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Short communication

Alpha and Beta amylase activity of *Fagopyrum esculentum* (Buckwheat): A Medicinal Plant

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ABSTRACT

Background and Objectives: *Fagopyrum esculentum*, common buckwheat popularly known as mithe fapar is one of the staple food crops of the mountain region. Traditionally, it is used to treat constipation and bowel upsets. It is also used by diabetic in different parts of Nepal and India. Due to its high nutritive and medicinal value, medical scientist and researchers are interested in developing this as pharmaceutical plant. In this regard department of biochemistry, College of Applied Education and Health Sciences, C.C.S. University, Meerut, India is working to analyse the biochemical composition and benefits of this plant. So, as a part of a multi-dimensional project of analyzing various components and their impact on health and diseases, here we are reporting the amylase activity during germination of seed in Buckwheat (*Fagopyrum esculentum*) plant.

Methodology: Common buckwheat (*Fagopyrum esculentum*) seeds were taken and germinated in dark at room temperature from 0 hours to 192 hours. Biochemical analysis for total amylase, alpha and beta amylase activities was measured by the standard method designed by Bernfeld (1955).

Results: The seeds of buckwheat showed high level of amylolytic activity during different stages of germination. At 0 hours, negligible amylase activity was found. The first amylase activity was found at 24 hours and increases up to 96 hours. After 96 hours the total amylase activity starts decreasing and becomes almost negligible at 192 hours. Alpha and Beta –Amylase activity is reported separately.

Conclusion: The amylases from the buckwheat showed different level of enzymatic activity during seed germination. Alpha amylase contributed a larger account to total amylase activity. The activity starts increasing and becomes maximum at 96 hours and starts decreasing and becomes lowest at 192 hours suggesting that alpha amylase plays a important role in starch metabolism in developing as well as geminating seeds which can be used for the drug discovery and treatment of several diseases like diabetes, polycystic ovary syndrome, constipation, bowel upsets, obesity and others.

Keywords: *Fagopyrum esculentum*; diabetes; polycystic ovary syndrome; alpha and beta amylase

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INTRODUCTION

Fagopyrum esculentum is common buckwheat (mithe fapar) which is one of the staple food crops of the mountain region of Nepal. It is the sixth most important crop after maize, rice, wheat, finger millet and barley, and the main crop in certain high mountain [1, 2]. Traditionally, it is used to treat constipation and bowel upsets. It is also used by diabetic in different parts of Nepal and India. Experiments with animal models have demonstrated that buckwheat and its nutrients inside may alleviate diabetes, obesity, hypertension, and hypercholesterolemia [3, 4, 5, 6]. In recent years buckwheat has regained interest as it has considerable health benefits, such as protein of high biological value [7, 8, 9] due to the relative high amino acid scores and high levels of lysine and tryptophan, when compared to cereals [10]. In addition buckwheat contains several antioxidants such as rutin, which has a hypotensive effect and is claimed to strengthen capillary blood vessels and suppresses the internal bleeding [11]. It also contains fagopyrins, which are claimed to reduce diabetes II [12, 13]. Buckwheat contains many flavonoid compounds, known for their effectiveness in reducing the blood cholesterol, keeping capillaries and arteries strong and flexible, and assisting in prevention of high blood pressure. In addition there are no glutein-like proteins in buckwheat, it is considered gluten free and can be consumed by those who suffer from mylas disease. Coeliac disease is a food induced immunological disease of the upper small intestine and results from gluten ingestion in genetically susceptible individuals. Buckwheat is also known as abundant source of minerals like zinc, copper and manganese [14]. It also contains large amounts of soluble and insoluble dietary fibres which have a positive effect on constipation and obese conditions [15]. Due to its high medical value, medical scientist and researchers are interested in developing this as pharmaceutical plant. In this regard biochemistry department of College of Applied Education and Health Sciences, C.C.S. University, Meerut, India is working to analyse the biochemical composition and benefits of this high medicinal value plants. So, as a part of a multidimensional project of analyzing various components and their impact on health and diseases, here we are reporting the amylase activity during germination of seed in Buckwheat (*Fagopyrum esculentum*) plant.

MATERIAL AND METHODS

The present investigation was carried out in the Department of Biochemistry, College of Applied Education and Health Sciences, C.C.S. University, Meerut, India. The experiments carried out were designed to study the activity of amylases during germination of seed in buckwheat (*Fagopyrum esculentum*). Five grams dry healthy seeds of *Fagopyrum esculentum,* were taken in 9 petridishes. The sample for the seed germination was drawn for every 24 hours interval from 0 to 192 hours. Each of 5 gram seeds were washed with distilled water followed by soaking seeds in 0.1% $HgCl_2$ for 3 minutes for mylaseingn and then again seeds were thoroughly washed under running tap water for 15 minutes to remove all the traces of mylaseing agent. The seeds were placed in sterilized petridishes containing moistened filter paper at 27±2ºC in dark for germination. The seed coat of the sprouted seedlings was released with the help of sterilized forcep. The weight of the endosperm was taken before grinding. Thirty endosperm of uniformly

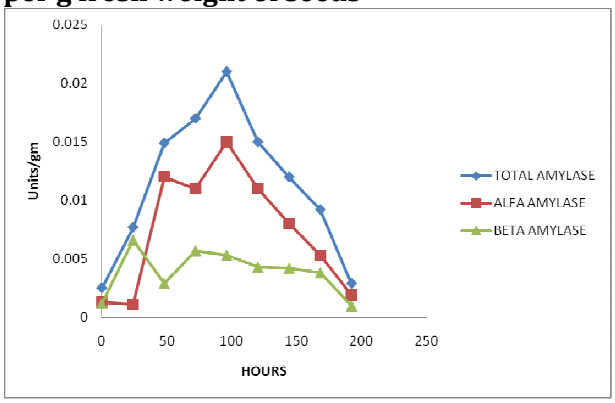
imbibed seeds were selected randomly for the extraction. The endosperm was macerated in mortar and pestle with minute amount of distilled water. Then 10 ml of 50mM of phosphate buffer (6.9) added for homogenation. The mixture was homogenized thoroughly and centrifuged at 8000 rpm for 15 minutes. The supernatant was taken with micropipette and was transferred to falcon tubes and then supernatant was taken for enzyme activity.

Biochemical analysis: Total amylase activity, alpha and beta amylase activity was measured by the standard method designed by Bernfeld (1955), wherein the reducing group liberated from starch were measured by reduction of 3,5,dinitrosalicylic acid [16].

RESULTS

The present paper reports the amylase activity in Buckwheat (*Fagopyrum esculentum*) seed during germination. The seeds of the buckwheat were germinated and the embryo was taken for the preparation of crude extract. The crude extract was then centrifuged and the supernatant was used for enzyme assays through DNS method. The first amylase activity was found at 24 hours and it increases up to 96 hours and was maximum at 96 hours. After 96 hours the total amylase activity starts decreasing and becomes almost negligible at 192 hours. At 192 hours starch in the seed embryo completely vanishes, so almost negligible amylases activity was found. Alpha and beta amylase activity was also calculated separately. It is clear from the figure 1 that alpha amylase contributed a larger account to total amylase activity. The detail amylase activity is shown in table 1.

Figure 1: Amylase activity expressed as units per g fresh weight of seeds



DISCUSSION

Buckwheat, popularly known as mithe fapar, is used traditionally to treat constipation and bowel upsets. It is also used by diabetic in different parts of Nepal and India. It has been considered as a healthy nutritious food and has been used by older and weak people to get quick recovery from illness.

Buckwheat as medical boon: It contains protein of high nutritional value, dietary fiber, resistant starch, rutin, D-*chiro*-inositol, vitamins, and minerals. They are also associated with the suppression of colon carcinogenesis by reducing cell proliferation and with the suppression of mammary carcinogenesis by lowering serum estradiol [17].

Buckwheat proteins extract may be used as a potential functional food additive to treat hypertension, obesity, alcoholism, as well as constipation [18]. D-chiro-inositol, a component of Buckwheat is a part of the secondary messenger pathway for insulin signal transduction found to be deficient in Type II diabetes and Polycystic ovary syndrome (PCOS) [19]. Starch and fiber are present in similar amounts, and buckwheat also contains a high

Table 1: Total amylases, alpha and beta amylases activity in units/gm

Hours	Alpha amylase unit (units/gm)		Beta Amylase unit (units/gm)		Total Amylase (Units/gm)	
	Dry Weight	Fresh Weight	Dry Weight	Fresh Weight	Dry weight	Fresh weight
0	0.0014	0.0013	0.0014	0.0012	0.0028	0.0025
24	0.00016	0.0011	0.000018	0.0066	0.0110	0.0077
48	0.01645	0.0120	0.00406	0.0029	0.0200	0.0149
72	0.1767	0.0110	0.00088	0.0057	0.0264	0.0170
96	0.0249	0.0150	0.00847	0.0053	0.0333	0.0210
120	0.0181	0.0110	0.0071	0.0043	0.0253	0.0150
144	0.0013	0.0080	0.0068	0.0042	0.0198	0.0120
168	0.0087	0.0053	0.0063	0.0038	0.0151	0.0092
192	0.0031	0.0019	0.0016	0.00099	0.0048	0.0029

level of polyunsaturated essential fatty acids such as linoleic acids, vitamins (B1, C, and E), minerals and well balanced aminoacid composition. Buckwheat has low content of prolamins which has prophylactic role in gastrointestinal tract diseases [20]. Coliac disease (also known as gluten-sensitive enteropathy) is a genetically-determined disease of the small intestine linked with gluten intolerance [21]. Buckwheat proteins show a strong supplemental effect with other vegetable proteins due to the well balanced amino acid composition [21]. The Lys/Arg and Met/Arg ratios in buckwheat proteins are lower than those in most plant proteins. This indicates that buckwheat should be characterized by the properties capable of lowering blood cholesterol level. Buckwheat proteins can exert a strong cholesterol-lowering effect and have a high biological value [22]. The addition of protein products of buckwheat to diets significantly lowers the levels of cholesterol in serum, liver, and gallbladder of hamsters and suppresses the formation of gallstones by altering cholesterol metabolism [23], whereas protein extracts are more efficient in lowering the blood cholesterol level, particularly that of LDL and VLDL [24]. The hypocholesterolemic effect in humans is linked with a lower digestibility of buckwheat proteins and the presence of fibre-like substances [24]. On the other hand, a low digestibility has been recorded, possibly due to tannins, phytic acid, and protease inhibitors that can act against human saliva amylase and affect the level of digestible starch [25] Foods with higher levels of resistant starch usually have low glycemic index (GI) and low GI foods are important in improving diabetic control and can be used according to their blood glucose raising potential [26].

Furthermore, Plant sterols (so-called phytosterols), although identified in buckwheat grains at low levels, also exert a positive effect on the blood cholesterol level. B-Sitosterol, which represents at least 70% of total sterols, occurs in the endosperm and embryo tissues of buckwheat grains. It cannot be absorbed in human body and displays a considerable competitive inhibitory effect on cholesterol absorption in vivo [27].Buckwheat protein has been found to bind cholesterol tightly. It is being studied for reducing plasma cholesterol in people with hyperlipidemia too [28].

It also contains a glucoside named rutin and quercetin which are the main antioxidants

in buckwheat and have been mentioned in the treatment of chronic venous insufficiency, a medicinal chemical that strengthens capillary walls, reducing hemorrhaging in people with high blood pressure and increasing microcirculation in people with chronic venous insufficiency [29].There is no doubt that this plant has high medicinal value.

This study reports the amylase activity in Buckwheat (*fagopyrum esculentum*) plant during its germination. It was observed that the amylase activity was low at 0 hours and starts increasing and becomes highest at 96 hours and starts decreasing again and becomes low at 192 hours. It was found that alpha amylase contributed a larger account to total amylase activity suggesting that alpha amylase plays a important role in starch metabolism in developing as well as geminating seeds. Amylases have activity such as physical form of starch, extent of retro degradation, amylase to amylopectin ratio and non starchy components may result in positive nutritional effects and health benefits which may be further basis of study for the pharmaceutical importance and drug discovery.

CONCLUSION

It was found that alpha amylase contributed a larger account to total amylase activity suggesting that alpha amylase plays a important role in starch metabolism in developing as well as geminating seeds.The activity starts increasing and becomes maximum at 96 hours and starts decreasing again and becomes lowest at 192 hours. Rich amylase content in buckwheat seeds and high levels of nutrients present in the seed embryo can be used in medical treatment for various diseases as well as for pharmaceutical purpose for drug discovery. Further elaboration of this study is needed to find the different composition and activity of the seeds, so that it may be used in drugs therapy of diseases like diabetes, constipation, pCOS, mammary carcinogenesis, obesity and others.

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