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SHORT COMMUNICATION

PROTEINASE OF *JARILLA CHOCOLA*, A RELATIVE OF PAPAYA

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Abstract—A papain-like proteinase is present in both fruits and tubers of *Jarilla chocola*, a wild species of the Caricaceae family.

THE GENUS *Jarilla* in the papaya family, Caricaceae, consists of three herbaceous species growing wild in north-western Mexico. *Jarilla heterophylla* (Llave) Rusby and *J. caudata* (Brge.) Standl. occur in the states of Sinaloa, Nayarit, Jalisco, and Guanajuato, where both roots and fruits are offered as food or medicine in the native markets.[1] *J. chocola* Standl. is known only from the Rio Mayo watershed in southern Sonora and adjacent Chihuahua, where it is limited to the tropical canyons in the short-tree forest.[2]

J. chocola is a rather succulent perennial herb with the male flowers and the female flowers and fruits borne on separate plants. Growth shoots up rapidly with the summer rains and by October the plants mature, quickly desiccate, and leave the ripe fruits lying upon the forest floor. The fruits are eaten by the natives and have a faint flavor suggestive of lemon. The tuberous starchy roots are baked and eaten. Both fruits and tubers possess a milky latex, as does the related papaya (*Carica papaya*), but no proteinase activity has been reported previously from *Jarilla*. Here we present evidence of a papain-like enzyme present in both fruits and male tubers of *J. chocola*.

The enzyme of *Jarilla* resembles papain in several respects. It hydrolyzes both proteins and certain esters of amino acids. It is inhibited by *p*-chloromercuribenzoate and is reactivated by sulfhydryl reagents. Activity of the fresh fruit juice is increased two-fold in the presence of 0·025 M to 0·05 M 2-mercaptoethanol.

At pH 6·5, *Jarilla* fruit juice hydrolyzes BAEE[3] at a rate of 3·50 μmoles/min/mg protein; it hydrolyzes TAME at 0·77 μmoles/min/mg protein; but it has no action on TEE or BTEE. The pH optimum against BAEE is 6·5 which resembles that of papain against the same substrate.[4]

* Agricultural Research Service, U.S. Department of Agriculture.

[1] P. C. STANDLEY, *Contrib. U.S. Nat. Herbarium* **23**, 853 (1924).

[2] H. S. GENTRY, *Carnegie Inst. Wash. Publ.* p. 527 (1942).

[3] The abbreviations used are: BAEE, N-α-benzoyl-L-arginine ethyl ester; TAME, tosyl-L-arginine methyl ester; TEE, L-tyrosine ethyl ester; BTEE, N-benzoyl-L-tyrosine ethyl ester.

[4] E. L. SMITH and J. R. KIMMEL, in *The Enzymes*, 2nd edition (edited by P. D. BOYER, H. LARDY, K. MYRBÄCK), pp. 149–161, Academic Press, New York (1960).

The *Jarilla* enzyme has a flat pH optimum against casein between pH 8·0 and 9·4. This optimum more nearly resembles that of trypsin[5] than the somewhat sharper pH optimum near pH 7 that has been reported for crystalline papain.[6] Half the *Jarilla* activity is lost by heating 5 min at 70°; complete destruction occurs at 80°. A preliminary test showed that 0·5 ml of tuber juice will clot milk (5 ml) in 13 min at 35°.

The majority of proteinase activity in the fruit is concentrated in the rind (pericarp) rather than in the pulpy interior (fleshy endocarp). Approximately 20 per cent of total activity is in the pulp, and 80 per cent in the rind. The pulpy portion of the fruit does not contain milky latex and, by analogy to the papaya, would not be expected to be rich in enzyme. Removal of seeds before crushing has little effect on enzyme recovery. No pattern of enzyme content in the whole fruit as a function of fruit maturity has been established, except that very immature fruits (sample 6 of Table 1) have the least enzyme. The smaller fruit (sample 1) contain as much enzyme as the larger fruit of the same maturity (samples 2, 3) because of the higher specific activity of the smaller fruit.

TABLE 1. VARIATION IN PROTEINASE ACTIVITY OF *Jarilla* FRUIT WITH AGE

Sample	Age since flowering (approx. months)	Wt. of fruit (g)	ΔA*/ml	ΔA*/mg protein	ΔA*/g fruit	ΔA*/fruit
1	4½ to 5	14·0	28·7	11·2	17·4	244
2		26·7	10·0	4·33	5·77	155
3		27·0	16·7	7·22	11·4	309
4	3½	27·0	5·50	2·10	3·62	122
5	2	12·0	14·8	4·75	7·25	93
6	1½	29·0	3·80	1·26	2·95	29

* ΔA = Increase in absorbance after 20-min incubation; 1 cm cell, $\lambda = 275$ nm. Enzymes activated with 0·05 M 2-mercaptoethanol.

The starchy tubers of *Jarilla* contain proteinase, but in lower concentration than the whole fruits (Table 2). This difference may be real or it may reflect the fact that the tubers were dug in December and stored 3 months before assays were performed.

TABLE 2. PROTEINASE ACTIVITY OF *Jarilla* TUBER JUICE

Sample	ΔA*/ml	ΔA*/mg protein	ΔA*/g tuber
1	7·30	0·65	2·72
1†	1·20	—	—
2	6·10	0·59	3·16
3	8·60	1·00	5·00

* ΔA = increase in absorbance after 20-min incubation; 1 cm cell, $\lambda = 275$ nm. Enzymes activated with 0·05 M 2-mercaptoethanol.

† Juice aged 7 days (refrig.).

[5] M. KUNITZ, in *Crystalline Enzymes*, 2nd edition (edited by J. H. NORTHUP, M. KUNITZ and R. M. HERRIOT), p. 12, Columbia University Press, New York (1948).

[6] H. LINEWEAVER and S. SCHWIMMER, *Enzymologia* **10**, 81 (1941).

The activities (against casein) of several commercial proteinases were determined under our conditions. Trypsin (1:300) produces an increase in absorbance of 9·00 per mg protein; purified papain concentrate (activated with 0·05 M 2-mercaptoethanol) produces an increase of 15·0 per mg protein; and dried papaya latex (activated with 0·05 M 2-mercaptoethanol) causes an increase of 11·7 per mg protein. In the case of sample 1, Table 1, the specific activity of whole fruit juice of *Jarilla* compares favorably with that of papain. The milky latex from the fruit rind might be a potential source of proteinase superficially resembling papain. An assessment of the plant's agronomic potential would be needed to determine feasibility of economic exploitation of *J. chocola*.

EXPERIMENTAL

Enzyme solutions were prepared by chopping either fruit or tubers into small pieces and pressing them at 5000–8000 lb/in^2 in a laboratory press. The juice was centrifuged (8000 × *g*) and filtered through paper. Juice of the fruits ranged from pH 3·6–4·0 depending on maturity; juice of the tubers was pH 5·7.

Esterase activity was measured by titration, at constant pH, of acids released from 0·04 M amino acid esters at 30°. The enzyme was activated by 0·05 M 2-mercaptoethanol.

Proteinase activity was measured by Kunitz' casein assay[7] with a 20-min incubation at 35°, pH 7·6, unless otherwise noted. The amount of amino acids released was estimated by the increase in the absorbance max. at 275 nm.[8] The development of absorbance during the 20-min incubation was proportional to time under the conditions tested. Care was taken to use small amounts of enzyme so that the response was nearly proportional to enzyme concentration. Activities reported were taken from the initial slope of curves of increase in absorbance plotted against volume of enzyme solution and calculated per milliliter of enzyme.

Thermal inactivation was studied by heating the enzyme solution 5 min at a stated temperature (pH 5·3, conc. 10 mg protein/ml), cooling, activating with 0·05 M 2-mercaptoethanol, and assaying against casein.

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[7] M. KUNITZ, in *Crystalline Enzymes*, 2nd edition (edited by J. H. NORTHRUP, M. KUNITZ and R. M. HERRIOT), p. 308, Columbia University Press, New York (1948).
[8] E. R. HOLIDAY, *Biochem. J.* **30**, 1795 (1936).