XLIX. THE NITROGEN METABOLISM OF THE KELSEY PLUM

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THE nitrogenous changes in mature Kelsey plums during storage appear to differ fundamentally from those in the apple. The most striking feature of the nitrogen metabolism of the apple is its phase of net synthesis of protein during the final stage of development on the tree [Hulme, 1936]. This drift towards protein synthesis is also characteristic of "mature", detached apple-fruits [Hulme, 1932]. Mature Kelsey plums, on the other hand, show a distinct drift towards protein breakdown when stored at either 35 or 70° F. [Donen, 1937, 2].

Hulme [1935] has shown, however, that the nature of protein changes in the stored apple depends upon its stage of physiological development: thus "young" apples display the same characteristics as "mature" plums and on storage at 1 or 12° C. show protein breakdown. It is possible then that the nitrogenous changes in the plum during its full development to maturity do not proceed further than the stage corresponding to "young" fruit in the apple. The most important steps in the nitrogen cycle of the growing plum have therefore been ascertained and are presented in this paper.

EXPERIMENTAL

Material and method of sampling. Two series of samples were collected. The object was to gauge whether fruit collected from trees grown under different environmental and cultural conditions showed any marked variation in the relationship between the estimated nitrogenous compounds.

Series I. The fruit was collected from 14 Kelsey trees worked on peach stock at Elgin, Cape. The trees were 9 years old and somewhat stunted in growth. Soil pebbly and poorly weathered. Petal fall on 20 October 1936.

Series II. The fruit was collected from 12 Kelsey trees on Mariana root stock at Somerset West, Cape. The trees were well grown and 10 years old. Petal fall on 5 October 1936.

To obtain a representative sample of plums Hulme's method of numbering the branches and fruit on each tree was adopted [Hulme, 1936]. The plums to be gathered for any one sample were then determined by a random selection of numbers. Each sample consisted of not less than 48 fruits. The collection of samples began about 20 days after petal fall and continued as long as the ripening fruit remained on the tree.

Preparation of material and methods of analysis. Only a brief outline of the methods employed will be given here, as they have been described in detail elsewhere [Donen, 1937, 1].

The fruit was brought to the laboratory within a few hours of picking. The plums were weighed and quartered. The stones were then removed and also weighed. Two opposite quarters of the pulp of each plum were then cut up finely by hand, thoroughly mixed and portions taken for extraction of soluble nitrogen

and for dry weight determination. Total nitrogen in the kernel and stone were estimated as outlined below for the fleshy portion of the fruit. Estimations of soluble, amino-, amide- and ammonia-nitrogen fractions were carried out on the pulp only, and in the discussion that follows all general references to nitrogen "in the plum" refer to nitrogen as estimated on the fleshy portions of the fruits only.

At intervals during the growing season triplicate samples were collected and used for the estimate of sampling error. Standard deviations were calculated by means of the formula:

$$\sigma = \sqrt{\frac{\sum v^2}{n-1}},$$

where σ = standard deviation, v = deviation from the mean, n = number of samples. Significant differences were taken as 2σ and are indicated by lines along the appropriate curves in the figures given in the text.

(1) *Total nitrogen* was estimated on material previously dried at 50° for 84 hr. Although tests showed nitrates to be absent, Ranker's modification of Kjeldahl's method to allow for nitrates was employed. The digest was made up to volume with ammonia-free water, and aliquots were used for determination of ammonia by a micro-distillation method.

(2) *Non-protein-nitrogen*. In a preliminary study of the distribution of N compounds in aqueous and alcoholic extracts of plums [Donen, 1937, 1], evidence was obtained suggesting that aqueous extracts of "young" fruit (up to about 20 g. per plum) contained N compounds (presumably of protein complexity) which are precipitated by colloidal ferric hydroxide [Thomas, 1927]. Water extracts of more mature plums were found to be unaffected by treatment with colloidal ferric hydroxide in that the total soluble N remained unaltered. Furthermore, in these larger plums 75 % alc. and water were found to extract equal amounts of the various N compounds estimated (soluble, amino-, amide- and ammonia-N). In the present instance, therefore, the non-protein-N extracts of the first six samples in Series I and the first four samples in Series II were obtained by grinding the pulp with ammonia-free water. The extract was conc. *in vacuo* at 25–30° and then treated with colloidal ferric hydroxide.

The alcohol extraction method was used for the remaining samples. Weighed portions of the material were frozen and kept at –10° until extraction could be carried out. The frozen material was ground and extracted with 75 % alc. at a low temp. The alcohol was distilled off *in vacuo* at 25–30° and the final extract was then made up to volume. The following estimations were carried out on aliquots of the extracts:

(a) *Non-protein-nitrogen*. 20 ml. extract were evaporated to dryness in a Kjeldahl flask at 40° under reduced pressure. Digestion and estimation of ammonia was carried out as for total-N.

(b) *Free ammonia-nitrogen*. This was estimated on 5 ml. extract by the method of Pucher *et al.* [1935]. A modified form of Parnas & Heller's [1924] distillation apparatus was employed. The ammonia was collected in *N*/50 acid and back-titrated with *N*/70 alkali, methyl red-methylene blue being used as indicator. The ammonia-N figure served as a blank for amide- and amino-N determinations.

Unexpectedly high values for ammonia were obtained during the early stages of growth of the plum. Since glutamine is known to be the cause of high results in this estimation, Chibnall & Westall's revised method for estimation of glutamine was employed [Vickery *et al.* 1935]. None was found.

(c) *Amide-nitrogen*. The method of hydrolysis employed was as laid down by Vickery *et al.* [1935]. These investigators have pointed out that urea and

allantoin may give rise to appreciable amounts of ammonia under conditions of ordinary glutamine or asparagine hydrolysis, and have suggested hydrolysis for 4 hr. at 100° and pH 6.5 as a test for this source of ammonia. Since the amide-N values of extracts of early samples of plums were found to be greater than the amino-N values, the above test was carried out in case the high amide-N values were due to interfering substances, but no rise in ammonia-N was observed.

(d) *Amino-nitrogen*. This was estimated by the Sørensen [1908] formaldehyde titration which was preferred to the usual Van Slyke method. A comparator was found satisfactory to compensate for the colouring matter in the extracts, which led to erratic results in the Van Slyke method.

(e) *Protein-nitrogen* = total N—non-protein-N.

Expression of results

It is usually assumed that the amide-N obtained on mild hydrolysis is due entirely to amides of the asparagine type. This requires the amino-N value to be at least equal to that of amide-N. Maskell & Mason [1929] have pointed out that many publications show the amino-N content of various plant tissues to be less than that of the amide-N. They emphasized the possibility of “amide-nitrogen” being derived from sources other than asparagine and suggested that in such cases the correct procedure should be to discuss amino-N and amide-N separately. This has been done in the present instance as it was found that during the early stages of development of the fruit amino-N was consistently lower than amide-N.

It is possible that low amino-N values were obtained because of the use of the formaldehyde titration method. But tests with the usual Van Slyke method also gave amino-N values lower than the amide-N.

The results obtained in this investigation are shown in the form of graphs (Figs. 1–9) and are expressed as mg. N per fruit, or in terms of % total N.

DISCUSSION OF RESULTS

In a discussion dealing with the development of a fruit like the peach or plum it is necessary to emphasize the importance of the stone. The stone reaches its full development early in the life of the fruit and marks a definite stage in its carbon metabolism [Donen, 1936]. During this period of “stoning” the rate of increase of total solids in the flesh of the fruit is very low and most of the incoming sugars appear to be utilized for the formation of cellulosic material of the stone.

Since it will be shown that the nitrogen metabolism of the plum is also greatly affected by the growth of the stone,¹ the most important features of the growth and nitrogen increase in the stone and kernel will be briefly considered. Fig. 1 shows that the stone reached its full growth about 50 days after petal fall. The kernel continued accumulating N for almost the complete duration of the growth cycle, but the N content of the stone reached a maximum as soon as the stone was fully developed, and then dropped rapidly to a minimum value (Fig. 2). If the pulp and stone be considered as one unit, then it will be seen from Fig. 3 that the stone absorbed a considerable proportion of the total N taken in by the fruit during its initial period of growth. This proportion reached a maximum at approximately the middle of the “stoning” period. It will be shown that this maximum coincided with a minimum concentration of amino-N in the pulp of the plum.

¹ The term “stone” as used here refers to the endocarp only.

Fig. 1. Growth curves of plum and stone for both series.

Fig. 2

Fig. 3

Fig. 2. Nitrogen content of stone and kernel during growth.

Fig. 3. Proportion of nitrogen, in stone plus pulp, accumulated by the stone alone. Note maximum values for Series I at day 35 and for Series II at day 30.

Fig. 4. Total, non-protein and protein-N content of pulp of Kelsey plums during growth (mg. N/fruit).

Fig. 5. The ratio of protein to non-protein-N in the pulp of the Kelsey plum throughout its development.

Fig. 6. [The amino-, amide- and ammonia-N content of the pulp of the Kelsey plum during growth (mg. N/fruit).

Fig. 7. The amino-, amide- and ammonia-N content of the Kelsey plum during growth (mg. N/fruit). Series II.

Fig. 8. Amide-, amino- and ammonia-N in the pulp as % of total N.

Fig. 9. The ratio of the rates of intake of protein- and non-protein-N in the pulp of the plum, per day, per fruit. This curve was calculated from the equations of curves fitted to total and non-protein-N accumulation curves, Series I, Fig. 4.

Protein- and non-protein-nitrogen. Except for a brief period during the early stages of its life (corresponding to the "stoning" period) the plum accumulated greater amounts of soluble N than of protein-N (Fig. 4). The curves shown in this figure are very similar in form to the growth curves (Fig. 1) and indicate that N compounds were being accumulated by the fruit as long as it remained on the tree. The plum differs from the apple in showing no fall of total N towards the end of the maturation period of the fruit. In the apple this fall may possibly be connected with the ripening of the seeds [Hulme, 1934].

The ratio of concentrations of protein-N to non-protein-N (P/NP) was very high in the first samples of each series, and by extrapolating the curves in Fig. 5 it may be shown that during the first few days after fruit "setting" as much as 80 % of the total N was stored as protein. The P/NP ratio fell rapidly during the "stoning" period and reached its lowest level at the beginning of the final stage of maturation, i.e. with the first dawn of colour in the flesh of the fruit. During this final stage the P/NP ratio remained practically constant. The remarkable phase of "net protein synthesis" which Hulme observed in mature apples appears to be absent in the plum. Nor has it been observed in grapes which also show a constant P/NP ratio throughout their ripening period although allowed to remain on the vine until the berries were almost "raisinified" [Douglas, 1937].

Non-protein-nitrogen fractions. The first stage in the development of the plum was remarkable for the high accumulation of ammonia which reached its maximum (in mg. N per fruit) at the end of the "stoning" period (Figs. 6 and 7). It then formed as much as 14 % of the total or 25 % of the soluble N in the pulp. In a study of the development of the wheat grain, Woodman & Engledow [1924] reported that the early sample contained as much as 20 % of its total N in the form of ammonia and concluded that a part at least of the entering N was in that form. It is highly probable that ammonia is also the primary nitrogenous substance entering the plum, for it would be most unexpected to have a considerable accumulation of ammonia as a result of down-grade processes at a time when synthesis of proteins proceeds at its maximum rate.

The high ammonia content appears to be intimately associated with the process of stone formation: the ammonia-N increased during its growth, but diminished rapidly as soon as the stone was completely developed. The period of "stoning" is characterized by the absence of sucrose, low rate of fructose and glucose accumulation and rapid hydrolysis of starch—all pointing to a high carbohydrate demand [Donen, 1936]. It is quite possible, therefore, that the high ammonia accumulation was due to a dearth of active carbohydrate which is, apparently, essential for conversion of ammonia into more complex nitrogenous compounds [Onslow, 1931; Murneek, 1935]. Hulme [1936] found no appreciable variations in the ammonia content of the apple throughout its growth.

Amino-N changes in the early stages of the development of the plum also appear to be influenced by the growth of the stone. In Series I a significant drop in amino-N (as % total N) was observed during the "stoning" period (Fig. 8). Unfortunately the picking of samples for Series II began too late to illustrate the same point, but it is rather significant that the minimum values for amino-N in both series should have occurred at the time when the nitrogen in the stone (as proportion of the total-N in pulp plus stone) was at its maximum and the influence exerted by the stone must have been at its highest (Figs. 3 and 8). If it may be assumed that the nitrogen supply of the stone came via the fleshy portion of the fruit, then the observed drop in amino-N might be explained by a migration of nitrogen into the stone.

The ammonia content of the plum fell very rapidly during the second stage of growth (Figs. 6, 7 and 8). At the same time the amide- and amino-N values rose steeply. The ammonia and amide fractions appear to be distinctly inter-related, for it was observed that, as percentage of total N, the amide-N reached its maximum value at the same time as the ammonia-N fell to its lowest level. In addition, when amide-N per fruit ceased to increase during the maturation period (Figs. 6 and 7) the ammonia-N value showed a distinct upward drift.

The cessation of amide-N storage mentioned above appears to be the only important event in the nitrogen metabolism of the plum that occurs during the

maturation period. The accumulation of amino-N (mg. per fruit) was in no way affected by the ripening process.

Here it is of interest to note that, like the plum, the apple has a stage of almost complete cessation of amide-N accumulation [Hulme, 1936]. In the apple this is preceded by a stage of rapid increase in asparagine (i.e. increase in amide-N = increase in amino-N), and, as in the plum, any appreciable rise in amino-over amide-N takes place only after the latter ceases to be stored by the fruit. This phase in the nitrogen metabolism takes place very early in the apple's life, and in this respect the original thesis that young apples and mature plums might, as far as nitrogen is concerned, be in the same physiological stage, seems to be substantiated.

During the maturation phases, however, the apple and the plum differ strikingly in their nitrogen metabolism. In the apple the tendency is towards increasing synthesis and with maturation a state of "net synthesis of protein" ensues. In the plum, on the other hand, slow inactivation of the processes responsible for protein synthesis appears to be the concomitant of maturation. This is suggested by the curve of the ratios of protein to non-protein-nitrogen accumulated per fruit, per day shown in Fig. 9. This curve was obtained by calculating the equations of lines of best fit to the experimental values for total and soluble nitrogen per fruit (Series I). From these the rates of accumulation were obtained and the calculated ratios plotted. It will be observed from Fig. 9 that with maturation there is a steady fall in the proportion of protein synthesized per unit of nitrogen taken in by the plum.

SUMMARY

1. The object of this investigation was to ascertain the most important stages in the nitrogen metabolism of the Kelsey plum.

2. Methods of sampling and analysis are briefly described. Samples were collected from two different sets of Kelsey trees.

3. Graphs are presented showing the changes in weight of fruit and stone, total N, non-protein-N, protein-N, ammonia-N, amide-N and amino-N throughout the course of development of the plum from petal fall to maturity.

4. There are three stages in the nitrogen metabolism of the plum. The first stage is greatly affected by the growth of the stone: the protein-/non-protein-N ratio falls very rapidly from 1.8 to 0.6 during this period, ammonia-N accumulates in the fleshy tissue of the plum up to 14% of the total N content, and the percentage of amino-N is diminished, presumably by migration into the stone. The second stage is marked by a rapid rise in both amino- and amide-N whilst ammonia-N falls to its lowest level. It is suggested that ammonia- and amide-N are interrelated. During the last stage of maturation the amide-N content of the plum remains constant.

5. It is pointed out that the nitrogenous changes that take place in the plum during its second and third stages of maturation also occur in the apple, but only when the latter is very "young". In mature apples the nitrogen metabolism tends towards increasing protein synthesis; in mature plums the tendency is towards increasing accumulation of non-protein-nitrogen.

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