Changes in Chemical Composition of Soybean Seeds during Ripening

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Soybean seeds contain protein, fat, and other important nutrients as a food. To examine how these components undergo transitional changes during seed ripening as related to their biosynthesis is very important for making clear the characteristics of seed ripening and for producing more these useful substances. Although there are several studies concerning the transitional changes of one or another component, attempts to grip an overall pattern of accumulation of these components by analyzing them simultaneously have been done only rarely. Therefore some of the experiments carried out by the author from such a viewpoint will be presented in this paper.

As the majority of seed dry matter (about 75% in case of a cultivar Norin-2) is accumulated after the flowering stage, production and chemical composition of seeds are greatly influenced by conditions of the ripening period. In the experiments, therefore, about ¼ of the number of pods per plant was thinned in order to increase amount of substances to be translocated to each pod (hereafter referred to the less-pod plot) in comparison with the untreated plant (the control plot).

Growth of pods

Length and width of pods increased rapidly at the very early ripening stage, and seed

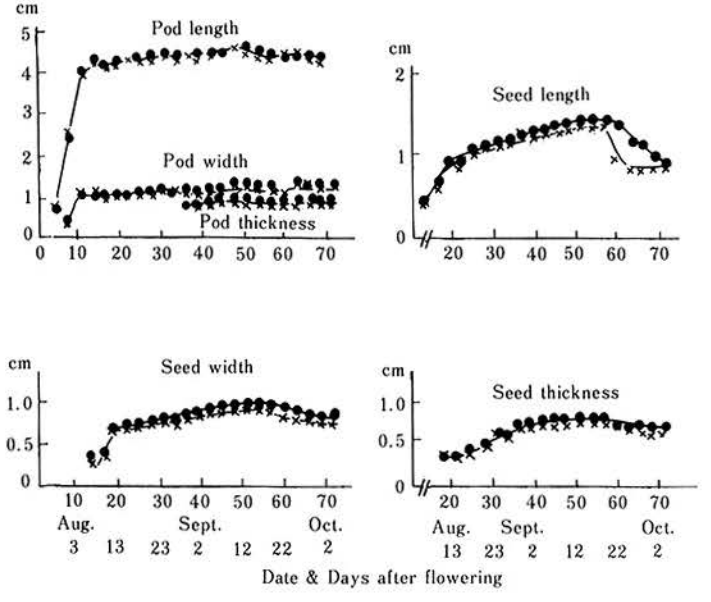

Fig. 1. Changes in pod and seed size during ripening.
● : limited in no. of pods × : control

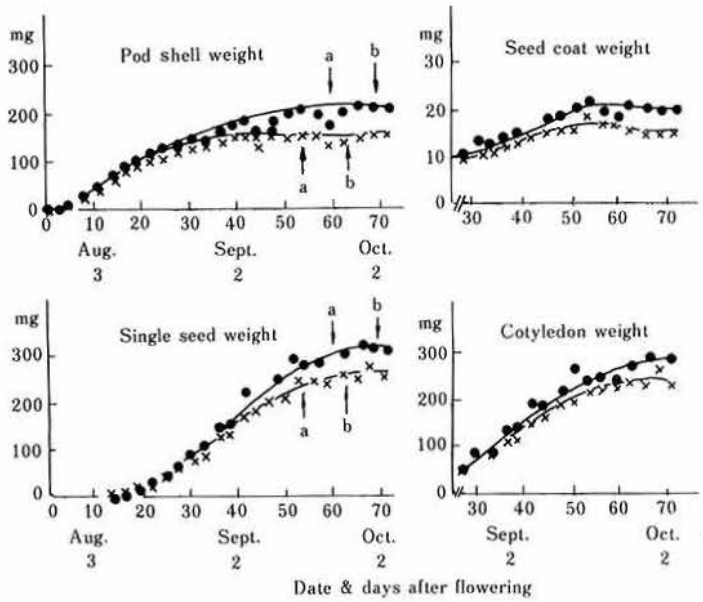

Fig. 2. Changes in weight of each organ of pod during ripening.
a : Leaf yellowing b : Defoliation
● : Limited in no. of pods × : Control

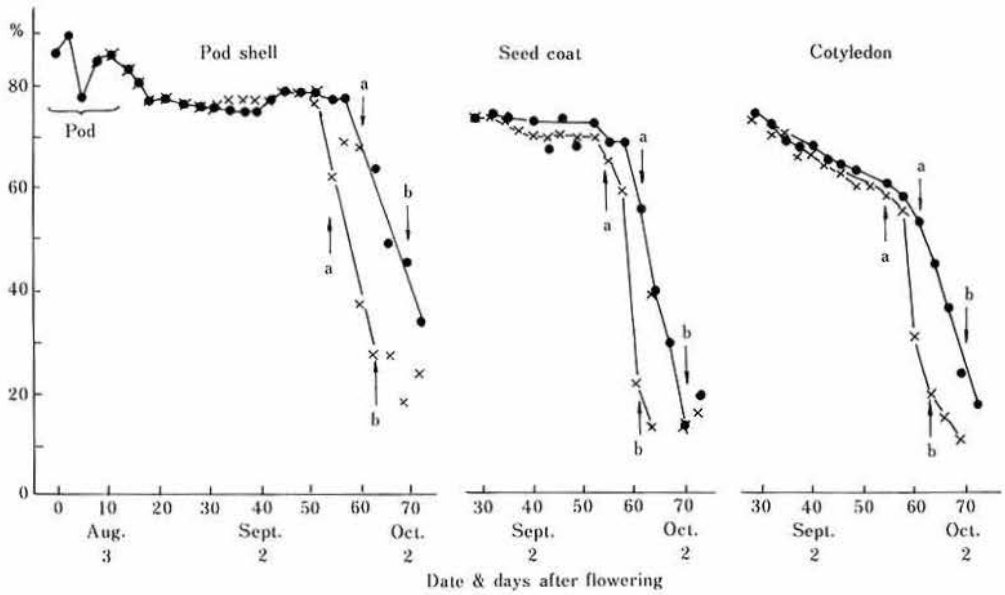

Fig. 3. Change in water content of pod shell, seed coat and cotyledon during ripening.
a : Leaf yellowing b : Defoliation
● : Limited in number of pods × : Control

filling started when the pod elongation was nearly completed. Size and weight of the seeds increased by the pod yellowing stage. Starting from about this stage, the seeds began to lose their moisture rapidly, resulting in reduced seed size, especially reduced seed length, and consequently a spherical shape of seeds at the maturity.

In the less-pod plot, in which more nutrients were translocated into each pod, the size and weight of seeds were greater than those of the control plot, and the ripening process was slightly prolonged with the delayed maturity (Fig. 1, 2, and 3).

The stage of seed filling initiation and the stage when seed moisture content begins to decrease rapidly are quite distinctive, and by taking these stages as borders the whole ripening process can be divided into 3 periods: the period of pod development, the period of cotyledon development, and the period of drying of pods and seeds.

Changes in chemical composition of pods during ripening

Pods were divided into pod shells, seed coats, and cotyledons, and contents of N, P, K, Ca, Mg, protein, carbohydrates (0.7N HCl soluble), starch, sugars and fat were determined.

In the cotyledons, content (in mg) of all these components increased during ripening, except carbohydrates including starch which decreased in the late ripening period. In the less-pod plot, all of these components showed higher contents than the control plot, without exception (Fig. 4).

In percentage of content in the cotyledons, P. Ca, Mg, etc. showed temporarily lower values due to dilutions caused by the rapid growth in size of cotyledons at the initial stage of ripening, but later their percentages increased with the progress of ripening except Ca and K. The content (%) of protein continued to increase by the time immediately before the maturity, while that of fat reached a final level at the middle of ripening period and remained at that level until maturity. Starch percentage increased by the middle of ripening period, and then decreased. In the less-pod plot, most components showed higher percentages than the control, but Mg, K, and sugars were similar to the control, while fat gave apparently lower percentage than the control (Fig. 5).

Based on this observation, it is suggested that at the middle of ripening period the mechanism for fat biosynthesis is established, and the final content (%) of fat is determined already at that time, so that in the later period fat is produced in proportion to the increase of cotyledon weight. On the contrary, protein is synthesized at a rate exceeding the rate of increase of seed weight, and its final content (%) is not determined until the late ripening stage, being influenced by nutritional conditions by the late ripening stage. Under the better nutritional condition of the less-pod plot, protein percentage was higher whereas fat percentage was lower than the control, although the amount of fat content was higher. The lower fat percentage is caused by the greater increases of protein and carbohydrates in relative proportion to fat.

The transitional pattern of starch indicates that starch is a temporary reserve substance in the cotyledons, and is used as a material for the production of protein, fat, sugars and others.

Content (%) of K was kept almost constant after the middle stage of ripening, suggesting that the translocation of K proceeds at the same rate (with a close relation) as the translocation of carbohydrates to the cotyledons.

In pod shells, P and N decreased from the early stage, and sugars decreased in the later period.

In seed coats, P, N, K, protein and sugars increased until the late ripening stage, and then decreased rapidly, suggesting their translocation to cotyledons. Particularly, sugars in pod shells and seed coats reached the maximum immediately prior to the pod yellowing stage, and then decreased rapidly (Fig. 6), suggesting a close relation with the conversion to the maturation.

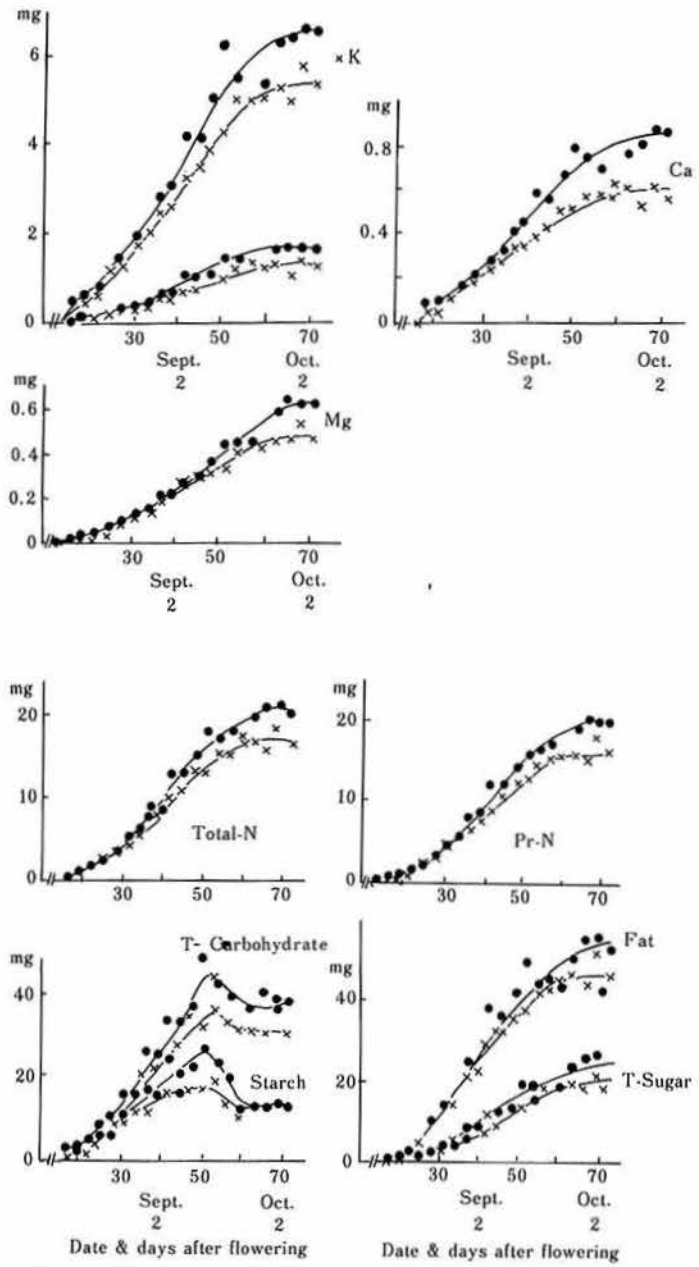

Fig. 4. Changes in content of chemical components in cotyledon during ripening.
● : Limited in number of pods × : Control
Seed coat was included up to 25 days after flowering.

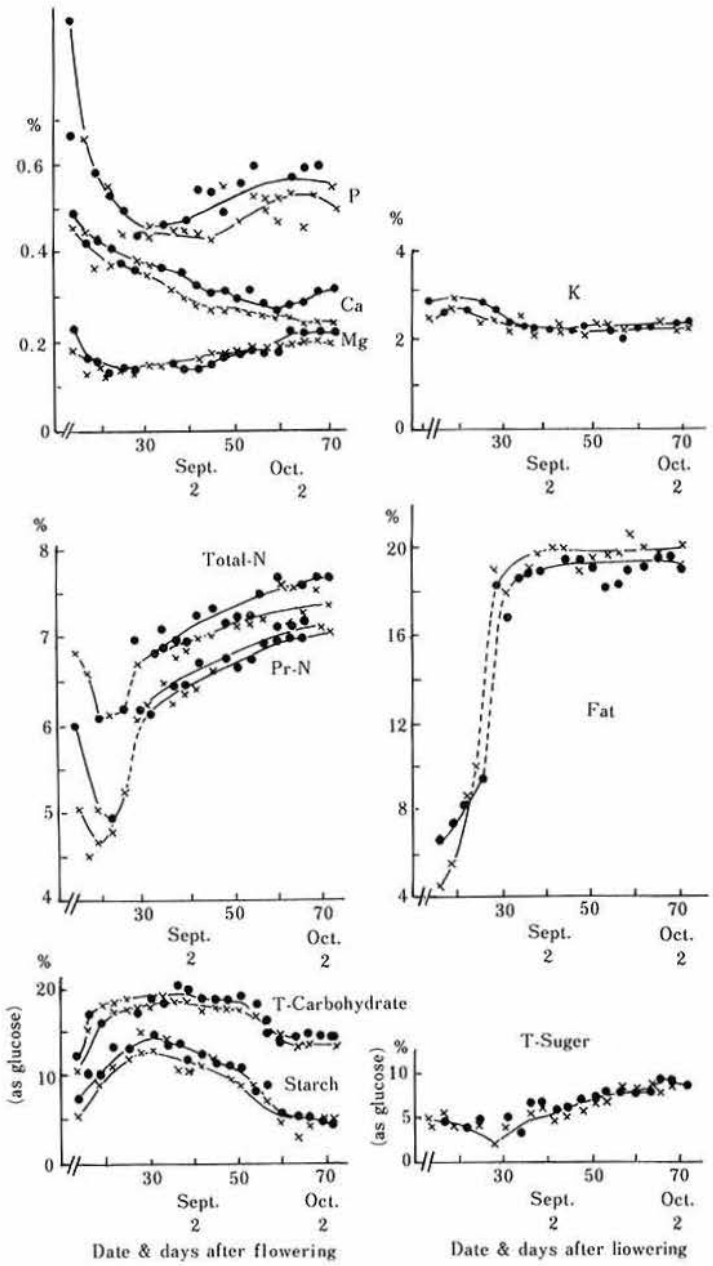

Fig. 5. Changes in content (%) of chemical components in cotyledon during ripening.
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Seed coat was included up to 25 days after flowering.

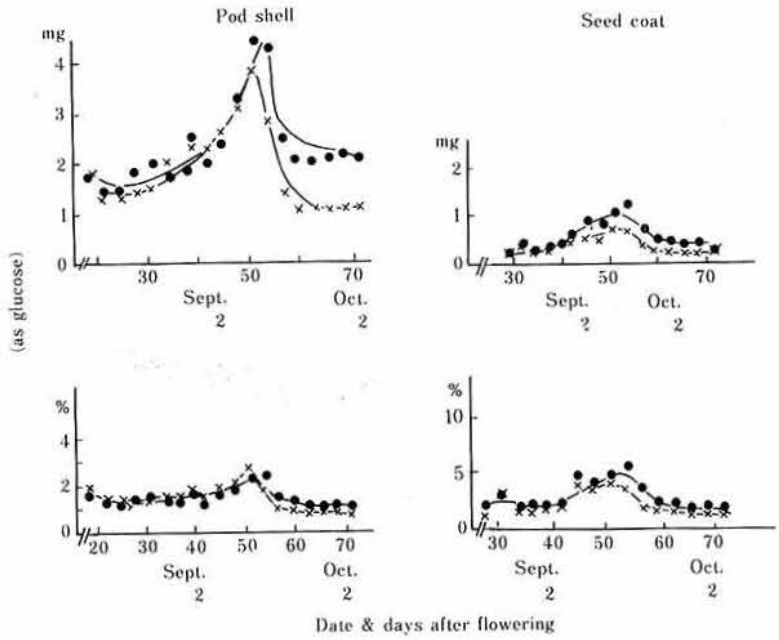

Fig. 6. Changes in total sugar contents in pod shell and seed coat during ripening.
● : limited in no. of pods × : Control

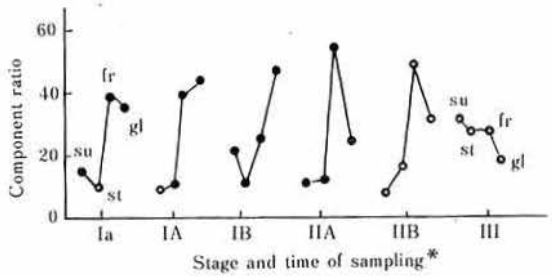

Fig. 7. Component ratio of sugars in leaf.
Su : Sucrose fr : fructose * : same as Table 5.
St : Stachyose gl : glucose

Changes in sugars during ripening

Composition of sugars was examined by thin layer chromatography and column chromatography. For comparisons, sugars in pod shells and leaf blades were also examined (Table 1–5, Fig. 7).

Flowers, very young pods, and very young seeds were rich in fructose and glucose, containing only small amount of sucrose and stachyose. Ratio of individual sugars in flowers and very young seeds resembles that of leaf blades, and ratio of fructose to glucose in very young pods resembles that of pod shells at the seed filling stage.

In the cotyledons, sucrose increased very rapidly from the time when cotyledons devel-

Table 1. Changes in sugar contents in seed during ripening (mg/seed)

weeks after flowering	Sucrose		Raffinose		Stachyose		Fructose		Glucose		Total	
	A	B	A	B	A	B	A	B	A	B	A	B
0		0.0042				0.0035		0.0320		0.0360		0.0757
0.5		0.0048				0.0096		0.0560		0.0140		0.0844
1		0.028				0.024		0.630		0.400		1.082
2		0.0007				0.0004		0.0160		0.0130		0.0301
3	0.500	0.320			0.030	0.023	0.310	0.260	0.260	0.200	1.100	0.803
4	4.50	4.48			0.08	0.05	0.20	0.26	0.36	0.10	5.14	4.89
5	7.22	7.56			0.17	0.09	0.14	0.22	0.32	0.24	7.87	8.11
6	13.86	9.79	0.04	0.02	0.11	0.09	0.36	0.24	0.50	0.29	14.87	10.43
7	21.15	11.36	0.03	1.40	0.18	0.48	0.37	—	0.38	0.45	22.08	13.69
8	19.60	14.00	1.20	1.12	2.52	2.52			0.20	0.80	22.12	18.44
9	13.14	10.26	2.40	2.40	6.48	5.76			0.09	0.18	22.11	18.60
10	12.87	11.70	1.49	2.40	6.12	6.27			0.17	0.09	33.65	20.46

A: limited in number of pods B: control

Table 2. Changes in sugar contents in seed during ripening (%/dry weight)

weeks after flowering	Sucrose		Raffinose		Stachyose		Fructose		Glucose	
	A	B	A	B	A	B	A	B	A	B
0		0.303				0.255		2.330		2.621
0.5		0.063				0.126		0.735		0.189
1		0.116				0.099		2.611		1.666
2		0.107				0.064		2.545		2.096
3	4.077	3.010			0.265	0.210	2.518	2.038	2.110	1.867
4	6.429	7.491			0.107	0.087	0.286	0.435	0.514	0.251
5	5.671	6.195			0.132	0.079	0.113	0.177	0.254	0.197
6	6.569	5.283	0.017	0.010	0.051	0.049	0.171	0.129	0.239	0.155
7	8.667	5.491	0.012	0.675	0.074	0.232	0.139		0.154	0.218
8	6.568	5.460	0.402	0.437	0.370	0.983			0.067	0.072
9	4.859	4.132	0.887	0.967	2.137	2.320			0.030	0.072
10	4.352	4.098	0.502	0.847	2.069	2.021			0.056	0.032

A: limited in number of pods B: control

Table 3. Changes in component ratio of sugars in seed during ripening

weeks after flowering	Sucrose		Raffinose		Stachyose		Fructose		Glucose	
	A	B	A	B	A	B	A	B	A	B
0		6				5		42		48
0.5		6				11		66		17
1		3				2		58		37
2		2				1		53		44
3	46	40			3	3	28	32	23	25
4	88	91			1	1	4	5	7	8
5	92	93			2	1	2	3	4	3
6	93	94	0.2	0.2	1	1	2	2	3	3
7	96	84	0.1	10	1	3	1		2	3
8	89	76	5	6	5	14			1	4
9	60	55	11	13	29	31			0.4	1
10	62	57	7	12	30	31			1	0.4

A: limited in number of pods B: control

Table 4. Sugar contents in pod shell

Stage	Sucrose	Stachyose	Fructose	Glucose	Total
mg/pod					
height of pod filling (6A)	1. 26	0. 18	2. 89	1. 00	5. 33
pod yellowing stage (9A)	0. 22	0. 16	0. 26	0. 06	0. 70
maturity	0. 09	0. 04	0. 18	0. 10	0. 41
%/fresh weight					
height of pod filling (6A)	1. 56	0. 22	3. 59	1. 24	6. 61
pod yellowing stage (9A)	0. 57	0. 39	0. 66	0. 15	1. 77
maturity	0. 63	0. 27	1. 23	0. 66	2. 79
Component ratio					
height of pod filling (6A)	24	3	54	19	
pod yellowing stage (9A)	32	22	37	9	
maturity	22	10	44	24	

( ) : weeks after flowering and treatment

Table 5. Sugar contents in leaf (%/dry weight, var. Shirodaizu)

	Stage and time of sampling		Sucrose	Stachyose	Fructose	Glucose
Ia	young leaf, pod filling stage,	6 am	0. 71	0. 50	1. 94	1. 80
IA	adult leaf, pod filling stage,	6 am	0. 20	0. 25	0. 88	1. 01
IB	adult leaf, pod filling stage,	3 pm	0. 55	0. 27	0. 66	1. 39
IIA	yellowed leaf at early stage	6 am	0. 21	0. 21	1. 03	0. 46
IIB	ditto	3 pm	0. 11	0. 23	0. 74	0. 46
III	fallen leaf		0. 11	0. 10	0. 10	0. 06

oped rapidly (about 3 weeks after flowering), reaching more than 90% of the total sugars at the most active growth stage of seeds. However, from the time prior to the pod yellowing stage, raffinose and stachyose increased while fructose decreased. In the seeds at maturity, sucrose, raffinose and stachyose accounted for 60, 10 and 20% of the total sugars respectively.

Such changes in the constituents of sugars correspond quite well to the 3 characteristic stages stated above; i.e. pod shell formation, accumulation of substances, and drying of pods. Particularly, the occurrence and increase of raffinose and stachyose are related to the start of yellowing of pods. In the control plot, where the yellowing occurred earlier than the less-pod plot, raffinose and stachyose appeared earlier and increased to more amount than the latter. Glucose and fructose observed in the early ripening stage are contained in tissues without reserve substances, sucrose observed in the middle stage of ripening is the sugar translocated from leaf blades and related to reserve substances, and raffinose and stachyose in the late ripening stage are considered to be a reserve form of sugars.

Raffinose and stachyose are consisted of sucrose combined with 1 or 2 molecules of galactose respectively. However, galactose in its free form was not detected in seeds, leaf blades or pod shells. As raffinose is known to be produced from UDP-galactose by an enzyme preparation taken from soybean[1], it is considered that galactose may exist in the form of combination with UDP, or UDP-galactose may be produced from UDP-glucose instead of galactose.

Under the better nutritional condition, sucrose which is closely related to substance accumulation was high in amount and percentage of content.

Immediately prior to the yellowing of pods,

sugars in pod shells decreased as stated above. In this case, the decrease was found to occur with each sugar, not with a particular kind of sugar. As similar observations were obtained commonly with several other cultivars, the above results are considered as expressing the general features of soybean plants.

Changes in free amino acids

Using Norin 2, T201 which does not produce root nodules, and its isoline T202, changes in free amino acids during ripening were examined in the less-pod plot in contrast to the control plot. Amino acid analyzer was used for the determinations.

Many kinds, nearly 40, of free amino acids and related substances were found in seeds during ripening. Their content per seed was greatest at the most active growth stage of seeds. Content (%) on dry weight basis was highest at the youngest stage of seeds.

The major amino acids were asparagine, α-amino butyric acid, histidine, glutamic acid and alanine, etc. Of them, asparagine, α-amino butyric acid and histidine were found to be at high proportion in very young seeds, and alanine, glutamic acid and arginine were predominant at the middle stage of ripening, while tryptophan and glucosamine became predominant in the late ripening stage. This pattern was observed similarly with other cultivars and at different nutritional conditions.

Differences observed between presence and absence of root nodules seemes to be simply resulted from the difference in nutritional levels. It appears that root nodules may not exert qualitative effects on free amino acids in seeds.

As relative quantities of individual free amino acids are different from those of protein, transamination must be occurred, in the process of protein synthesis.

Conclusion

Based on the morphological changes of pods during ripening, the ripening process could be divided into 3 periods: pod shell development, seed development, and drying of pods. Chemical composition of seeds was found to show characteristic changes corresponding to these 3 periods. Particularly, the occurrence or biosynthesis of glucose and fructose in the early stage of ripening, sucrose in the middle stage, and raffinose and stachyose in the late stage was quite distinct. Occurrence of glucosamine was also a characteristic of the late stage.

It was recognized that fat content (%) of seeds is determined as early as in the middle stage of ripening, while protein content (%) continues to be influenced by nutritional conditions until the late ripening stage. The better nutritional condition caused by pod thinning gave increased contents (%) of carbohydrates and protein, with the consequent decrease of fat content (%).

Further studies on the transitional changes of these chemical components and various conditions effecting them are needed to obtain more protein and fat with better qualities.

It is suggested that further investigations on the occurrence of raffinose, stachyose and glucosamine may find a clue to the problem of physiological mechanism of ripening.

References

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