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Research Article

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Study of Biochemical Changes in *Solanum tuberosum* Due to Infection and Storage

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ABSTRACT

This paper presents the pathological and biochemical changes among the freshly harvested potato tubers, freshly harvested infected tubers, cold stored healthy tubers and cold stored rotted tubers. In the study, fresh and infected samples of potato tubers were taken from the farmer's field to understand the pathological and biochemical diversity. After the examination, potatoes were kept in the cold storage and studied again for pathological and biochemical changes. The potatoes were found infected by the Alternaria solani fungus during the season and Aspergillus niger fungus after storage. Biochemical parameters like vitamin c, fat, starch, gallic acid, chlorophyll, xanthophylls, carotene except oxalic acid indicates the significant reduction in nutrients value in stored and infected potato tubers.

Key words: Vitamin C, Fat, Starch, Gallic acid, Oxalic acid, Potato, cold storage,

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INTRODUCTION

Potato *(Solanum tuberosam*) popularly known as the 'King of vegetables' and native of South America, has now become indispensable part of Indian cuisine. Potatoes use for several purpose including starch production, alcohol fermentation and recultivation. Potato component use in biomedical, pharmaceutical, fermentation field and engineering applications [1]. During the harvesting as well as storage, various pathological agent like fungus, bacteria and viruses attack on the potatoes [2-6].These tubers suffer from post harvest losses as a result of physical, physiological or pathological factors or combination of all three factors [7].The nutrient value of potato is excellent as it has carbohydrate in abundance, protein of superior quality, minerals and fibers in appreciable amount, vitamin C in sufficient quantity and vitamin B in reasonably good quantity [8]. Potatoes also a good source of starch. According to [9] starch has a wide range of applications ranging from being a thickener, gelling agent, to being stabilizer for making snacks, meat products and fruit juices. During recent years, starch has been taken as a new potential biomaterial for pharmaceutical and functional characteristics [10, 11].

A number of post harvest diseases attack on tuber during the harvesting and storage time which deteriorate its nutritional value. Undesirable storage conditions leads to physical and chemical quality loss in stored potatoes which affects their consumer acceptability.
The present investigations were conducted for finding out the fungal infection on the above mentioned pre and post stored potato tubers and biochemical changes altering the nutritional status of the tuber during the storage and due to pathogen.

EXPERIMENTAL SECTION

Potato variety Kufri jyoti which are extensively cultivated in Rajasthan were collected from farmer's field for the pre storage biochemical and pathological study. The incubated potatoes were used for the study. Uninfected potatoes served as control. The organic chemicals ascorbic acid, gallic acid, oxalic acid, fat, starch, chlorophyll, xanthophyll and carotene were estimated. After the study, tubers were kept in the cold storage for about 3 months at 10˚C and 85-90% relative humidity. After 3 months the same samples were used for pathological and biochemical estimation.

Isolation of fungi: In pathological study 10 gm of samples was taken and blended with 100 ml of buffered peptone water. In initial suspension 1 ml was taken aseptically and transferred to the sterile petri dishes. 15 ml of Yeast extract-dextrose chloramphenical-agar medium was poured (previously melted and maintained at 45$\pm$1˚C in water bath from culture bottle) into each petri dish. Inoculums was carefully mixed with medium and allowed to solidify by leaving petri dishes to stand on cool horizontal surface of bio safety cabinet (Indian standard method for yeast and mould count of food stuff IS 5403:1999). A separate controlled plate was made with 15 ml of medium to check its sterility. Petri dishes were placed inverted in the BOD incubator at 25$\pm$1˚C for five days. After 5 days a loop full of fungus was taken from petri dishes on slide. Staining was done by cotton blue stain and observed under microscope.

Biochemical study:

Vitamin C-- Vitamin C or ascorbic acid was estimated by 2, 6 Dichlorophenol visual titration method [12]. 10 gm of sample blended with 3% HPO_3 and made up to the volume 100 ml with HPO_3. 10 ml of aliquot of HPO_3 was taken and titrated with the standard dye until a pink end point. Standard dye was previously standardized by standard ascorbic acid solution.

Oxallic acid--Oxallic acid was estimated by Permanganate titration method [13]. Slurry of sample was made with hot distilled water. 30 gm of this slurry was taken for examination. 55 ml of 6M HCl and 2 drops of caprylic alcohol was added to slurry and boiled for 15 minutes. It was kept in a 500 ml flask overnight and mixed, filtered through filter paper. After discarding first 100 ml filtrate, 25 ml filtrate was taken into 50 ml Erlenmeyer, 5 ml tungstophosphoric acid reagent added and kept for 5 hrs. After filtration, 20 ml filtrate was taken into centrifuge tube and NH_4OH drop was added to pH 4-4.5. Further, 5 ml acetate buffer solution was added and kept for overnight. It was centrifuge at 1700 rpm for 15 minute, precipitate collected, supernatant discarded. Precipitate was washed by cold water, 5 ml H_2So_4 added, and titrated with 0.002M $KMno_4$ until first pink colour persist for minimum 30 second.

Fat--Fat was extracted by the Soxhlet extraction apparatus [14]. 10 gm of dried sample was taken in an extraction thimble and covered by wad of fat free cotton and connected to the flask. Thimble was placed in extraction tube of Soxhlet. 100 ml of anhydrous ether poured through tube (extraction) to flask. Sample was extracted for 16 hours on water bath. The ether volatilized, condensed and dropped continuously upon the sample without any appreciable loss. After the end of extraction period, ether was evaporated on a steam bath at low heat and dried at 100˚C for 1 hour. Flask was left for cooling and weighed. The difference in the weight indicates the ether soluble material (fat) in the sample.

Starch—Starch was detected by Anthrone method [15]. 0.5g of the sample repeatedly washed with hot 80% ethanol. Add 5ml of water and 6.5 ml of perchloric acid. After 20 min. extract was centrifuged. The volume of supernatant made up 100 ml with water. Pipet out 0.1 and 0.2 ml volume in tubes and added 1 ml of water.4 ml of anthrone reagent was added in each tube heat for 8 min. in water bath, cooled and absorbance was measured at 630nm.

Gallic acid--For the detection of Gallic acid, a suitable amount of sample was taken to get 5 gm of dried powder after grinding. Then sample was vortex for 2 min in 25 ml hexane and the mixture was filtered. The hexane extraction was evaporated to dryness at 50˚C using vacuumed oven. The residue after hexane extraction was extracted 2 times with 25 ml of acidified methanol (7%.acitic acid in 80% methanol) to obtain the hydrophilic fraction. The final volume of the hydrophilic fraction was made up to 50 ml with acidified methanol. 0.5 ml of this hydrophilic extract was diluted to 50 ml with distill water, 0.5 ml folic-ciocaltue reagent was added and allowed to react at room temperature for 3 minutes. 1 ml of 1N Na_2Co_3 was added and the mixture was incubated at room temperature for 1 hour. The absorbance was measured at 725 nm. Chlorogenic acid was used as standard. Total

phenolic content was reported as mg of chlorogenic acid equivalents per gram fresh weight sample (mg GAE/gfw) which was converted into mg of gallic acid by multiplying by a factor of 0.445 [16].

Chlorophyll--Chlorophyll of potatoes was determined by the Spectrophotometric Determination method [17]. About 10 gm of sample was taken and a small amount of calcium carbonate was added in it. Sample was extracted with the help of acetone in pestle and mortar. Supernatant liquid was decanted. Extraction was repeated till the residue become colourless. Acetone extract was filtered in to a 250 ml volumetric flask. 50 ml of ether was taken in a separating funnel and 30 ml of acetone extract was added. Water from the sides of separating funnel was added until the water layer was apparently free of all fat soluble pigments. Water layer drained off and ether layer was washed with 10 ml distilled water until the ether layer was free from acetone. After washing, ether extract was transferred to a 100 ml vol. flask. This volume was diluted with ether and transferred to a 100 ml Amber colored flask with Na_2So_4 and left until the solution became cleared. Pipette an aliquot of this solution into another dry bottle and OD was taken at 660/642.5 nm.

Xanthophyll--Total Xanthophyll was detected by the Spectrophotometric method [18].2 gm of grinded sample was taken into 100 ml vol. flask. 30 ml of extractant (Hexane + Acetone + Toluene + absolute alcohol, 10:7:7:6) were added into the flask and swirled. 1 ml of H_2O_2 was added into the flask and swirled again. 2 ml of 40% alcoholic KOH was also added and placed on water bath at 56˚C for 20 minutes. Mixture cooled in dark place and 30 ml hexane added into the flask Volume was made up by 10% Na_2So_4 solution. Mixture was shaken vigorously for 1 min. and left in dark place. Separation was done by column (glass wool + 12 cm adsorbent I + 7 cm adsorbent II + 2 cm anhydrous Na_2So_4) absorbent using vacuum by taking 25 ml of extract. Monohydroxy pigments (MHP) eluant added immediately after addition of extract. After completion of elution, elutant placed in dark and volume was made up by MHP. Absorbance was taken at 474 nm.

Carotene--Carotene was separated and estimated by chromatography [19]. 25 gm of sample was grinded with acetone. After 2-3 extraction residues form a resinous matter. 1 or 2 ml of water was added for further grinding. It was filtered in a conical flask until the residue became colourless. This filtrate was transferred to a separating funnel. 10-15 ml of petroleum ether was added and diluted with water containing 5% Sodium sulphate. Petroleum ether extract was filtered through anhydrous Na_2So_4 and volume was made up to 25 ml. Intensity of colour was measured at 452 nm using 3% acetone in petroleum ether as blank

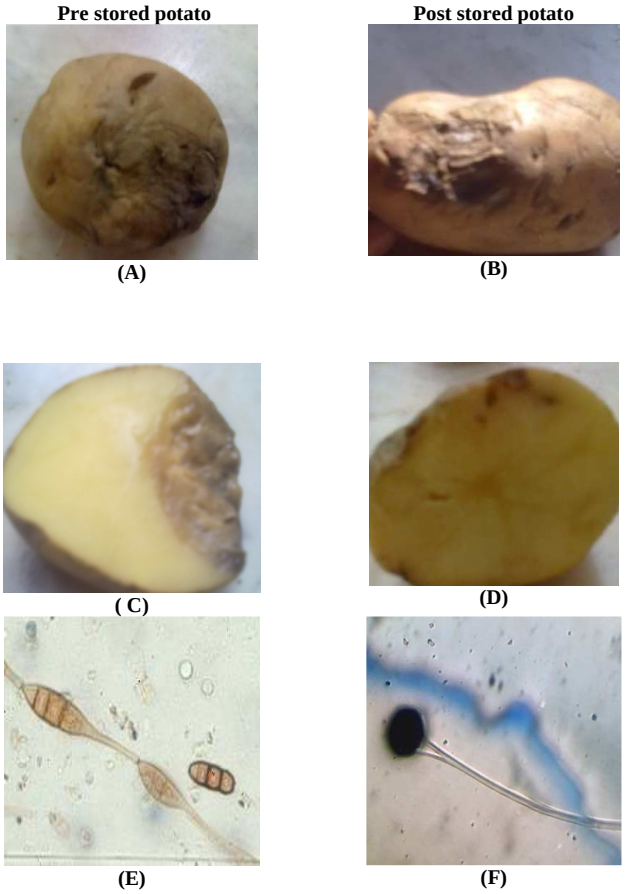
For Chromatographic Separation, absorption tube was attached to a flask and plugged by glass wool. Vacuum was applied and added enough absorbent (Magnesium oxide + Supercel, 1:3) to made the column approximately 10 cm. Na_2So_4 was added for 1 cm column. Column was washed with 25 ml of petroleum ether. Vacuum was disconnected and transferred the adsorption column to a flask. Pipette 5-10 ml of extract to the column and suction applied. Column was washed continuously with eluent (3% acetone in petroleum ether) until the desired pigment moved off the column and the eluent was colourless. Content was transferred to a vol. flask and dilute to volume with eluent. Intensity was taken at 452 nm using 3% acetone in petroleum ether as blank.

RESULTs AND DISCUSSION

After the pathological study *Alternaria solani* and *Aspergillus niger* fungus was founded in pre and post stored infected potatoes (Fig.1 E, F). *Aspergillus niger* caused stored rot disease in potatoes. The change in relative humidity and temperature caused fungus infection in potatoes. Previous study of [20] has reported that the environmental factors such as temperature and low relative humidity could minimize the infection of fungus.

Freshly harvested infected tubers as well as stored infected potatoes shows less biochemical content as compare to the fresh sample (Fig-2). Observations of [21] also indicate that the nutrients are used by fungus for its successful establishment, cellular growth, reproduction and survival within the tissues of the potato tubers.

The precursor of fat like triglyceride and glycerol declined due to increase rate of respiration in infected tubers as well as stored tubers [22] also reported that fat was drastically decreased in the chili powder due to breakdown of fat by lipases present in *Aspergillus flavus.*



(Fig-1)

Fig-1 A. Infected pre stored potato, B. Infected post stored potato, C. Cut portion of infected pre stored potato, D. Cut portion of infected post stored potato, E. *Alternaria solani*, F. *Aspergillus niger* (100 X).

Potatoes are excellent source of Vitamin C and it is helpful for healthy bones, muscles teeth, gums and blood vessel. Vitamin C decreased in post-stored potatoes as compared to fresh potatoes. Temperature and storage period may be decreased the level of vitamin C in tubers because of spurt in respiration or popularly called respiratory burst upon shifting the tubers to the lower temperature. Vitamin C was also decreased in infected potatoes due to the increased rate of oxidation in infected potatoes. Similar finding were made by [23-26] because certain oxidative enzymes produce by the pathogen or the host pathogen complex oxidation.

The fungus produces different types of toxic substances in the cell which may reduces the amount of chloroplast or pigments like chlorophyll, carotene and xanthophylls in infected potatoes. Result of [27] were also showed that during microbial growth there is increase respiration rate which generates some amount of heat in the micro atmosphere which may have effect on stability of carotenes. During the storage period, potatoes were not exposed to sun light therefore amount of pigments get reduced.

Gallic acid is a phenol compound and it works as an antioxidant. It was found high amount in fresh potatoes and it is very useful bioactive component in lymph-node carcinoma of the prostate (LNCaP) and prostate cancer-3 (PC-3) [28]. It was found in very less amount in infected and stored potatoes because of the higher rate of oxidation. Study of [29] also revealed that phenols level decreased in infected fruit due to higher activities of phenol oxidizing enzymes in auxin metabolism.

Fresh potatoes has sufficient amount of starch and it has found wide use in the food, textiles, cosmetics, plastics, adhesives, paper and pharmaceutical industries. In pharmacy starch appears indispensable; it is used as recipients in several medicines. Starch has also been used to produce a novel and satisfactory artificial RBCs with good oxygen carrying capacity [30] also used in the making of nanoscales, sensors, tissues, mechanical devices and drug delivery system [31].

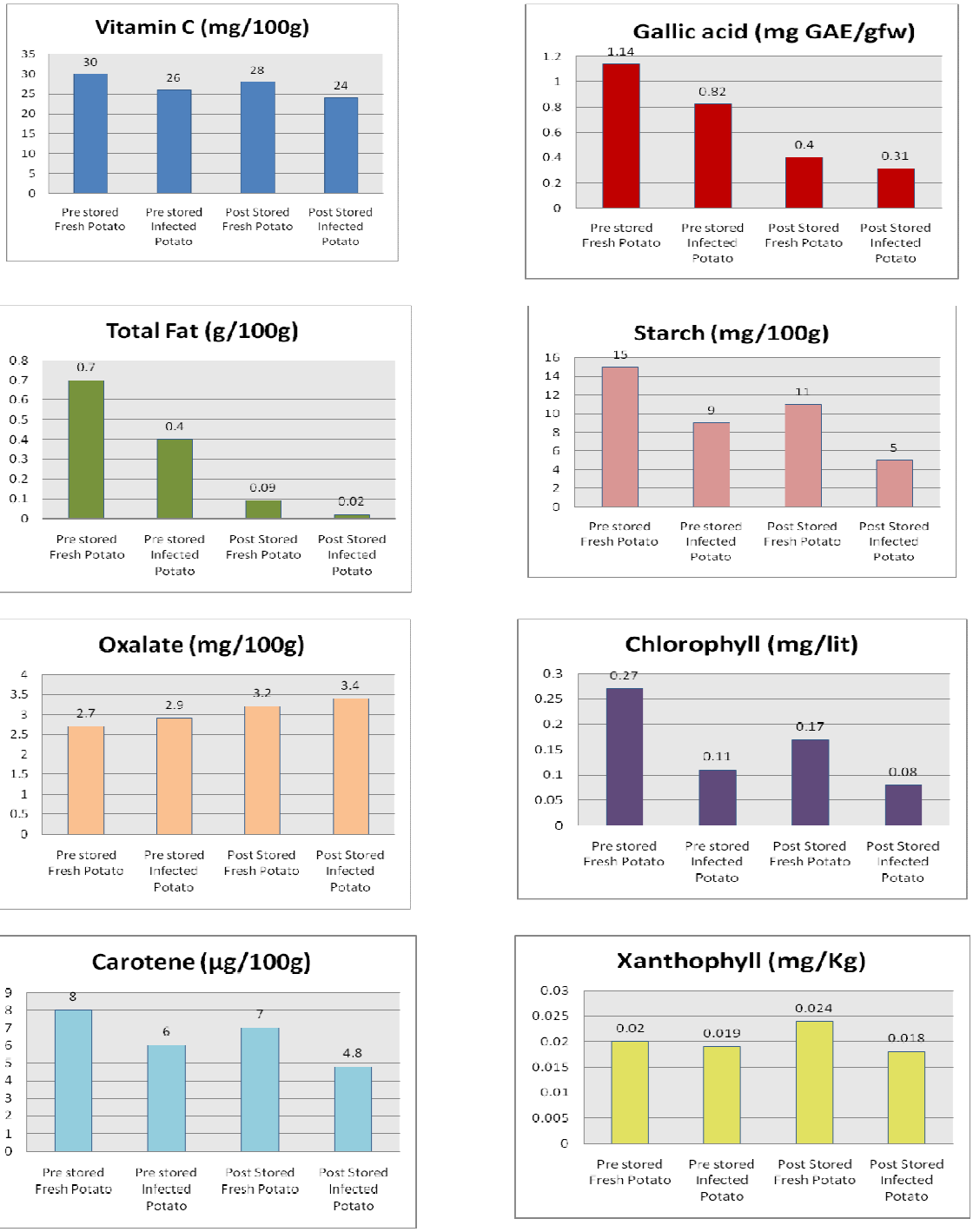


Fig-2: Biochemical changes in pre stored fresh, pre stored infected, post stored fresh and post stored infected potatoes

Starch show less amount in infected tubers because fungus produce glucoamylase enzyme which hydrolyzes alpha-1, 4 glycosidic bonds and form glucose as the end product [32]. Starch decrease after storage because during storage sucrose breakdown in cell and its triggers starch breakdown and respiration in stored potatoes tubers [33].

Oxalic acid increased in infected potatoes because fungus produces a small amount of oxalic acid [34]. It is also increased in stored potatoes. This may be due to higher amount of carbohydrate in stored potato [35]. Previous studies of [36] indicated that pathogenic infections affect the overall level of nutritional component in a plant. The nutrient component generally decreases due to disease pressure, plant responds quickly to disease pressure by showing a decrease in the nutrient composition.

CONCLUSION

The present findings suggest that the potato tubers are in rich of starch, gallic acid, vitamin c etc. The infection as well as storage reduces the useful content of the tubers therefore tubers should be free from infection and storage should be free from microbes.

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