FUNDAMENTAL STUDIES ON THE UTILIZATION OF OLIVE FRUITS

Ⅱ. Identification of the Amino Acids in the Protein Hydrolyzate of Ripe Olive Flesh by Paper Chromatogrphy

By Teiichi NARASAKI and Kenji KATAKURA

(Laboratory of Agricultural Products Technology)

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In the previous paper[1] it has been shown that olive flesh contains only 1—3% of protein on fresh basis and the level of it was maintained almost constant during the whole period of the experiment. The low content and constant level of the protein seem to indicate that the protein exsists in olive fruits not as accumulative but as constitutive component.

On the practical point of view the protein content is so small, that it may be of no importance to consider the significance of it. However, the protein should rightly participate somewhat in the nutritional value of pickled olives with the oil and the carbohydrates.

Thus the authors studied the protein components by paper chromatography and the results are given in the present paper.

EXPERIMENTAL

Material: Ripe fruits of the *Mission* variety were used in the present studies.

Preparation of Sampls: Flesh was separated from seeds, cut into small sections, and then defatted with ether in a SOXHLET apparatus for 30 hrs. The dried and defatted flesh was taken for protein extraction. The protein extracts were prepared by extracting from about 2gr. of the defatted flesh (ca. 50 mg. of protein) with 90% formic acid according to the direction of R.J. BLOCK & D. BOLLING[2]. After removing the carbohydrate by precipitation with two volumes of ethanol the extracts were evaporated to dryness *in vacuo*, aud then hydrolyzed under reflux with 20 ml. of 6 N hydrochlolic acid for 20 hours. The excess hydrochlolic acid was removed by evaporation to dryness *in vacuo* on a water bath and the resulting thin film of the hydrolyzate was taken up in 5 ml. of 1% acetic acid. The mixture, afrer filtration, was passed slowly through a column of sulfonated polystylene resin (Amberite IR-120, in the H^+ cycle, 100×10 mm.) [3][4][5], washed with 20 ml, of distilled water, and the amino acid mixture was eluted with a sufficient volume of 4 *N* aqueous ammonia. The eluate was again evaporated to dryness,and finally taken up in about 2 ml. of 10% isopropanol.

Circular Paper Chromatography:[3] This method is very simple and speedy, but gives only unsufficient separation. In spite of its poor separation it was conveniently used preliminarily to inspect the applicability of samples and to determine the quantity to be applied. Filter paper Tōyō No.6 (dia.11 cm.) was employed, and the chromatograms were run with *n*-butanol : acetic acid : water (4:1:5,v/v).

One-Dimensional Paper Chromatography: One-way chromatograms were run ascendingly with *n*-butanol: acetic acid: water or collidine saturated with borate buffer of pH 9.3, prepared from 200 ml. of 0.1 *M* boric acid and 113.5 ml. of 0.1 *M* sodium hydroxide, on filter paper strips Tōyō No.50 (2×40 cm.) using chromatographic apparatus Tōyō-B (Tōyō Filter Paper Co., Japan). The experimental detailes were essentialy the same as that described by E.F. MCFARREN[6] and K.OTOZAI[7]. It is reported[3] that elaborate thermal control does not improve the resolution of each amino acid, and thus the chromatograms were run at room temperature (ca. at 25° C.) without special thermal control.

Two-Dimensional Paper Chromatography: Ascending two-way method described by M. MUTŌ[8] was employed,and the improved methed of A. L. LEVY & D.CHUNG[9] was also refered in precise techniques. Chromatography was carried out by using *n*-butanol : acetic acid : water, previously described,as the first solvent, and *m*-Cresol : phenol (1:1,v/v) saturated with borate buffer of pH 9.3 for the second run according to A. L. LEVY & D. CHUNG. Collidine of pH 9.3, mentioned above, was also employed for the first run, but, in this case, double development was necessary, for this solvent gives too low Rf values for each amino acid. Filter paper sheets Tōyō No. 50 (20×20 cm.) were used.

Color Development of the Chromatogram: The dried paper was sprayed with 0.2% ninhydrin in water-saturated *n*-butanol solution and developed about 100° C. for few minutes as usually. Iodine,[10] alkaline permanganate,[11] diazotized sulfanilic acid,[12] p-dimethylaminobenzaldehyde,[13] and isatin[14] were also employed to detect individual spots on paper.

RESULTS AND DISCUSSION

The one-way chromatograms obtained are shown in Fig. 1, and the two-way chromatograms are given in Fig. 2 (by BuOH : AcOH : H_2O —— mCresol : PhOH system) and Fig.3 (by Collidine —— *m*-Cresol : PhOH system).

The identification of each spot on the chromatograms was carried out by addition test with standard amino acids, determination of *Rf* values, detection with color reactions, or the relative positions on the chromatograms. The results are summarized in Table I.

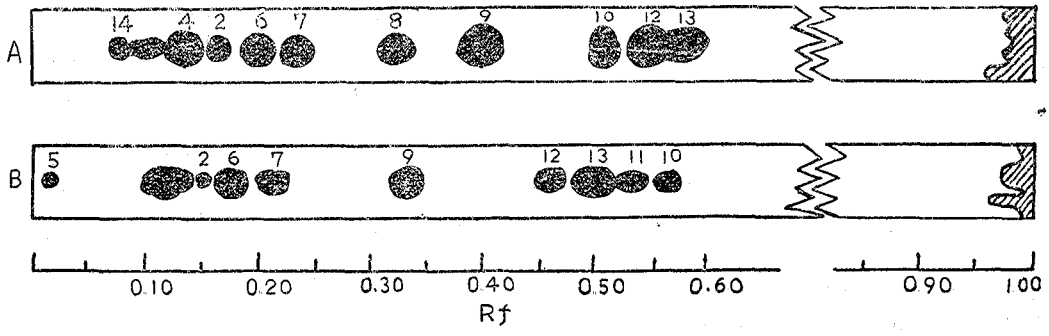

Fig .1 One-dimensional paper chromatograms of amino acids in the olive hydrolyzate
A : by BuOH : AcOH : H_2O ; B : by Collidine , pH 9.3(double development)

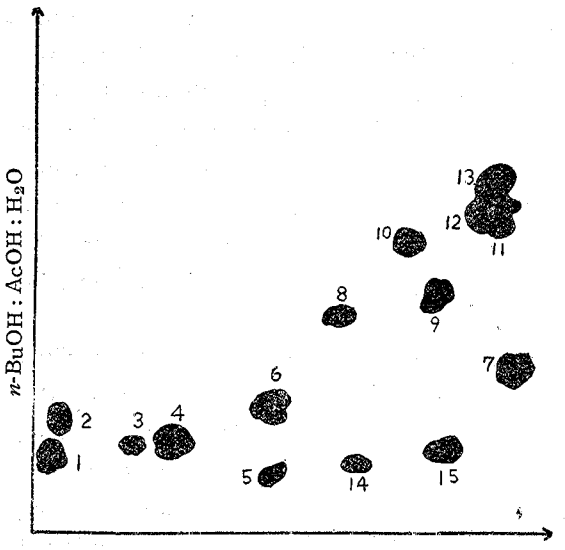

Fig. 2. Two-dimensional paper chromatogram of amino acids in the olive hydrolyzate by the BuOH : AcOH : H_2O — *m*-Cresol : PhOH , pH 9.3 , system

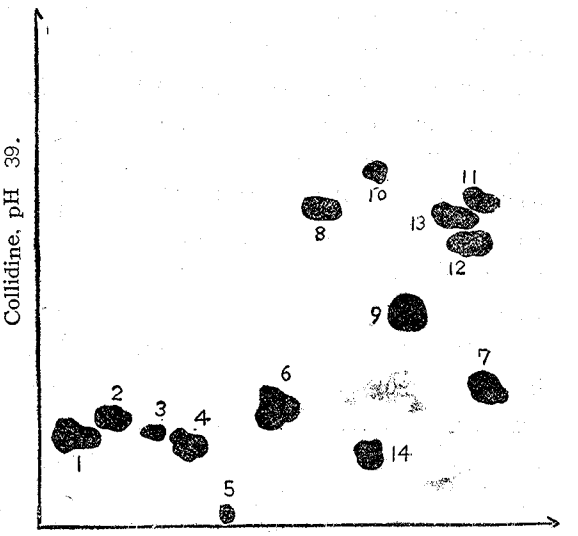

Fig. 3. Two-dimensional paper chromatogram of amino acids in the olive hydrolyzate by the Collidine , pH 9.3 — *m*-Cresol : PhOH, pH 9.3, system

Table I. Identification of Each Spot on Chromatograms

No. of Spots	*Rf* One-way Butanol-Acetic acid	*Rf* One-way Collidine	*Rf* Two-way Butanol-Acetic acid	*Rf* Two-way Collidine	*Rf* Two-way *m*-Cresol-Phenol	Color Reaction Ninhydrin Color	Intensity	Others	Correspond to
1	( )	( )	0.14	0.18	0.04—0.06	B.P	##		aspartic acid
2	0.16	0.14	0.21	0.20	0.05—0.14	P.	##		glutamic acid
3	( )	( )	0.16	0.18	0.19—0.22	Y.R.orR.P.	+		serine
4	0.13	( )	0.16	0.15	0.26—0.29	Br.R	##		glycine
5	( )	0.01	0.10	0.01	0.36—0.45	R.orP.	+		lysine
6	0.20	0.17	0.22	0.21	0.45—0.46	P.orR.P.	##		alanine
7	0.23	0.21	0.29	0.25	0.87—0.91	Y.	##	isatin +,B.	proline
8	0.32	( )	0.39	0.59	0.55—0.58	L.	+		tyrosine
9	0.40	0.33	0.44	0.39	0.72—0.76	P.	##	(iodine —, A.B.A. —)	valine
10	0.51	0.57	0.53	0.67	0.66—0.71	L.	±		tryptophan
11	——	0.53	(0.57)	0.59	0.85—0.89	B.P.orB.	+		phenylalanine
12	(0.56)	0.46	(0.59)	0.52	0.84	P.	##		isoleucine
13	(0.59)	0.50	(0.66)	0.57	0.82	P.	##		leucine
14	0.08	( )	0.12	0.13	0.61—0.64	L.	+	D.S.A. +,R.Y.	histidine
15	( )	( )	0.14	——	0.77	L.	±		arginine

( ) : overlap of spots, D.S.A. : diazotized sulfanilic acid, A.B.A. : *P*-dimethylaminobenzaldehyde ; B. : blue, P. : pink, Y. : yellow, R. : red, Br. : brown, L. : lavender; ± : not sure, + : small, ## : middle, ### : large

As shown in Table I about 15 amino acids were detected in the hydrolyzate of olive flesh, but serine, tyrosine, histidine, and phenylalanine were very small in amount, and the presence of tryptophan and arginine was not sure. Methionine was not detected either with the iodine or with the alkaline permanganate test. Another essential amino acid, threonine, was also undetectable. From these results it will be easily concluded that olives are inferior as protein food both in the content and quality.

As shown in figures leucine, isoleucine, and phenylalanine were more clearly separated with the collidine solvent than with the butanol and the phenol solvents. The spot No. 10 was determined as tryptophan according to its positions on the chromatograms (in Figs. 2 and 3), but could not be ascertained with *P*-dimethylaminobenzaldehyde. Proline, in two-way chromatograms, was easily detected by blue color with isatin from brown coloratin at the solvent front, but isatin was not so useful for phenylalanine, tyrosine, and tryptophan as described by A. SAIFER & I. ORESKES[14].

SUMMARY

(1) Olive flesh was studied for amino acids by preparing one- and two-dimensional paper chromatograms.

(2) The protein hydrolyzate was prepared by the formic acid extraction as provided by R. J. BLOCK & D. BOLLING[2], and deionized by cation-exchange resin, and then used for chromatography.

(3) The amino acids found are as follows : aspartic acid, glutamic acid, serine, glycine, lysine, alanine, proline, tyrosine, valine, tryptophan, phenylalanine, leucine, isoleucine, histidine, and arginine; the presence of tryptophan and arginine was uncertain.

(4) The two essential amino acids, methionine and threonine, were not found.

(5) Isatin was effective only for proline as a color reagent.

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オリーブ果実利用に関する基礎的研究

2. 完熟果実果肉蛋白中のアミノ酸のペーパークロマトグラフイーによる検出

楢崎丁市・片倉健二

前報に於て，筆者等は，完熟オリーブが乾燥果肉の1—3%の粗蛋白を含有し，且この含量は実験期間中殆ど一定である事を報告した．この事は，オリーブ蛋白は構成蛋白であり貯蔵蛋白ではない事を示している．

本報に於ては，蛋白加水分解物中のアミノ酸のペーパークロマトグラフイーによる検出実験の結果に就いて報告した．

1） 検出されたアミノ酸は約15種，即ち，アスパラギン酸，グルタミン酸，セリン，グリシン，リジン，アラニン，プロリン，チロシン，バリン，トリプトフアン，フエニールアラニン，ロイシン，イソロイシン，ヒスチジン，アルギニンであるがこの中トリプトフアン及びアルギニンは明確でなかつた．

2） 必須アミノ酸の中，メチオニン及びスレオニンは全然検出されなかつた．

3） 以上の結果より，オリーブ蛋白は栄養的にすぐれているものでない事が結論された．

猶クロマトグラフ実施上の問題についても二，三考察を加えた，特にクロマトグラフ用試料の調製法については詳細に報告した．

終りに臨み本研究を行うに当り引続き有益な助言を与えられつゝある本学川村教授，並びにアミノ酸標品を供与された三野講師に感謝する．