PAPERCHROMATOGRAPHIC EXAMINATION OF GROWTH-INHIBITING SUBSTANCES WITH SPECIAL RESPECT TO FLESHY FRUITS

(Résumé of thesis for Cand. Sc. Biol.)

By

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The presence of growth- and germination inhibitors in fruit juices is a well-known fact for a long time, and there are numerous publications regarding this problem. However, studying and comparing the results of the works appearing since the years of 1890, and dealing with the subject, it became clear that in spite of the many experimental works it is very difficult to arrive at definite conclusions and the problem of the existence and mechanism of the »blastocholines« is actually not dissolved.

The numerous contradictions of the literary data suggested first of all to attempt again to approach the problem with modern methods. Further investigations were reasonable also by the fact that several natural growth inhibitors from very different parts of plants have been demonstarted in the recent years, and it seemed to be very interesting to see, if there be any relation between the growth inhibitors in fleshy fruits and the above mentioned substances? The inhibitors of fruit juices seemed to be worth while to submit to a more detailed examination because of their great theoretical interest and several practical connections too.

Material and method

The examinations were made with 26 different fleshy fruits with very diverse morphological structure, belonging to 10 families. The ether-extractable, acidiferous inhibiting substances of fruit juices were chromatographed with isopropanol: ammonia: water (10 : 1 : 1) solvent, on Sch & Sch paper No. 2043 b. The growth regulator contents in the eluate of the 1 cm strips of the 20 cm chromatograms were analysed with straight growth wheat coleoptile sections and Papaver seeds. These biological tests were carried out as described in our earlier papers (8, 9).

Results and evaluation

I. The results of the bio-assay fairly resemble each other. According to the experimental data, generally five active zones were separated on the chromatograms obtaiend from the ether extract of the fruit juices examined, and four of them have usually inhibitory effect.

In the sector between Rf 0,0 to 0,3 of the chromatograms generally two inhibiting zones of a milder activity could be observed which in many cases were replaced by slightly stimulatory spots. On some of the chromatograms growth promoting action due to indole-3-acetic acid at Rf 0,35 to 0,40 is discernible. However, in most of the fruits examined in full maturity state no auxin is detectable (9).

A strong inhibition appeared on the chromatograms of all the fruits between Rf 0,60 to 0,85. The very effective substances located here and playing the main role in the inhibition of fruit juices were summarized under the name of »β-inhibitor complex« after BENNET CLARK and KEFFORD (3). On the chromatograms great inhibitory effect is shown immediately behind the front line (R 0,9 to 1,0) too. Since the β-inhibitor complex and the inhibitors at the front line are present in all the fruits examined, it may be rightly supposed that the well-known inhibiting effect of the fleshy fruits is to be attributed first of all to these substances.

Now the question arises, by which substances are caused the inhibiting zones observed in the various chromatograms? Therefore, in the course of the successive examinations the chemical identification of the inhibiting substances, separated from fruit juices, were attempted, so far as this can be realized in the frame of the method of paper chromatography.

II. The identification of the ether-extractable, biologically active substances of fruit juices was attempted with three fleshy fruits: lemon (*Citrus medica* L.), apricot (*Prunus armeniaca* L.), and straw berry (*Fragaria ananassa* Duch). In addition to the numerous chromatograms obtained from the ether extracts of these three fruit juices, some chromatograms of other fruits were also available for the chemical identification. The estimation of the inhibiting spots was carried out on the basis of the relative and absolute Rf values observed in various solvent system, the UV fluorescence, the biological effect, and the qualitative reactions given by spraying reagents, furthermore by comparison with the properties of the control synthetic compounds.

1. As the results proved the inhibiting zones near the start line are caused mainly by the well-known short chain carboxylic acids (malic-, tartaric-, succinic-, citric acid etc.) and the ascorbic acid, as well as the tannic acids in fruit juices.

As to the biological effect, it became sure that the short chain organic acids in higher concentrations exert an inhibiting effect whereas in lower concentrations they promote the growth of coleoptile sections and *Papaver* embryos, however, in similar concentrations the biological activity of the single acids is different *(Fig. 1. and 2.)*.

Consequently, the inhibiting effect of the aliphatic acids is considerably only in acidic fruits, even in these cases it is less significant than that of other inhibitors. On the other hand, in less acidic fruits the effect of these acids may be stimulatory. According to the experiments the mixtures of aliphatic acids exert greater inhibiting activity than the single acids participating in it *(Fig. 3)*. There is no relation between the pH of the solution of acids and acid mixtures respectively, and their biological activity.

Tannic acids also inhibit growth in higher concentrations, however, in lower concentrations their effect turns into promotion *(Fig. 4)*. No significant

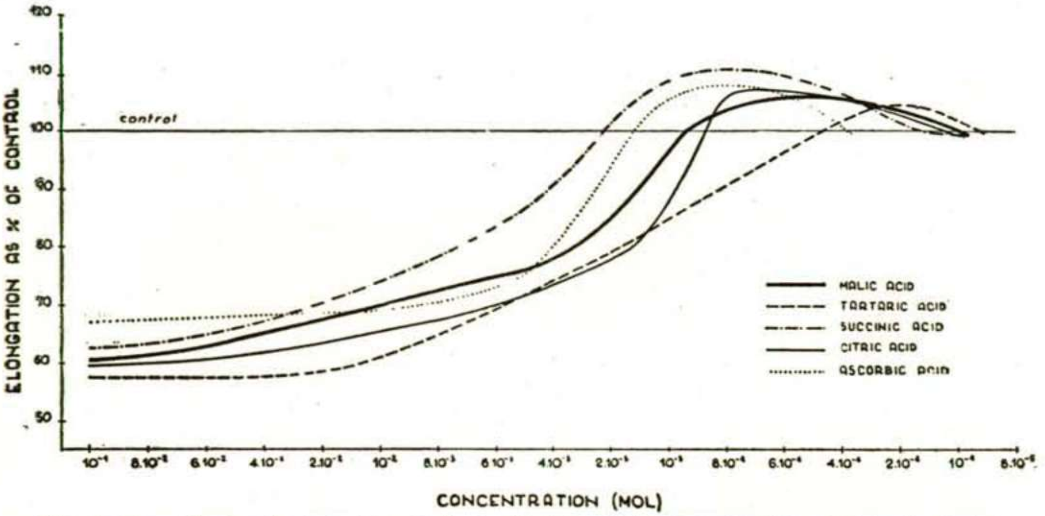

Fig. 1. Elongation of coleoptile sections in different concentrations of short chain organic acids (Average of 3 parallels).

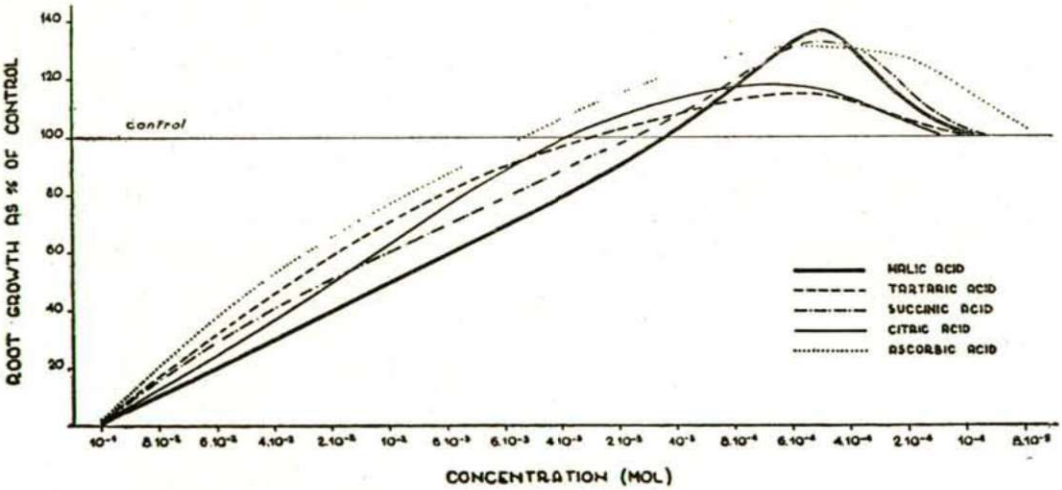

Fig. 2. Root-growth of Papaver seedlings in different concentrations of short chain organic acids (Average of 3 parallels).

role can be attributed to these substances in the inhibition of fruit juices, even in some cases, owing to their fairly low concentration, they rather stimulate than inhibit growth and germination. Because of their relative slight effectiveness chlorogenic- and caffeic acids — to which a remarkable role was ascribed earlier (1) — can in no case be considered to be one of the main inhibitory factors in fruit juices *(Fig. 5).*

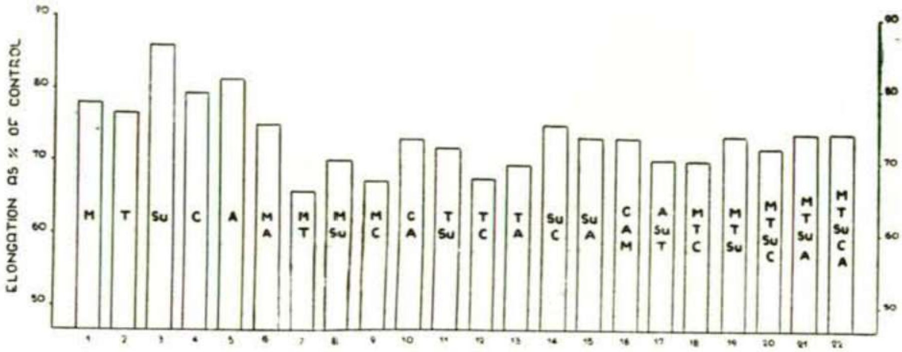

Fig. 3. Elongation of coleoptile sections in mixtures of aliphatic acids (5.10^{-3} M). Average of 3 parallels.
M = malic acid, T = tartaric acid, Su = succinic acid, C = citric acid, A = ascorbic acid.

In addition to the aliphatic and tannic acids, amino acids and other substances may also contribute, to a certain extent, to the inhibiting effect appearing on the lower sector of the fruit chromatograms (R_f 0,0—0,3), however, they can not be regarded as important inhibitors.

2. The examinations of quality and properties of the substances of the β-inhibitor complex yielded the following results:

The substances located in the β-inhibitor zones, in consequence of their solubility, thermostability, sensitivity to alkalines and peroxides, further their weak acidic character and strong biological activity, can be identified with the KÖCKEMAN's »blastocholine« (6). It was also demonstrated that the activity of the β-inhibitors is by no means attributable to the acid effect. On the other hand, even if the eluate of the β-inhibitor zone is highly diluted it does not promote prowth *(Fig. 6),* thus this group of compounds do not contribute to the stimulatory effect exerted by highly diluted fruit juices.

As regards the chemical determination, the β-inhibitor complex proved to be a mixture of several closely located aromatic acids and their derivatives, respectively. The combination of this inhibitor complex and the quantitative proportion of its components are not the same in the various fruit species, but in all the cases the inhibitors involved proved to be related compounds belonging to benzoic- and cinnamic acid derivatives. According to the results no indolic compounds take part in the β-inhibitor complex of fleshy fruits.

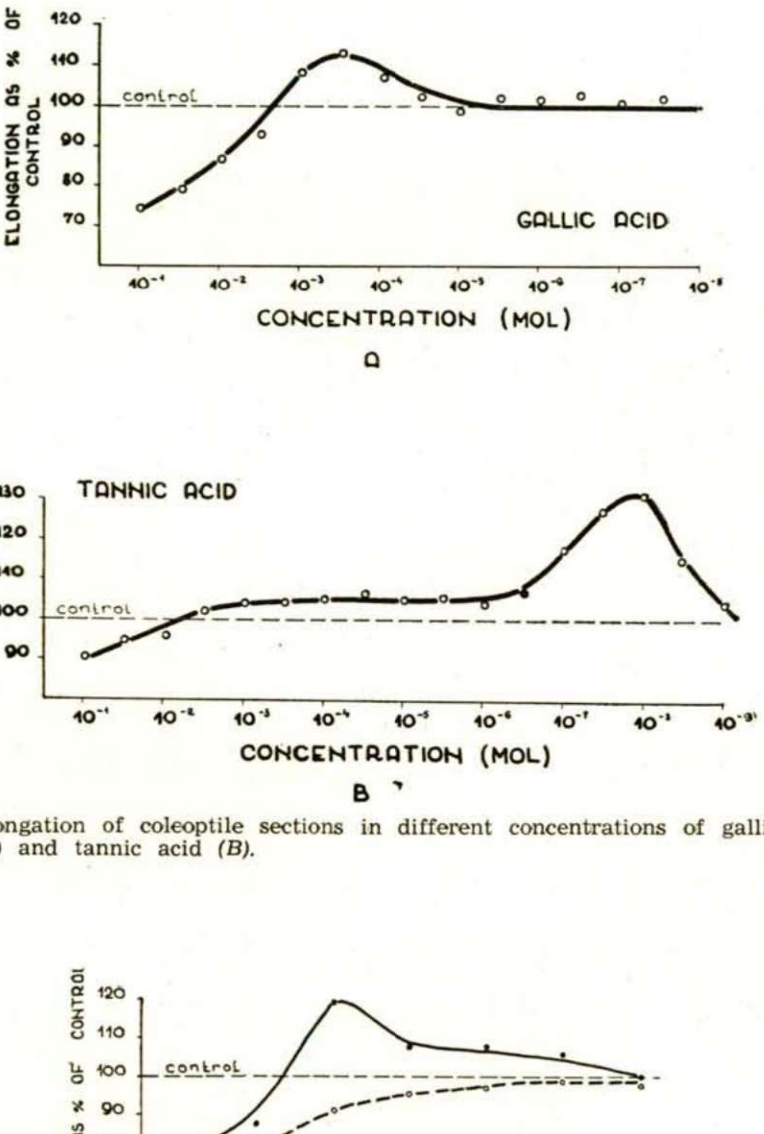

Fig. 4. Elongation of coleoptile sections in different concentrations of gallic acid *(A)* and tannic acid *(B)*.

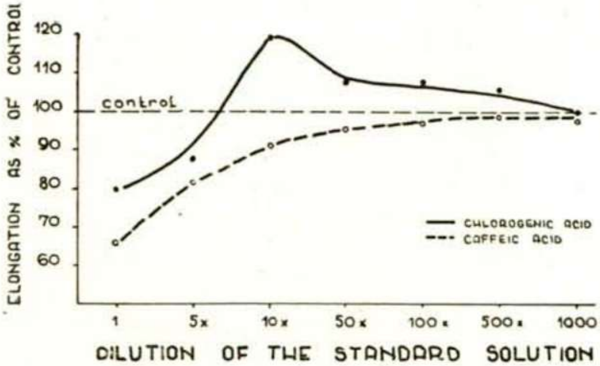

Fig. 5. Elongation of coleoptile sections in different concentrations of chlorogenic- and caffeic acid. (Standard solution: 3 g fresh weight of coffee beans per ml).

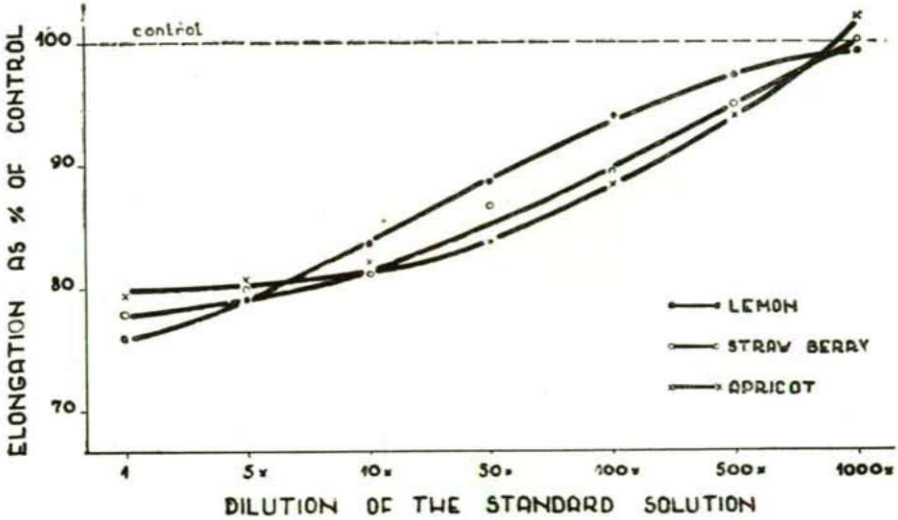

Fig. 6. Elongation of coleoptile sections in different dilutions of the eluate of the β-inhibitor spot (Average of 2 parallels).

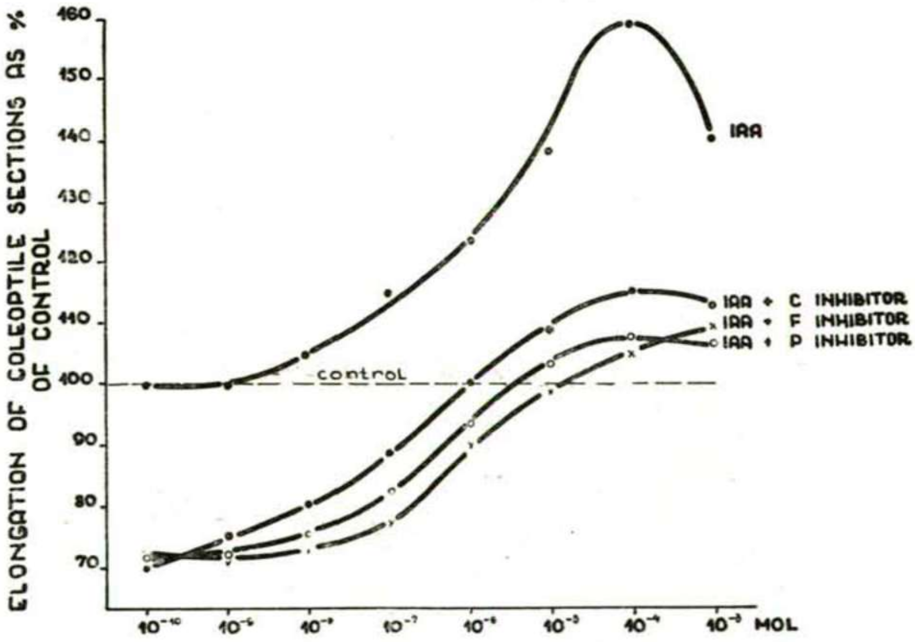

Fig. 7. Growth reactions of coleoptile sections in range of IAA concentrations, without and with β-inhibitor of *Citrus medica (C)*, *Fragaria ananassa (F)* and *Prunus armeniaca (P)*. (Average of 2 parallels).

In the β-inhibitor zone of the three fruits analyzed in detail (lemon, apricot, and straw berry) the following substances could be detected: coumarin, o-coumaric acid and presumably ferulic- and cinnamic acid as coumarin derivatives, whereas salicylic acid and possibly m-oxy-benzoic acid as benzoic acid derivatives. From the β-inhibitor complex of apricots besides two still unknown inhibitors were sperated. It is likely, that in addition to the above-enumerated substances other compounds may also occur. The action of these inhibitors is synergistic.

Investigating the mechanism of action of these substances it became evident that the inhibitors prevailing in the eluate of the of the β-inhibitor complex are auxin-antagonists. *Fig.* 7 shows that the strong (about 30 %)

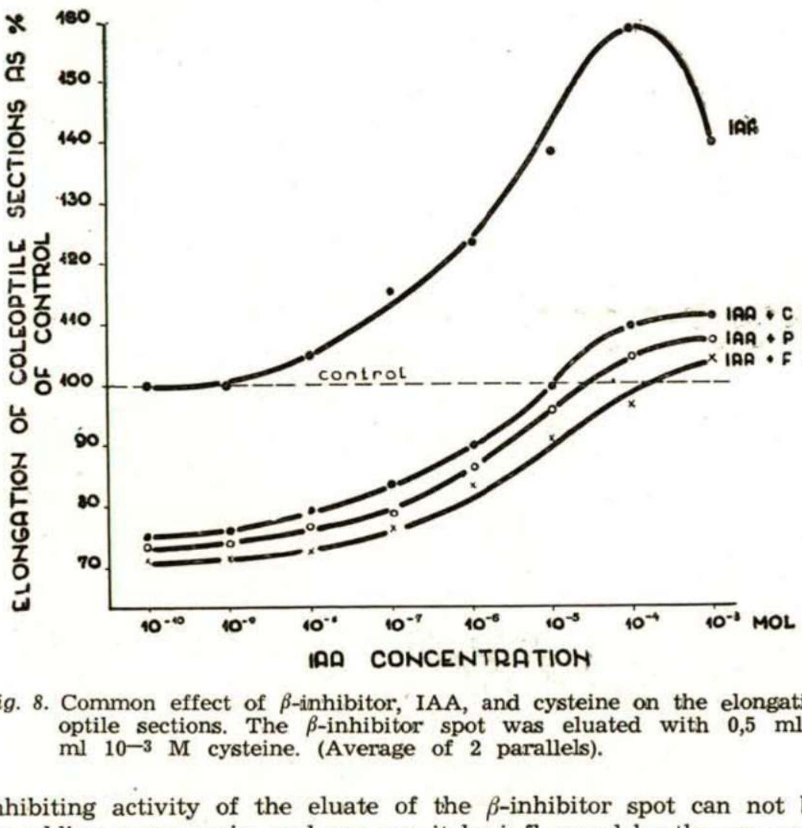

Fig. 8. Common effect of β-inhibitor, IAA, and cysteine on the elongation of coleoptile sections. The β-inhibitor spot was eluated with 0,5 ml IAA + 0,5 ml 10^{-3} M cysteine. (Average of 2 parallels).

inhibiting activity of the eluate of the β-inhibitor spot can not be stopped by adding more auxin, and nor can it be influenced by the presence of —SH compounds *(Fg. 8).* Accordingly, the inhibiting activity of the β-inhibitor complex is not attributable to —SH blocking.

Comparing the results with the literary data, it seems very likely, that the β-inhibitor complex demonstrated in fruit juices and the compounds of the β-inhibitor zones observed in the same position on chromatograms of other parts of plants (2, 3, 4, 5, 7, 8) are related.

3. Estimating the substances of the inhibiting zone along the front line it became evident that it contains the essential oils of the fleshy fruits. Accord-

ing to the bio-assays the effectiveness of the volatile oils in fruit juices is about equal to that exerted by benzoic- and cinnamic acid derivatives, therefore they are one of the main inhibitory agents in fleshy fruits.

III. Further examinations proved that in the juice of lemon fruits containing germinated seeds and seedlings respectively, the concentration of β-inhibitor complex and essential oils is decreased in a great extent (12).

IV. Ether extract of fleshy fruits in different stage of maturity were also chromatographed and bio-assayed to study the change in concentration of their growth-regulating substances in the course of the ripening. The results are the follows:

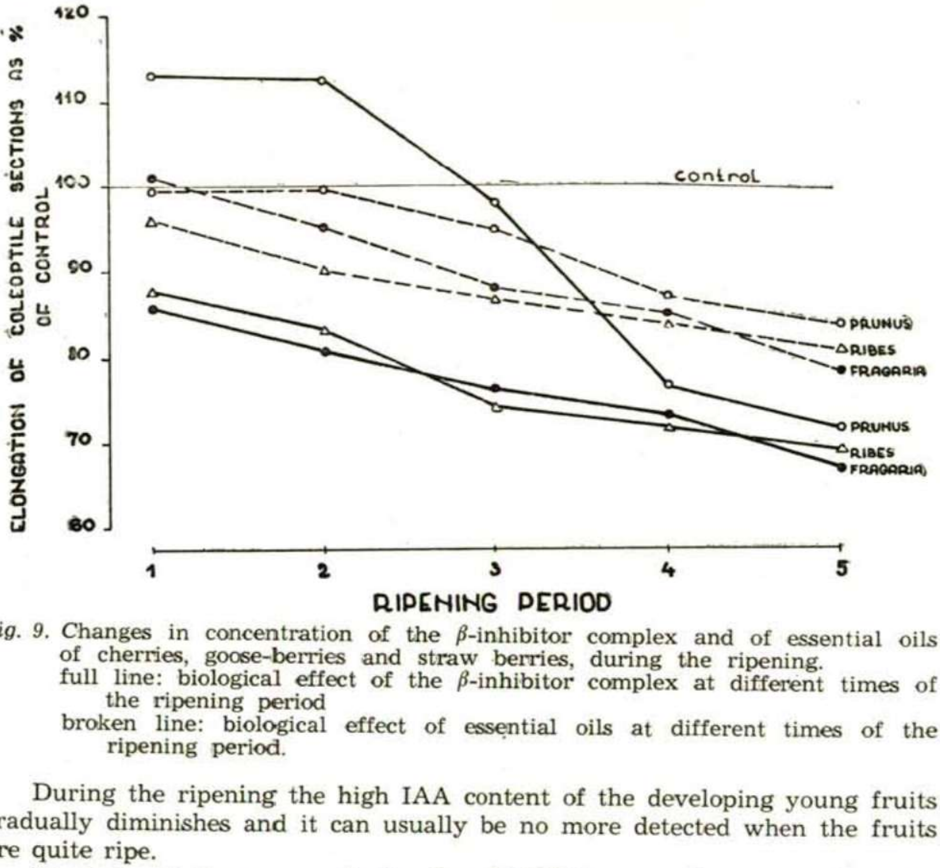

Fig. 9. Changes in concentration of the β-inhibitor complex and of essential oils of cherries, goose-berries and straw berries, during the ripening.
full line: biological effect of the β-inhibitor complex at different times of the ripening period
broken line: biological effect of essential oils at different times of the ripening period.

During the ripening the high IAA content of the developing young fruits gradually diminishes and it can usually be no more detected when the fruits are quite ripe.

In some of the young fruits the β-inhibitor complex accumulates in inhibitory amounts relatively earlier and in others later, and reaches its maximum in the entire ripe fruits *(Fig. 9)*. Hence, the separation of these inhibitors is most adventageous when the fruits are in the state of full maturity. The amount of the essential oils in the fruits also begin to accumulate in inhibitory concentrations towards the middle of the ripening period and the largest amount may be found in full maturity state *(Fig. 9)*.

The detailed analysis of the β-inhibitor complex of some fruits is in progress.

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