



**ORIGINAL RESEARCH PAPER**

**Botany**

**EFFECT OF WHEY ON THE QUANTITATIVE CHANGE IN RESERVE FOOD OF THE GERMINATING SEEDS**

**KEY WORDS:** Effect, Whey, Reserve Food, Germinating Seeds

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**ABSTRACT**

The percent starch in the germinating paddy seed was found gradually less faster due to treatment with whey than the control. Percent protein from germinating lentil seed was found to be gradually reduced with faster rate due to treatment with whey than the control. There appeared increase in soluble sugars in the endosperm of paddy during germination both in treated and control seeds. Also it was found that there is increase in total free amino acid in the cotyledons of lentils due to the treatment of whey. The decline in the amount of total soluble sugars in the endosperm of paddy after 3rd day of germination and in the cotyledon of lentil after 2nd day points out that soluble carbohydrates are more rapidly transferred to the embryo in lentil than to the paddy. Also decline in the amount of total free amino acid was observed in the endosperm of paddy after 3rd day while same on the 3rd day in lentil indicates the speed of the transfers of amino acid to the growing embryo. The unequal or varying speed might be due to the nature of seed as they belong to two unrelated group of plants.

**INTRODUCTION:**

The percent germination and the rate of germination was observed more due to treatment of cereals and pulses seeds with whey. This paper is in continuation of the work on the effect of whey on the Hydrolytic Enzyme activity of some cereals and pulses seeds and their germination. Whey as waste material is considered as useless after removal of cheese from milk. In present work starch and total soluble sugar was determined in paddy and similarly protein and total free amino acid was determined in lentil during their germination. The percent starch in the germinating paddy seed was found gradually less with faster rate due to treatment with whey than the control. Percent protein from germinating lentil seed was found to be gradually reduced with faster rate due to treatment with whey than the control. There appeared increase in the soluble sugars in the endosperm of paddy during germination both in treated and control seeds. Also, it was found that there is increase in total free amino acid in the cotyledons of lentil due to the treatment of whey.

**MATERIAL AND METHODS**

Procedure mentioned in the paper on "Effect of whey on the Hydrolytic Enzyme activity of some cereals and pulses seeds and germination" by Kumar A & et. al. (2005) was adopted in the preparation of whey for the experimentation. The hydration of the seeds due to soaking of water activates the dormant hydrolytic enzymes of the seeds that initiates hydrolysis of the polymeric food reserve of the seed which is transferred to the growing region, radicle, hypocotyle, epicotyle and the shoot and part of it is used up in the respiratory metabolism of the seed, various syntheses, amination, deamination, decarboxylation etc. In such condition it is highly expected that the reserve food materials in the form of starch, proteins and lipid would be consumed after their simplification resulting in reduction in the dry weight of the seed. This aspect have been worked in Vigna sesquipedalis ( Oota et. al., 1953), rice (Palmiano and Juliano, 1972) and maize (Ingle et. al., 1964; 1965).

In the present work the starch and total soluble sugar was determined in paddy. Similarly protein and total free amino acid were determined in lentil during their germination. Both the seeds lots possessing 98 and 99% germination respectively were taken 30 g of each-in triplicate. Seeds of the noted lots were treated with whey maintaining untreated water soaked control. The seeds were set for their germination.

**Germination of the Seeds:**

Seeds were germinated in petri dishes. Petri dishes made of corning, India and having 12 cm diameter were taken and

smaller one of the pair was covered with three layers of blotting sheets. The condition of germination was maintained at 30°C temperature and 100% RH and 12 hour diffused sunlight and 12 hr darkness. Seeds just showing the emergence of the radicle were considered germinated ones. The germinated seeds were cultured in the sand medium.

**Determination of Starch**

It was estimated calorimetrically following the method of Snell and Snell (1961). The germinating seeds of paddy in triplicate of 10g was harvested everyday at 10AM. The embryo were cautiously removed and 2g endosperm was weighed in triplicate in two sets. One set was kept for dryness at 80°C for 24 hr and desiccated for next 48 hr over fused CaCl<sub>2</sub> in the sealed desiccators. Another one set was put in 50ml of 80% warm ethanol. The endosperm was ground with the help of mortar and pestle and put over the sand bath. The volume of evaporated ethanol was maintained by adding it time to time. The ground tissue was stored over night. The ethanol extract was decanted and 25ml of ethanol was added in the residue every time, warmed and again decanted. This procedure was repeated six times. The ethanol free extract was centrifused at 10,000 rpm for 20 min and supernatant was separated using pipettes. This clear liquid was saved and stored at -10°C for the determination of total soluble sugar and total free amino acid. The residue was saved without loss and stored at -10°C for the determination of starch and insoluble protein.

The residue was mixed with 5ml of water and 8 ml of 52% perchloric acid. The suspension was centrifused at 10,000 rpm for 10min. The supernatant was removed and saved four times after repeating the process. All the supernatants were pooled together. The volume was made to 200 ml in volumetric flask and the resulting sugar due to dissolution of the starch was determined with anthrone reagent.

$$\text{Amount of starch was calculated by: } \frac{(\text{Amount of Sugar})}{0.9}$$

In this way total amount of starch was known in the germinating seed.

**Determination Of Protein:**

Protein in the residue of germinating lentil seed was determined after evaluating total nitrogen content. The value of protein was determined as follows:

$$\text{Protein: Amount of nitrogen X } 6.25 \frac{X}{X1}$$

$$\% \text{ of Protein in cotyledon on dry weight basis: } X 100$$

$$X = \text{Weight of protein in dry cotyledon}$$

$$X1 = \text{Weight of dry cotyledon}$$

**Determination of Total Soluble Sugar:**

Glucose phosphate is the product of the activity of starch phosphorylase on amylase. Therefore, it was thought worthwhile to determine the amount of total water soluble sugars in germinating seeds.

From the ethanol free extract of the endosperm of paddy the value of total soluble sugar was determined by Anthrone method. Per cent value of total soluble sugar in enthrone on dry weight was calculated as follows:

Amount of total soluble sugar = X g

Total dry weight of endosperm = X<sup>1</sup> g

% Value of total soluble sugar = X/X<sup>1</sup> x 100

**Determination of total free amino acid:**

Total free amino acid is the product of hydrolysis of proteins by protease. Calculation of percent total free amino acid in cotyledon:

Amount of total free amino acid= x g

Total dry weight of the cotyledon= x<sup>1</sup>

% value of total free amino acid = (x/x<sup>1</sup>) X 100

**RESULTS AND DISCUSSION**

1. Percent starch content in the endosperm of paddy during germination due to treatment with the whey:

The percent starch in the germinating paddy seed was found gradually less with faster rate due to treatment with whey than the control (Refer Table 1).

**Table 1 : Percent starch content in the endosperm of paddy during germination due to treatment with whey**

	Day of germination				
	0	1	2	3	4
Treated	49.08	46.24	44.01	41.63	39.65
Control	49.08	47.75	45.81	43.64	41.92

2. Percent protein from germinating lentil seed was found to be gradually reduced with faster rate due to treatment with whey than control (Refer Table 2).

**Table 2 : percent protein content in the cotyledon of lentil during germination due to treatment with whey**

	Day of germination				
	0	1	2	3	4
Treated	20.63	18.89	17.62	16.24	15.02
Control	20.63	19.55	19.43	18.71	18.25

3. There appeared increase in the total soluble sugars in the endosperm of paddy during germination both in treated and control seeds. Total soluble sugar in the endosperm of paddy increased with lapse of days of germination and reached to the maximum on the 3rd day due to the effect of whey. Thereafter, it declined in its quantity. The rate of increase of total soluble sugar in the control seed failed to reach to the extent as shown due to the effect of whey (Refer Table 3).

**Table 3 : Percent total soluble sugar in the endosperm of paddy during germination due to the treatment of the whey**

	Day of germination				
	0	1	2	3	4
Treated	2.15	2.86	3.25	3.76	3.68
Control	2.15	2.15	2.25	2.38	2.27

4. There was increase in total free amino acid in the cotyledons of lentil due to the treatment of whey. The rate increase in total free amino acid in the cotyledons of lentil was faster due to the effect of the whey. In control seeds

the percent amount of total free amino acid was least. Total free amino acid in lentil seeds was found to decline on the 3rd day of germination (Refer Table 4).

**Table 4 : Percent total free amino acid in the cotyledons of lentil during germination due to the treatment of the whey**

	Day of germination				
	0	1	2	3	4
Treated	2.15	2.87	3.46	4.05	3.66
Control	2.15	2.22	2.40	2.53	1.87

5. The gradual decrease in the starch from endosperm of paddy and protein from cotyledons of lentil both in treated and untreated control has been observed which points out that these polymers are being consumed after their gradual hydrolysis to their respective simplest units of which they are constituted. Concomitantly the total soluble sugar and total free amino acid were found to increase besides stimulated activity of amylase, starch phosphorylase and proteolytic enzyme due to the treatment of the seeds with the whey. The treatment of the whey to the seed acts in stimulation of the hydrolytic activity of amylase, starch phosphorylase proteolytic enzyme due to which the stored food material in the form of starch and protein are simplified and there appears more and more soluble sugars in amino acid.

The loss of the starch and protein of the endosperm/ cotyledon with rapid rate and increase of the water soluble product there with same speed by the treatment of whey gives an impression that the two phenomena are hastened a result of the effect of whey transferring more and more soluble carbohydrates and nitrogen to the growing embryo. This might be conjectured a factors in rapid growth of the root and shoot, leaf area and dry weight of the seedlings.

The decline in the amount of total soluble sugars in the endosperm of paddy after 3rd day of germination and in the cotyledon of lentil after 2nd day points out that soluble carbohydrates are more rapidly transferred to the embryo in lentil than to the paddy. Also decline in the amount of total free amino acid was observed in the endosperm of paddy after 3rd day while same on the 3rd day in lentil indicates the speed of the transfers of amino acid to the growing embryo. The unequal or varying speed might be due to the nature of seed as they belong to two unrelated group of plants.

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