growth at dilutions lower than 20～200 times.

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