

EFFECT OF WHEY ON THE HYDROLYTIC ENZYME ACTIVITY OF SOME CEREALS AND PULSES SEEDS AND THEIR GERMINATION

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The activity of amylase and starch phosphorylase in germinating cereals and pulses seeds was higher due to their treatment with whey in contrast to slower in untreated control. The starch content in whey treated seeds was less and consequently total soluble sugar was more in amount while reverse was the case of untreated control. The activity of protease in pulses seeds was stimulated due to treatment and as a result remaining protein in the seed was less and total free amino acid more. The per cent germination and the rate of germination were more due to treatment of both categories of seeds with whey.

Keywords : Cereal and pulses seeds; Germination; Hydrolytic enzymes of seeds; Whey.

Introduction

Several workers¹⁻³ have observed beneficial effect of the treatment of seeds with ascorbic acid, IAA, thiourea, CaCl₂, KCl and MgSO₄ solution on the stand of the crop plants and have explained its mechanism as a attenuation of physiological deterioration of seeds. The present paper deals with soaking of some cereals and pulses seeds in whey, a waste material produced during manufacture of cheese and sweets, and containing water soluble content of the milk including cations, anions, proteins⁴ such as beta - lactoglobulin, lacto albumin, serum albumin and immuno globulin and vitamins, on the activity of hydrolytic enzymes simplifying stored starch and proteins during germination. Per cent seed germination and the rate of germination were also worked out.

Materials and methods

Preparation of whey : Whey was prepared adopting the method of confectioners. One litre of cow's milk was boiled with 200 ml of whey, previous stock taken from the confectioner, till curdling. It was cooled to 30°C and filtered with three layers of cheese cloth. The pH of resulting whey was adjusted to 7 using 0.1 N NaOH solution by vigorous stirring. One hundred seeds each of paddy (*Oryza sativa* L) var Sita, wheat (*Triticum aestivum* L.) var Kalyan Sona, lentil (*Lens culinaris* Medic) var Local, bengal gram (*Cicer arietinum* L) var Local and green gram (*Vigna radiata* L) var Local were soaked in 100 ml of whey separately for 18 hr at 8°C. The beakers containing seeds in whey were shaken time to time. Distilled water was used for the control in place of whey. The seeds treated as above were plated on sterilized moist blotter at the rate of 10 seeds per blotter and incubated at 30±1°C for four days. Activities of amylase and starch phosphorylase that catalyze the stored starch of the seeds were determined on the fourth day after soaking the seeds besides estimating the remaining starch as

substrate and total soluble sugar as hydrolytic product.

Assay of amylase : Five g each of the treated and control seed lots were chilled and ground with mortar and pestle using 25 ml of phosphate buffer containing 0.72% KH₂PO₄ and 0.045% NaHPO₄. This was filtered through three layers of cheese cloth. The filtrate was centrifuged at 10,000 rpm for 10 min. The supernatant was used as enzyme preparation (EP). The activity of amylase in the seeds noted above was assayed.⁵

Assay of starch phosphorylase : It was assayed adopting Gibbs and Turner⁶ method, grinding and filtering 5 g of the seeds as treated earlier with 25 ml of distilled water at 2°C. Supernatant was used as EP. The phosphate released from glucose-phosphate was estimated using 1.0% Na₂HPO₄.⁷

Assay of protease : Protease activity was determined taking 5 g of pulses seeds each, chilling at 0°C and extracting with 20 ml of 0.1M phosphate buffer. The filtrate was centrifuged at 4°C at 10,000 rpm for 10 min. The supernatant served as EP. Amino acids released from casein as substrate was estimated⁸ with ninhydrin reagent by calibration curve using serial dilution of glutamic acid.

Estimation of remaining starch after amylase activity and resulting soluble sugar : The seeds that were used for the assay of the activity of amylase earlier, were used for the quantitative estimation of starch⁹. Per cent starch content was determined on drying 10g of seeds in triplicate at 80°C for 24 hr on fourth day after soaking in whey and cooling over fused CaCl₂ for next 72 hr in sealed desiccators. Per cent soluble sugar resulting due to the activity of amylase was estimated by extracting 5 g of seed in 80% warm ethanol. Total soluble sugar was estimated¹⁰ and its per cent value was recorded on dry weight basis by the formula

$$\frac{X}{Xi} \times 100$$

where x is the amount of total soluble sugar and xi = total

dry weight of the seed in g.

Germination of the seeds: The seeds were washed thoroughly and adhering water was soaked with clean dry towel. One hundred seeds of each crop was separately soaked overnight in 100 ml of whey. Distilled water was used for the control. Seeds were set for germination in sterilized moist blotter at $28 \pm 1^\circ\text{C}$ for 7 days. Per cent germination was calculated pooling total germinated seeds together set in all the ten blotters and total seeds set for germination. The rate of germination was also calculated.¹¹

Results and Discussion

The activity of amylase and starch phosphorylase in cereals and pulses seeds were higher due to treatment of seeds with whey slower in untreated control (Table 1). The starch content in whey treated seeds was less and total soluble sugar was more in amount while reverse was the case of untreated control (Table 2). The activity of protease in germinating pulses seeds was also stimulated due to treatment (Table 1) and consequently the remaining protein in the seed was less and total free amino acid more (Table 3). Per cent germination and the rate of germination were observed higher in the whey treated seeds (Table 4).

Starch, the reserve food of the seed is hydrolysed by amylase, alpha-glucosidase, limit dextrinase and starch phosphorylase¹⁰. Stimulated activity of the first and last enzymes indicates that whey might quicken simplification of starch due to presence of cations, anions, organic compounds, water soluble proteins serving as promoter of enzymes quoted above, providing enough raw materials for more enzyme synthesis as stated earlier¹². This is true for the activity of protease also. The stimulation of the

activity of the noted enzymes is further evinced by decrease in starch and protein and increase in total soluble sugar and total free amino acid in the seeds respectively. The per cent germination and the rate of germination indicate beneficial effect of whey treatment. Paddy and wheat were more favoured in enhancing the germination. The rate of germination was also considerably higher. Both the phenomena point out that the biochemistry and physiology of seed germination are appreciably favoured.

References

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Table 1. Activity of amylase (expressed as mg starch digested) and starch phosphorylase (expressed as mg phosphate released) in cereals and pulses seeds, and protease (expressed as mg amino acid released) in pulses.

Seeds		Amylase		Starch Phosphorylase			Protease			
		Time in min		Time in min			Time in min.			
		30	60	90	30	60	90	30	60	90
Cereals										
Paddy	T	5.52	6.86	8.17	0.27	0.41	0.62	-	-	-
	C	3.57	4.72	6.36	0.18	0.22	0.29	-	-	-
Wheat	T	5.72	7.13	9.25	0.32	0.46	0.60	-	-	-
	C	4.27	4.85	5.62	0.17	0.19	0.23	-	-	-
Pulses										
Lentil	T	2.92	3.22	3.85	0.28	0.48	0.69	3.73	4.25	4.86
	C	2.34	2.68	3.06	0.08	0.11	0.13	1.86	3.21	3.78
Bengal Gram	T	2.73	2.95	2.48	0.21	0.39	0.52	3.62	4.12	4.82
	C	2.22	2.31	2.48	0.05	0.08	0.11	1.73	2.45	3.48
Green Gram	T	2.86	3.14	3.62	0.28	0.49	0.58	3.82	4.18	4.96
	C	2.33	2.57	2.89	0.06	0.09	0.12	2.08	2.83	3.64

Table 2. Starch and total soluble sugar in cereals and pulses seeds on the 4th day after soaking the seeds in whey.

Seeds	Starch (%)		Total soluble sugar (%)	
	T	C	T	C
Paddy	39.65	41.92	6.15	5.18
Wheat	32.67	34.93	4.89	3.57
Lentil	26.83	28.61	3.96	3.16
Bengal gram	28.75	29.4	4.17	3.43
Green gram	25.84	27.07	4.73	4.05

T = Treated, C = Control

Table 3. Protein and total free amino acid content in pulses on the 4th day after soaking the seeds with whey.

Seeds	Protein (%)		Total free amino acid (%)	
	T	C	T	C
Lentil	22.02	24.77	3.66	1.87
Bengal gram	15.23	17.62	3.15	1.93
Green gram	22.86	24.27	3.74	2.32

T = Treated, C = Control

Table 4. Per Cent germination* and rate of germination of cereal and pulses seeds due to the treatment of whey

Seeds	Per cent germination		Rate of germination	
	T	C	T	C
Paddy	94	82	17.98	16.11
Wheat	96	85	18.12	16.23
Lentil	95	88	19.94	17.04
Bengal gram	94	87	19.89	16.98
Green gram	98	89	20.11	17.23

CD = 6.063 at 5%

*= Figures were rounded to the whole number.

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