THE RELATION OF MATURITY TO THE COMPOSITION OF BROAD BEAN SEEDS

II On the Carbohydrates

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(Received May 25, 1955. Accepted as received.)

INTRODUCTION

The broad bean, *Vicia faba*, is an important legume in Kagawa Prefecture. It is very suitable as a winter crop in the rice field, since it can maintain soil fertility. However, its cultivation is decreasing gradually owing to the lack of specific uses. Studies should be made in order to develop some new uses of broad beans. It is necessary to investigate the relation of maturity to the composition of broad beans as one of the fundamental studies in this connection.

The first object of this study is to find out the most suitable time of harvest. As described previously[1], it is most suitable to harvest broad beans toward the end of May (about May 26) in utilizing them as green vegetable, while it is most suitable to harvest them in early June (about June 7) in utilizing as dried cereal. These conclusions are made on the measurement of the yield of seeds and the analysis of general crude components.

In this paper the result is reported on the analyses for carbohydrates, especially starch and amylose. A part of the cost of investigation is defrayed by a grant (1954) from the Ministry of Education to KAWAMURA who is a member of the group of fundamental studies on the utilization of starch headed by Prof. Ziro NIKUNI, Osaka University.

The second object of this study is to know of the mode of biochemical formation of starch in broad beans.

In 1899 EMMERLING[2] analyzed various parts (including seeds and hulls) of broad bean plants harvested at intervals of 14—20 days to investigate the variation of nitrogenous constitituents. Using *Vicia faba minor* as the sample, BLAGOVYESHCHENSKI[3] showed that starch content was 8.53, 21.24, 48.08, 47.87, 47.91% based on dry weight as the maturity increased. He calculated starch content by hydrolyzing first with diastase and then with hydrochloric acid, and determining glucose by the BERTRAND method. Recently MORIYA, MORIMOTO, and KURATA[4] analyzed broad beans harvested at Tiba. They analyzed various parts of broad bean plants at 5 maturities, but they analyzed seeds only at two later harvest times. Their data on broad beans harvested at May 29 and June 7 were, respectively, moisture on fresh basis 85.7, 75.2%; crude protein on dry basis 30.3, 29.0%; crude fat 4.1, 0.5%; nitrogen-free extract 39.2, 54.5%; crude fiber 21.8, 11.6%; and ash 4.6, 4.6%.

Concerning the maturity studies with other starchy legumes than broad beans, there are several studies on snap beans (*Phaseolus vulgaris*)[5-7], wrinkled peas (*Pisum sativum*)[8-12], and smooth peas (*P. sativum* var. *arvense*)[13].

EXPERIMENTAL

Material The brosd beans, variety *Sanuki nagasaya* (*nagasaya* means "long pods"; *Sanuki* is an old name for Kagawa Prefecture), were cultivated at Kagawa Agricultural Experiment Station.

The seeds were sown on October 31, 1953, the flowers bloomed from March 21 to April 21, 1954. The seed samples were taken 13 times at intervals of about 5 days from April 21 to June 16. However, very young seeds could not be collected sufficiently to make analyses possible. The analyses were made on dried samples of seeds which were collected from three successive points from the lowest point, where the first blossom bloomed, attached to the main stem (which often withered out) and the 4 primary branched stems. They were considered to correspond to the seeds from the flowers which were in bloom about the same time[14].

The Method of Analysis The reducing sugar was determined as follows. Extract the dried and pulverized sample at 85° with 50% ethanol two times each for 1 hour. Wash the residue with water at 85°. Determine the reducing sugar in the combined solution of the extracts and washing by the BERTRAND method and express as glucose. (Broad beans contain small amounts of reducing sugars which are glucose and fructose[15].)

The nonreducing sugar was determined as follows. Hydrolyze the above solution obtained by extraction with 50% ethanol by heating 2 hours with 2% hydrochloric acid on boiling-water bath. Determine the reducing sugar as glucose. Subtract the direct reducing sugar from total sugar. Multiply the difference with 0.95 and express as saccharose. (Broad beans contain saccharose, raffinose, and stachyose[15].)

Starch was determined by the method of MCCREADY et al.[16] after a small modification. Sugars were removed by 2—4 extractions with hot 80% ethanol by the original method, but we carried out 2 extractions with 80% ethanol at the room temperature and then 4—5 extractions with cold water to remove sugars and soluble proteins. The last extract was confirmed to be free from soluble sugars by the anthrone reaction. Treat centrifuged residue with 52% perchloric acid at 0° twice to dissolve starch. Add anthrone reagent to the starch solution, heat at 100° for 7.5 minutes, and determine the color intensities at 620 mμ after cooling. Find out the glucose value by a calibration curve for standard glucose solution containing the same amount of perchloric acid. Multiply glucose found by 0.90 to convert to starch. This determination required only 0.2 g of the sample dried broad beans.

Amylose or more correctly blue value was determined by the MCCREADY method[16]. Add iodine-potassium iodide solution to starch solution in perchloric acid, and determine the color intensities at 660 mμ. The blue value was divided by the amount of starch present in the aliquot used and comparable value was calculated.

Table 1. Analyses of broad beans harvested during growth.

Date of harvest		Age from beginning of bloom days	Moisture % on fresh basis	% on dry basis									
				Crude protein	True protein	Crude fat	Ash	Crude fiber	N-free ext.	Reducing sugar	Nonreducing sugar	Starch	Amylose (approx.)
April	30	41	86.8	41.2	22.0	0.8	3.8	8.0	46.2	—	—	8.0	1.6
May	6	47	81.3	34.8	19.7	0.9	3.7	12.8	47.8	1.2	6.8	11.0	1.6
	11	52	80.8	33.4	22.5	1.0	3.6	7.7	54.3	—	—	27.8	3.6
	17	58	75.6	31.7	26.9	1.1	3.5	8.4	55.3	1.1	4.1	36.9	4.1
	21	62	72.7	31.0	23.1	—	—	—	—	—	—	—	—
	26	67	61.1	29.5	24.6	1.1	3.8	7.5	58.1	—	—	33.1	12
June	1	73	42.2	28.2	24.5	1.4	4.8	7.4	48.2	1.1	4.1	39.4	14
	7	79	26.2	29.0	24.7	1.4	5.3	7.2	57.1	—	—	40.8	12
	11	83	26.5	29.3	24.2	1.5	5.3	7.5	56.4	1.2	4.8	38.1	13
	16	88	22.8	28.2	24.6	1.8	6.8	6.5	56.7	—	—	42.8	11

The Result of Analysis The result of analyses for reducing and nonreducing sugars, starch, and amylose is shown in Table 1, together with the data on crude components reported previously[1].

Table 2. Starch and amylose of broad beans.

Date of harvest		Starch found % on air-dry basis (a)		Starch, mg in 10 ml (b)	Color intensity as detd. (c)	Color intensity/ 10 mg starch (d)	Approx. amylose content of starch (e)	
April	30	4.1 9.8	7.0	0.81 1.52	0.010 0.021	0.123 0.132	19.1 22.4	20
May	6	12.2 7.2	9.7	2.43 1.44	0.017 0.017	0.070 0.118	10.7 18.3	15
	11	23.6 24.7	24.2	5.58 4.95	0.043 0.044	0.078 0.089	12.1 13.8	13
	17	38.9 37.7	33.3	6.75 7.74	0.058 0.085	0.086 0.110	13.4 17.1	15
	26	27.8 29.0	28.4	5.58 7.38	0.134 0.177	0.240 0.241	37.3 37.4	37
June	1	35.3 32.3	33.8	6.75 7.02	0.134 0.176	0.199 0.251	30.9 39.0	35
	7	35.2 34.5	34.9	7.38 7.20	0.137 0.137	0.186 0.191	28.9 29.7	29
	11	30.8 34.2	32.5	6.89 7.47	0.151 0.153	0.218 0.204	33.8 31.7	33
	16	36.7 —	36.7	6.34 —	0.123 —	0.167 —	25.9 —	26

Notes to Table 2.

(a) The experiments carried out in duplicate were not satisfying in giving considerable discrepancy in the data. The data of starch content in Table 1 are based on the average value.

(b) This value was calculated by the equation, (d) = [(c)/(b)] × 10.

(e) This value was calculated by putting 0.644 as 100. The color intensity of amylose-iodine complex was 0.322 when 5.000 mg potato amylose was used. Thus, the value (d), or the color intensity per 10 mg starch becomes 0.644. Amylose of each starch at different maturities was not isolated, but this value was calculated to obtain approximate amylose content of satrch.

Table 2 shows starch and amylose data in more detail. When expressed as mg of each component per seed, the increase according to growth can be shown as in Fig. 1, where the ordinate (amount of each component) is in logarithmic scale.

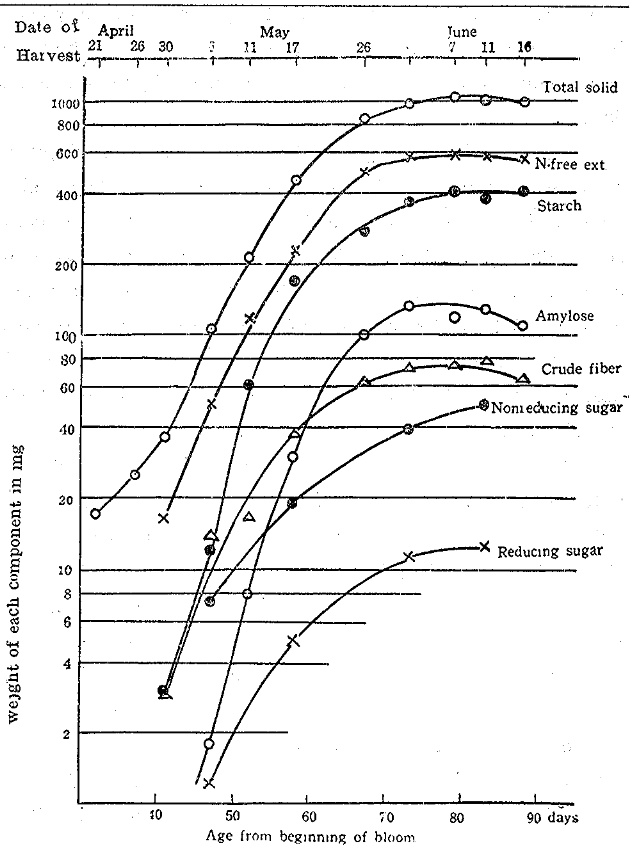

Fig. 1. Increase in the weight of carbohydrates per seed during growth

DISCUSSION

Concerning the suitable time of harvest mentioned in the introduction of this paper, it should be noted that Kagawa Prefecture is located in warmer part (a little north of 34° N.) of Japan. Thus it may not be applicable to other places of much different climate. It is unnecessary to change the conclusion obtained previously[1] by the result of carbohydrate analyses.

The sugar content showed no prominent change when compared in percentage on dry basis. The nonreducing sugar content was higher (6.8%) for the seeds harvested earlier (May 6) than for those harvested later (4—5%). Similar tendency was obtained by BISSON and JONES[10] with wrinkled peas. The reducing sugar content of broad beans remained similar in percentage, namely it increased in parallel with total solid during growth.

The change of starch content agreed well with that reported with a Russian small variety of broad beans [8], but it was totally different from the change with peas, where starch content is relatively high in unmature stage, decreases, and then increases steadily[10].

As described in the note for (e) in Table 2, amylose content of starch is only preliminary and approximate. However, relative tendency may be seen from the calculated values.

The amylose content of the starch of immature broad beans seemed to be lower (about 15%) than that of mature ones (about 30%). It might be interpreted that the increase of amylose was less rapid than the increase of amylopectin. In earlier time up to May 17, amylopectin might have increased more rapidly than amylose, though the total starch increase was small up to May 11. McCREADY et al.[16] reported that the ratio of amylose to amylopectin in wrinkled pea starches increased from 44 to 69% as the pea matured. They did not collect the pea samples at various harvest dates, but reached this conclusion by analyzing peas of different sizes harvested on the same day.

It seemed that amylose per seed decreased slightly in the later time of growth or when the seeds were overripe. If this decrease did occur, there should be a conversion of amylose into amylopectin, since starch per seed steadily increased (except the data for June 11). However, another possibility exists that amylose was catabolized in some way and disappeared, and amylopectin was newly anabolized. In any case, it is to be noted that the formaton of starch in broad beans is not simple and that the increase of amylose does not go parallel with that of amylopectin.

It was found under microscope that starch granules of unripe broad beans were very small in size, about one fifth of the size of starch granules of ripe broad beans. See another paper[17] on the starch granules of various legumes.

The acid-hydrolyzable polysaccharides were determined on the residue of ethanol extraction obtained in determining sugars. They were expected to show a little higher values than starch determined by the McCREADY method.[16] However, they showed a little lower values on the contrary. We wished to determine so-called hemicellulose as the difference between the acid-hydrolyzable polysaccharides and starch, but it was unsuccessful. Anyhow the hemicellulose content of broad beans may not be so large. (Studies on broad-bean hemicelluloses are in progress[18,19].)

It is to be noted that the unmature broad beans harvested on May 6 contained more than twice of undetermined component compared to the broad beans of advanced maturities. From Table 1, the date of harvest and the undetermined component i.e. N-free extract−(sugars+starch) calculated are, respectively, May 6, 28.7%; May 17, 13.2%; June 1, 13.6%; and June 11, 12.3%. Nothing can be said on the nature of the component giving this difference.

ACKNOWLEDGMENT

Sincere thanks are due to Dr. Ziro NIKUNI of Osaka University, who is the head of the group of fundamental studies on the utilization of starch. Experimental works were made by Mr. Hisasi KAGAMIHARA, Mr. Yosimi TUBOI, and Mr. Teiichi NARASAKI of Kagawa Agricultural College. The authors wish to express their sincere thanks to all of them.

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ソラマメ種子の生育と成分との関係

II 炭水化物について

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1953年10月31日に播種したソラマメ（品種サヌキナガサヤ）につき翌年4月21日から6月16日まで約5日おきに13回試料をとつたものについての種子の生育過程と一般分析については既に報告した[1]．その結果青ソラマメとして利用するには5月26日頃，乾燥雑穀として用いるには6月7日頃が収穫の適期であることを示した．

この同じ試料を用いて還元糖，非還元糖，澱粉，アミロースの定量を行つた（第1表及び第2表）．

糖類の含有率には大差はないが，非還元糖が未熟種子にやや多いことはエンドウの場合[10]に似ている．

澱粉は順次増加するが，6月7日頃でほぼ一定となる．澱粉の中のアミロースは未熟の間は少くて15%内外であり，熟すと30%内外となる．これはアミロースの増加速度がアミロペクチンよりおそいと解釈されよう．生育後期に過熟の状態になると1粒当りのアミロースが減少するようである．もしそうであるとすれば，澱粉の量は順に増しているから，そのときアミロースからアミロペクチンへの変化が行われる（或はアミロースが消費され，アミロペクチンがそれ以上に新しく合成される）ことになる．1粒当りの成分の増加は第1図に示した．

なお未熟種子の澱粉粒は顕微鏡で見ると小さくて，成熟したものの½程度であることを観察した．

この研究の経費の一部は文部省総合研究費（澱粉の利用に関する基礎的研究）により支払われた．代表者の二国二郎教授（大阪大学産業科学研究所）に深謝する．実験に協力された鏡原寿君，助手壺井好君，助手樫崎丁市君に感謝の意を表する．

要旨は1955年5月21日吹田市における日本農芸化学会の関西，中部両支部合同の大会で報告した．

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