CCCXX. THE INFLUENCE OF FERTILIZERS ON THE CAROTENE AND VITAMIN C CONTENT OF PLANTS.

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THE estimation of carotene and vitamin C in vegetables has generally been carried out without paying attention to the different conditions prevailing during growth of the plants analysed. Therefore, the results obtained under these circumstances can only be looked upon as average values. Although these average values are sufficient for practical use, it is interesting, with a view to the quality of the crop, to examine if the carotene and vitamin C contents can be influenced by soil treatment and particularly by fertilizers.

The purpose of the investigations was to decide if the concentration of the elements necessary in plant-growth had any influence on the carotene and vitamin C contents of the plants.

EXPERIMENTAL.

The investigations were carried out by means of pot experiments, because only by this method of working can equal distribution of the salts be secured, this being essential to produce a crop of sufficient uniformity. In a number of the experiments the pots contained pure washed quartz sand, in other cases an exactly analysed sandy soil, which besides K and P deficiency also showed a low pH.

The pots were supplied with a salt solution according to Krüger of the following composition:

MgSO ₄ , 7H ₂ O	0.688 g.
KCl	0.552 g.
NH ₄ NO ₃	0.752 g. or
NaNO ₃	1.650 g.
Distilled water	1000 g.
CaHPO ₄ , 2H ₂ O	1.200 g.
pH = 7.2	

One or more components of this solution were omitted or administered in increased amounts, so that by the disturbance of the conditions of life an insight could be gained into the conditions of the formation of vitamins in plants.

K, Mg or N deficiency was produced by omitting the corresponding salt from the solution. In order to get Ca deficiency Na₂HPO₄ was used instead of CaHPO₄, 2H₂O and PO₄ could be substituted by SO₄ to give P deficiency.

Pots of indifferent material were used, such as glass, enamelled iron and glazed earthenware.

Spinach was used as a test plant; it has the advantage of growing rapidly and containing considerable quantities of carotene and vitamin C.

The plants were kept in a glasshouse. The pots were weighed every day during the experiment and the loss in weight was compensated by the addition of distilled water.

The full-grown plants were cut with scissors. To avoid errors caused by differences in weight of the stalks, which contain only a very small quantity of ascorbic acid, the latter were discarded and only the leaves were analysed.

Table I.

	mg. of ascorbic acid per 100 g. of fresh material
Stalks	8
Leaves	19

The plants had to be analysed rapidly, because at room temperature and even at ice-box temperature the leaves show a rather rapid decrease in vitamin C content.

Table II. *Storage of spinach leaves in the ice-box (4°) in stoppered glass jars, with a piece of moist cotton-wool to keep the leaves fresh.*

No. of days of storage	mg. of ascorbic acid per 100 g.	
	Fresh small leaf	Fresh large leaf
0	35	46
1	29	43
3	4	18
5	5	8

Table III. *Storage of spinach leaves at room temperature and in the ice-box.*

No. of days of storage	mg. of ascorbic acid per 100 g.	
	Fresh leaf at room temp.	Fresh leaf in the ice-box
0	60	60
1	49	45
3	23	49
5	8	21

Methods.

The vitamin C content of leaves was determined by the titration method of Birch *et al.* [1933], with 2:6-dichlorophenolindophenol in acid medium.

The acid extract of fresh spinach leaves shows the same vitamin C content before and after treatment with H₂S or with mercuric acetate as used first by Emmerie [1934]. Therefore this method (Emmerie) has only been used when estimating the loss of ascorbic acid during storage of the leaves, because in this case it is possible that substances other than vitamin C are present which reduce the indicator or that, on the other hand, a certain amount of vitamin C is reversibly oxidized and cannot be determined by titration according to the method of Birch *et al.*

Carotene was extracted with ether, after saponification of the chlorophyll etc. by boiling the plant material with a saturated solution of KOH in 96% alcohol (20 ml. per g. of plant material) for half an hour [Guilbert, 1934]. Besides carotene and xanthophyll the ether contained saponified chlorophyll and flavones. The latter were removed by washing the ether with water. Then the

ether was evaporated *in vacuo* and the residue dissolved in light petroleum; the xanthophyll was then removed from the light petroleum by extraction with 85 and 90 % alcohol.

This method can be simplified by extracting the carotene with light petroleum immediately after saponification. Thus, no chlorophyll or flavones and only a little of the xanthophyll dissolve in the light petroleum; the xanthophyll can be removed by washing once with 85 % methyl alcohol. The saponification must be carried out with only half of the amount of alcoholic KOH used by Guilbert. In this case, the concentration of the alcohol is reduced to about 90 % by the water in the plant material; at this concentration carotene dissolves sparingly and is extracted easily, while xanthophyll dissolves better in the alcohol than in the light petroleum. By 4 to 5 extractions all the carotene can be removed from the alcohol; further light petroleum extractions contain xanthophyll only, which can be washed out again with 85 % methyl alcohol. Therefore the extraction of carotene is complete when in the light petroleum extract all the yellow colouring matter can be removed with 85 % methyl alcohol.

The amount of carotene was determined with the Zeiss Stufenphotometer.

Results.

A. The influence of Ca, K, N and Mg deficiencies on the carotene and vitamin C contents of spinach. The experiment included 23 pots, filled with quartz sand.

Table IV.

Treatment	g. of leaf per pot	Type of leaf produced	Carotene γ /g. of fresh leaf	Vitamin C mg./100 g. of fresh leaf
Ca deficiency	4	Normal	29	31
K deficiency	1	Small, dark, shrivelled margin	67	46
N deficiency	1.1	Small, light colour, erect	7	29
Mg deficiency	2	Light-coloured spots	34	31
Normal	4	—	31	33

B. The influence of added nitrogenous fertilizers on the carotene and vitamin C contents of spinach grown in pots filled with a sandy soil and supplied with increasing quantities of P_2O_5 .

Each result is the mean of two analyses.

Table V. Vitamin C in mg. per 100 g. of fresh leaf.

Organic N mg. per pot	mg. P_2O_5 per pot				
	0	15	30	45	60
0	71	80	80	68	59
25	107	87	63	69	70
75	119	95	102	92	117
125	119	116	103	125	130

Table VI. Carotene in γ per g. of fresh leaf.

Organic N mg. per pot	mg. P_2O_5 per pot				
	0	15	30	45	60
0	26	30	26	33	26
25	65	38	33	44	41
75	71	77	63	65	63
125	95	87	75	71	87

Table VII. *Vitamin C in mg. per 100 g. of fresh leaf.*

Inorganic N mg. per pot	mg. P ₂ O ₅ per pot				
	0	15	30	45	60
0	56	72	74	58	61
25	73	74	72	76	86
75	90	94	75	90	113
125	90	81	116	105	92

Table VIII. *Carotene in γ per g. of fresh leaf.*

Inorganic N mg. per pot	mg. P ₂ O ₅ per pot				
	0	15	30	45	60
0	37	36	34	35	27
25	65	40	46	51	49
75	80	107	73	88	73
125	67	96	103	63	71

C. The influence of added nitrogenous fertilizers on the carotene and vitamin C contents of spinach grown in pots filled with a sandy soil and supplied with increasing quantities of potassium.

Each result is the mean of two analyses.

Table IX. *Vitamin C in mg. per 100 g. of fresh leaf.*

Organic N mg. per pot	mg. K ₂ O per pot			
	0	40	80	120
0	64	—	—	—
25	100	124	113	114
75	92	124	126	122
125	81	148	150	140

Table X. *Carotene in γ per g. of fresh leaf.*

Organic N mg. per pot	mg. K ₂ O per pot			
	0	40	80	120
0	100	—	—	—
25	124	86	45	49
75	93	101	94	104
125	100	92	82	94

Table XI. *Vitamin C in mg. per 100 g. of fresh leaf.*

Inorganic N mg. per pot	mg. K ₂ O per pot			
	0	40	80	120
0	—	—	—	—
25	—	105	119	122
75	121	121	136	134
125	—	70	142	140

Table XII. *Carotene in γ per g. of fresh leaf.*

Inorganic N mg. per pot	mg. K ₂ O per pot			
	0	40	80	120
0	99	—	—	—
25	113	86	62	65
75	80	106	96	81
125	113	104	108	113

D. Addition of phosphate had no effect.

E. From the results of the experiments recorded under C, the influence of added potassium fertilizer at different concentrations of nitrogen in the soil also becomes evident (Tables IX–XII).

DISCUSSION.

The results of the analyses show that the carotene and ascorbic acid contents of the test plants largely depend on the amounts of nitrogen and potassium in the soil.

A larger amount of nitrogen results in greater carotene and vitamin C contents.

An increasing potassium content of the soil causes a decrease in carotene and an increase in vitamin C. Diminution of the carotene content is only obvious in the plants grown in pots with little nitrogen. Generally the increase in vitamin C content is most distinct in plants grown with a high concentration of nitrogen. Under the conditions prevailing in the experiments, the influence of P, Ca and Mg salts is small.

Apart from the practical results, it is interesting to make an attempt to draw some physiological conclusions and to view the data in the light of what is known about the influence of fertilizers on photosynthesis and chlorophyll content.

Briggs [1922] was the first to investigate the influence of K, Mg and Fe. Gregory & Richards [1929] stated that the assimilation of nitrogen-deficient plants of *Hordeum* is "subnormal". Müller [1932] found the same for *Sinapis alba*. Gaszner & Goeze [1934] stated that larger additions of nitrogen resulted in an increased assimilation and transpiration and in increased chlorophyll and albumin contents both with rye and wheat; the difference between differently treated plants became greater when the plants grew older.

From the data of the experiments with N fertilizer described in this paper, it can be observed that nitrogen has an influence on the carotene and vitamin C contents similar to its influence on assimilation and chlorophyll content. It seems plausible to suppose that there exists a direct relationship between photosynthesis and vitamin C content, in view of the fact that the vitamin C content of leaves increases when they are irradiated with neon-light and that etiolated plants contain no vitamin C. Another argument in support of this theory can be found in the experiment of Randoin *et al.* [1935], who found less vitamin C in white than in green portions of plants.

This connexion with photosynthesis does not hold good in the case of carotene. Etiolated plants show no assimilation and possess carotene. Moreover Willstätter & Stoll [1913], Scherz [1929], Pfützer & Pfaff [1935] state that the carotene content of plants fluctuates with the chlorophyll content. Furthermore, Karrer & Helfenstein [1931] pointed out that carotene is derived from phytol or phytolaldehyde, establishing a chemical relationship between the nitrogenous chlorophyll and the N-free carotene.

From the data concerning the influence of nitrogenous fertilizer it seems that there exists a relationship between photosynthesis and vitamin C content on the one hand and between chlorophyll content and carotene on the other.

The data obtained from the experiments with potassium fertilizer confirm this supposition.

Gaszner & Goeze [1934] find an increased photosynthesis under the influence of more potassium fertilizer in wheat and rye, 25 days old and grown in a medium of comparatively high N content. Plants grown with little N show practically no difference in assimilation under influence of K fertilizer.

At a low N level the chlorophyll content of K-deficient plants is much higher than of plants richly supplied with potassium; at a high N level this difference does not exist.

Concerning the influence of potassium on photosynthesis, similar results were obtained by Briggs [1922], Gregory & Richards [1929] and Lundegårdh [1932]; the decrease of green colouring matter as a result of increasing quantities of K fertilizer is a well-known fact to every farmer and is frequently mentioned in the literature [see Maiwald, 1923; Remy & Dhein, 1932; Remy & Liesegang, 1926].

From the data concerning the influence of K fertilizer on the carotene and vitamin C contents of spinach, grown on a sandy soil with increasing quantities of nitrogen, it can be readily seen that carotene shows the same fluctuations as chlorophyll, whereas obviously vitamin C is a product of assimilation or at least closely connected with the process. Carotene content decreases at low N levels as a result of increasing quantities of K, whereas this decrease is practically zero at high N levels; vitamin C content increases but little at low N levels as a result of the addition of K fertilizers, whereas at high N levels a rapid increase can be observed.

From the results of the experiments it can also be concluded that nitrogen and potassium stand in close interrelation physiologically; K deficiency has the effect of N excess and K excess acts like N deficiency.

Therefore, fertilizer experiments concerning one of these two elements only give accurate results if made at different concentrations of the other element.

SUMMARY.

1. Details are given of the methods of growing spinach for analysis.
2. Tables are presented showing loss in vitamin C content during storage.
3. A modified method for estimating the carotene content of vegetables is given.
4. Tables are presented showing the influence of K, N, Ca and Mg fertilizers on the carotene and vitamin C contents of spinach. A higher level of nitrogen results in a greater carotene and vitamin C content. An increasing potassium content of the soil causes a decrease in carotene content and an increase in vitamin C.
5. An attempt is made to show a relationship between chlorophyll and carotene; on the other hand the suggestion that ascorbic acid only can be a product of photosynthesis is discussed.

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