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Inhibition of proteolytic activity in alfalfa leaf extracts

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All plant tissues are very rich in proteolytic enzymes. Proteolysis creates a major problem in processing of plant material because protein biosynthesis stops soon after harvesting, and proteolysis continues for some time both in the plant material and in the juice obtained from this material. Proteolysis of proteins by endogenous leaf proteases occurring between harvesting and the early stages of processing accounts for up to 50% loss in protein recovery from alfalfa leaves [1, 2]. Such a great loss of protein seriously affects the economics of plant material processing. This is why determination of proteolytic enzyme activity in the juice and identification of the factors that inhibit proteolysis is of great significance.

This communication presents preliminary results on effects of some substances on proteolytic activity and content of reducing sugars and chlorophyll in alfalfa juice. The following additives were tested: Ag^+, Hg^{2+} (0.5 mmol/l), acetic aldehyde (1 ml/l), simazine (1 mg/l), juice expressed from buckwheat biomass (1:4, v/v), and supernatants obtained either after thermal (85°C) coagulation of leaf protein concentrate (LPC) or after addition of anionic flocculant Magnafloc LT-26 (1:10, v/v).

Proteolytic activity was measured in 0.5 ml of alfalfa juice by adding 4 ml of casein (5%) in 0.1 M phosphate buffer, pH 5.5, and incubation for 18 h at 40°C. Next, the unhydrolysed protein was precipitated with 4 ml of 24% trichloroacetic acid and the mixture left at 4°C for 24 h. After filtration the hydrolysed protein was determined in the filtrate by the method of Lowry *et al.* [3]. Absorbance was measured against a control in which hydrolysis was stopped with the acid immediately after addition of casein. Standard curve was prepared for tyrosine and the proteolytic enzymes activity was expressed in micromoles of tyrosine released by 1 ml of juice per 1 h. Total nitrogen was determined by the Kjeldahl method in the automatic Kjel-Foss apparatus and the total protein was calculated by using the factor (N% × 6.25). High-molecular mass protein was determined as the fraction insoluble in 10% trichloroacetic acid.

It was found that in the juice from alfalfa the activity of proteolytic enzymes amounted to 1.3 μmol/ml per h. Addition of juice from buckweat caused the highest reduction of activity, by 26%, while reduction by 17% and 15%, respectively, was obtained when Ag^+ or acetic aldehyde was added.

Feed grade preparations of leaf protein concentrate from alfalfa press juice contain mainly high-molecular polypeptides because oligopeptides are filtered off after precipitation of the chloroplasmatic fraction. The supernatant (so called "brown juice") used in our experiments contained all of the soluble material from the leaves which was not coagulated either by heat or by polyelectrolytes. The "brown juice", as the result of proteolysis occurring in the press juice, could be additionally enriched in small peptides and amino acids.

Low-molecular compounds present in the "brown juice" may specifically affect the susceptibility of some proteins to proteolysis [4, 5] thus influencing this process. On the other hand, it is possible that the "brown juice" contained a bulk of inhibitors of proteolytic enzymes.

It can be seen from Table 1 that the high-molecular protein content in the juice ranged from 42% to 50% of total protein. The high-molecular protein content in alfalfa juice without addi-

Table 1

Effect of additives on proteolytic activity and protein content in alfalfa juice

Alfalfa juice	Total protein (% of dry mass of juice)	High-molecular mass protein (% of dry mass)	High-molecular mass protein (% of total protein)	Activity of proteolytic enzymes (μmol/ml per h)
Substance added:				
Ag^{+}	35.90	16.73	47	1.05
Hg^{2+}	36.68	18.06	49	1.18
acetic aldehyde	37.21	17.61	47	1.07
supernatant from:				
flocculating LPC	37.02	15.87	42	1.11
heated LPC	37.76	15.47	42	1.11
simazine	38.18	16.29	43	1.23
juice from buckwheat	38.94	16.47	43	0.93
Control	37.62	18.92	50	1.26

Table 2

Effect of additives on chlorophyll and reducing sugars content in alfalfa juice

Alfalfa juice	Reducing sugars (% in 100 g of juice)	Chlorophyll "a" (mg/kg of dry mass of juice)	Chlorophyll "b" (mg/kg of dry mass of juice)	Chlorophyll "a" (% of total chlorophyll content)
Substance added:				
Ag^{+}	2.27	4.44	1.87	70
Hg^{2+}	2.06	4.41	1.96	69
acetic aldehyde	2.48	4.43	1.87	70
supernatant from:				
flocculating LPC	1.43	4.13	2.01	67
heated LPC	1.45	4.18	1.47	71
simazine	1.45	4.25	1.71	71
juice from buckwheat	-	3.84	2.13	64
Control	1.46	4.66	1.96	71

tives reached 18.9% of the dry mass. All the substances added decreased this parameter. The most efficient were supernatants derived from LPC after thermal coagulation or flocculation which decreased this parameter to 15.87% and 15.47%, respectively.

The addition of Hg^{2+}, Ag^{+} or acetic aldehyde to the alfalfa juice increased the reducing sugars content (determined with 3,5-dinitrosalicylic acid according to [6]) as compared to the control (Table 2); the other additives had no effect. In the total chlorophyll content (determined by the method of Arnon [7] a negligible decrease was seen after addition of the substances studied. No isomerization of the two forms of chlorophyll was observed. The fraction of chlorophyll "a" in total chlorophyll was similar in all cases.

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