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THE VOLATILE ISOTHIOCYANATES OF FRESH CABBAGE.

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Because of the importance of dehydrated cabbage as a fresh salad substitute to the soldier in the field, the Army Quartermaster Corps is interested in the nature of the flavor components of fresh cabbage. The volatile isothiocyanates, which are released by the action of enzymes on the thioglucosides present in cabbage (Slide 1) and which are lost in the process of dehydration, are believed to be of particular importance to the taste and odor of fresh cabbage.

The presence of allyl isothiocyanate in the head of white cabbage and of allyl isothiocyanate and 3-butenyl isothiocyanate in the head of red cabbage (Slide 2) has been indicated, on the basis of paper chromatographic evidence, by Kjaer and coworkers.

One hundred pounds of cabbage (*Brassica oleracea* var. *capitata*) of known history, obtained from the Waltham Experiment Station of the University of Massachusetts through the courtesy of Professor Robert E. Young, was extracted with normal-hexane after shredding and allowing enzymatic action to take place (Slide 3). The hexane was removed by careful fractionation, and the residue was vacuum steam-distilled at 35-36° and 36-38 mm. pressure. The distillate was extracted with peroxide-free ether, and the extract was treated with methanolic ammonia, in order to convert the isothiocyanates to thioureas. Concentration yielded 900 mg. of crude product.

An ultraviolet absorption spectrum of this crude product showed a maximum at 243 millimicrons characteristic of substituted thioureas. The extinction indicated that there was 150 mg. of thiourea, calculated as allylthiourea, present in the original 100 lbs. of cabbage. This represents approximately one part per 350,000 of allyl isothiocyanate.

After the crude mixture of thioureas had been further purified by extraction with water, it was examined by paper chromatography in the chloroform-water system described by Kjaer and Rubinstein for the identification of substituted thioureas. Grote's nitroprusside reagent was used as a spray. The following spots were obtained: (Slide 4)

		R_{Ph}
(1)	Strong blue spot	0.25 - 0.27
(2)	Blue spot	0.74
(3)	Weak blue spot	0.61
(4)	Red-purple spot	0.0

The R_{Ph} value expresses the ratio of the distance travelled by the unknown spot to the distance travelled by phenylthiourea. Known compounds to which the spots could be assigned are shown in the following table: (Slide 5)

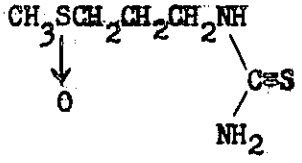
		R_{Ph}
Spot (1)	Cabbage Extract	0.25 - 0.27
	Allylthiourea	0.26
Spot (2)	Cabbage Extract	0.74
	sec-Butylthiourea	0.74
	α-Methallylthiourea	0.74
	iso-Butylthiourea	0.76
	3-Methylthiepropylthiourea	0.82 (or 0.76)
	trans-Crotylthiourea	0.78
	cis-Crotylthiourea	0.76
Spot (3)	Cabbage Extract	0.61
	3-Butenylthiourea	0.61
	β-Methallylthiourea	0.62

The intensity of the spot at R_{Ph} 0.25 - 0.27, which corresponds to allylthiourea, is possibly as much as 25 times that of the spot at R_{Ph} 0.74.

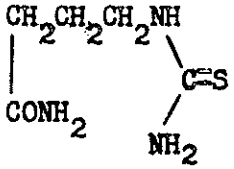
The spots at R_{Ph} 0.74 and 0.61 were tentatively regarded as representing sec-butylthiourea and 3-butenylthiourea, since the corresponding isothiocyanates are known to occur in similar plant sources.

At the suggestion of Professor Martin Ettlinger of The Rice Institute, a chromatogram was also run in a butanol-toluene-water system (Slide 6) in order to study the spot that remained at the origin in the chloroform-water system. The spots obtained, together with known compounds of corresponding R_{Ph}, are shown in the following table (Slide 7).

		R_{Ph}
(1)	Cabbage Extract (Strong Blue Spot)	0.89
	Allylthiourea	0.90
(2)	Cabbage Extract (Blue Spot)	0.32
	3-Methylsulfinylpropylthiourea	0.38



	N-(3-Carbamylpropyl)-Thiourea	0.35



(3)	Cabbage Extract (Faint Orange Spot)	0.17
(4)	Cabbage Extract (Pink Spot)	0.07

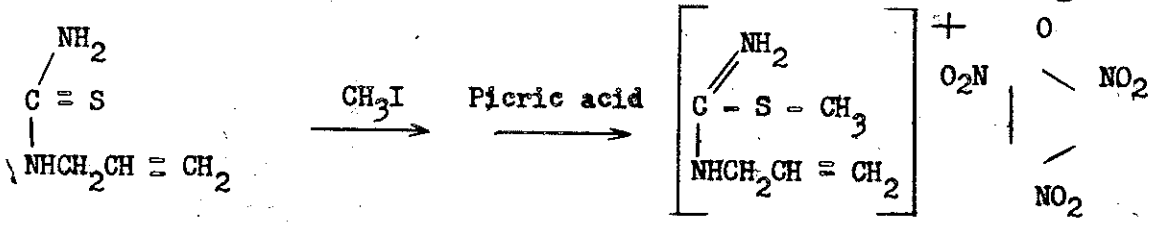
Thus, the chromatogram in the butanol-toluene-water system indicates the presence of one blue spot in addition to the three observed in the chloroform-water system, and the paper chromatograms indicate the presence of four thioureas in the mixture. (The orange and pink spots may indicate the presence of sulfur compounds of other types.)

On the assumption that the mixture consisted primarily of allylthiourea, sec-butylthiourea, and 3-butenylthiourea, a countercurrent distribution in a chloroform-water system was carried out in a 60-transfer Craig apparatus on a known mixture of these compounds after theoretical curves had been drawn. (Slide 8) The curve of the distribution shows that allylthiourea can be separated from the other two components (Slide 9). Although sec-butylthiourea and 3-butenylthiourea are not separated, paper chromatographic examination of the tubes, as well as the theoretical curve, indicates that the maximum of sec-butylthiourea occurs first in the distribution.

A 109 mg. sample of the unknown mixture was similarly distributed in a 60-transfer chloroform-water system. The concentrations of the tubes were determined spectrophotometrically, and the contents of the tubes were also examined by paper chromatography. From these results (Slide 10) it was considered that the large peak in the curve at tube 39 corresponded to allylthiourea and that the peak at tube 20 and shoulder at tube 27 corresponded to sec-butylthiourea and 3-butenylthiourea respectively.

The rise in the curve at tubes 58 and 59 and chromatographic results on these tubes indicate the presence of an additional thiourea, with an R_{Ph} of 0.88 in the butanol-toluene-water system.

The tubes comprising the fraction believed to contain allylthiourea from the countercurrent distribution (tubes 36-45) were combined and concentrated. By treatment of the concentrate with methyl iodide and picric acid, S-methyl-N-allylisothiuronium picrate was obtained as a crystalline derivative, m.p. 145.5 - 147°C. (Slide 11)



This derivative was demonstrated to be identical to an authentic sample by mixed melting point, X-ray diffraction analysis, and infrared analysis. The predominant isothiocyanate of cabbage is thus unequivocably proved for the first time to be allylisothiocyanate.

It has been recently suggested to us by Dr. Ettlinger on the basis of his study of numerous samples of the seeds of Cruciferae that the spot at R_{Ph} 0.74 in chloroform-water might well be due to 3-methylthiopropylthiourea rather than to sec-butylthiourea, which we had previously considered to be the most likely possibility. A sample prepared by elution chromatography was rechromatographed in three other solvent systems. A comparison of the R_{Ph} and R_F values indicates that it is indeed not sec-butylthiourea and may well be 3-methylthiopropylthiourea. (Slides 12, 13, and 14)

In toluene - acetic acid-water (5:2:4)

	R_{Ph}
Eluted unknown	0.46
3-Methylthiopropylthiourea	0.46
sec-Butylthiourea	0.64

In heptane-butanol-formic acid (1:1:1)

	R_F
Eluted unknown	0.12
3-Methylthiopropylthiourea	0.12
Sec-Butylthiourea	0.26

In benzene - ethanol - water (5:1:2)

	R_{Ph}
Eluted unknown	0.67
3-Methylthiopropylthiourea	0.67
sec-Butylthiourea	0.74

An ultraviolet absorption spectrum was obtained from a sample that had been similarly eluted from the spot at 0.74. (Slide 15) It showed the strong maximum at 244 mμ characteristic of substituted thioureas and was similar to 3-methylthiopropylthiourea. The extinction indicated that there was a total of 7.4 mg. of 3-methylthiopropylthiourea obtainable from the original 100 lbs. of cabbage. This corresponds to 1 part in 7,000,000 of 3-methylthiopropylisothiocyanate.

In summary, paper chromatography and countercurrent distribution have indicated the presence of five thioureas in the mixture from cabbage. The predominant component has been proved to be allylthiourea. Two of the remaining are considered to be 3-methylthiopropylthiourea and 3-butenylthiourea. These results show that a number of isothiocyanates contribute to the flavor of fresh cabbage.

SLIDES

Slide 1 Release of isothiocyanates from thioglucosides

Slide 2 Estimate of isothiocyanates in cabbage per A. Kjaer

Slide 3 Flow sheet of the isolation of isothiocyanates from cabbage

Slide 4 Paper chromatogram of thioureas from cabbage in the chloroform-water system

Slide 5 List of compounds with R_{Ph} values in the chloroform-water system similar to those of spots from cabbage extracts

Slide 6 Paper chromatogram of thioureas from cabbage in the butanol-toluene-water system

Slide 7 List of compounds with R_{Ph} values in the butanol-toluene-water system similar to those of spots from cabbage extracts

Slide 8 Countercurrent distribution - theoretical curve

Slide 9 Countercurrent distribution - known mixture

Slide 10 Countercurrent distribution - cabbage extract

Slide 11 Formation of S-methyl-N-allyl-isothiuronium picrate

Slide 12 Paper chromatogram of a re-chromatographed portion of the cabbage extract in the toluene-acetic acid-water system

Slide 13 Paper chromatogram of a re-chromatographed portion of the cabbage extract in the heptane-formic acid-butanol system

Slide 14 Paper chromatogram of a re-chromatographed portion of the cabbage extract in the benzene-ethanol-water system

Slide 15 Ultraviolet absorption spectrum of a portion of the cabbage extract chromatographed in the chloroform-water system and eluted by chromatographic irrigation