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Assessment of biochemical parameters in Chilli under storage conditions

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Abstract
The present study on biochemical parameters in chilli were carried out for the samples collected from storage during November 2016. The analysis on nutritional information, about the changes in important biochemical constituents of red chillies powder during mould infection and storage is of utmost need, as most of these parameters are major quality criteria's for pepper export and consumption. It was evident from the study, that except for total proteins all other constituents showed remarkable losses due to fungal growth. Red pepper proved to be an ideal substrate for growth of many species of *Aspergillus* and *Penicillium*. It can be seen that maximum nutritional losses were encountered for total carotenoids, sugars and fat. In all the biochemical constituent studied at initial incubation days there was no significant drop in the values, but after 20 days of infection due to increase in fungal biomass, there is a considerable drop in all the constituents. The growing fungus utilizes the constituents of the substrate for its growth may be the reason for same.

Keywords: Chilli, biochemical constituents, pepper, sugars, carotenoids, etc.

Introduction
Capsicum (*Capsicum annuum* L.), a fruit vegetable, belongs to the family Solanaceae and is believed to have originated in South America. Christopher Columbus who discovered America in 1493 brought chilli to the rest of the world. Today, the sharpest and valued varieties of chillies are grown in Asia only. Currently the largest producer of chillies in the world is India accounting for 13.76 million tonnes of production annually. In India, its introduction is believed to be through the Portuguese in the 17th century. The Portuguese brought *Capsicum* from Brazil to India during the year 1584. India is the most popular for chilli production because it has the best-suited climate, soil, irrigation facilities, skill, types of chillies required to various markets and the intensive cultivation practices adopted by the farmers.

The *Capsicum* is commercially high valued crop due to its high nutritional and medicinal properties. Both fresh and dry chillies are used in cooking. The *Capsicum* fruit is rich in vitamin 'C' content which is about 118.6 mg/100 g. Other vitamins like vitamin 'A,' 'B6', 'B12' and 'E' are also present (Anon., 2011). *Capsicum* has medicinal properties too such as antioxidant and antimicrobial properties; improves the immune system, enhanced metabolism and even for cancer treatment (Anon *et al.*, 2004) [2]. The detailed nutritional value is presented in Table 1.

The medicinal importance of chilli pepper has been recognized long back by Mayan and Aztec civilizations in America, who used them in the treatment of Asthma, coughs and sour throat and in the modern era, it is included in the American Illustrated Medical Dictionary. The Merck Manual and Meteria Medica, where it is referred to as a rubefacient, local stimulant and diaphoretic apart from Analgesic activity, Anti-cancer properties, Capsicin as neurotransmitter, Anti-obesity properties, Cardiovascular benefits, Anti-epileptic agent, Anti-psoriatic agent, Gastrointestinal benefits and Hair follicle production.

The maintenance of the quality of fruits and vegetables depends upon the storage conditions, before sale and consumption. Many compositional changes that occur during storage of vegetables, influence their appearance, texture, flavour and nutritional content. Some changes can be desirable, while others can be detrimental to the quality of the commodity (Kader, 1986; Maguire *et al.*, 2004) [14, 18]. Chillies are highly hygroscopic and continuously undergo changes in moisture content either losing moisture or gaining moisture from the surrounding atmosphere. A number of studies have been carried out to establish a relationship between equilibrium moisture content (EMC) and equilibrium relative humidity (ERH) (Karon and Hillary, 1949) [15]. The equilibrium moisture is important for ensuring whether the produce will

gain or lose moisture under a given set of temperature and relative humidity conditions determining the fate of moisture loss and establishing a lower limit to which the products can be dried.

Narayanan *et al.* (1964) conducted packaging and storage studies and determined the stability of black pepper oleoresin packed in 30 and 125 ml aluminum bottles at different temperature and humidity. Govindarajan *et al.*, (1986) [12] reported that with good storage methods, the quality of oleoresin was maintained for fairly long periods. Gopalakrishna and Babylatha (2000) [11] conducted storage studies on raw and roasted samples of chillies (*Capsicum annuum*) whole and deseeded at 5 ^{0}C in the refrigerator and in an incubator at 37 ^{0}C. They reported a loss of 8 percent in oleoresin in whole chillies of Guntur variety. In case of raw and roasted chilli pericarp powder, the loss of oleoresin was more in samples stored at 37 ^{0}C than the samples stored at 5 ^{0}C.

The objective of this study was to analyse the biochemical changes that occurs in the chilli samples with infestation of fungi under natural conditions that were collected from cold storage in the month of November 2016. Samples were analyzed for biochemical constituents such as protein, total sugars, reducing sugars, total phenols ash content crude fibre, total carotenoids, total capsaicin and ascorbic acid. The analyses of biochemical components of the dried chilli sample before storage were taken as control.

Material and Methods

The biochemical constituents such as protein, total sugars, reducing sugars, total phenols ash content crude fibre, total carotenoids, total capsiacin and ascorbic acid were analysed for the chilli samples collected from storage during Nov' 2016. The analyses of biochemical components of the dried chilli sample before storage were taken as control.

Protein content was determined by method of Lowry *et al.* (1951) [17]. Chilli sample (100 mg) was hydrolyzed by adding 5 mL of 2.5 N HCl. This solution was boiled for 3 h and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased. Volume was made to 10 mL and centrifuged. The supernatant was used for the total sugars' estimation. Total sugars in chilli samples were assessed by phenol-sulfuric acid method suggested by Dubois *et al.*, (1956) [9]. The chilli sample of known amount was weighed and the sugars were extracted with 80% alcohol twice. Reducing sugars present in the extract were determined by dinitrosalicylic acid method (Miller 1959) [20]. Concentration of total phenols and ascorbic acid present in the cold-stored chillies was estimated as per standard methods described by Sadasivam and Manickam (1997) [24].

Proteins: The protein content of stored chillies was estimated by the method of Lowry *et al.* (1951) [17]. 500mg of sample material was ground in 10 ml of distilled water and centrifuged at 1800 x g for 30 min. Proteins of the supernatant were precipitated by adding 30% trichloro acetic acid (TCA) and centrifuged at 1800 x g for 15 min. The sediment thus obtained was washed 2-3 times with distilled water and dissolved in known volume of 0.1 NaOH and used for protein estimation. To 1 ml of test solution, 0.5 ml of Folin-Ciocalteu's (FCR) reagent C (4% Sodium carbonate, 0.2 N Sodium hydroxide and 1% Copper sulphate in 5:5:0.2 ratio) was added. The intensity of blue colour thus developed after incubating for 15 min at 37 ^{0}C was read at 540 mm. The amount of protein was calculated from standard curve prepared using bovine albumin.

Reducing sugars: The reducing sugars of chilli sample were estimated by the anthrone method as suggested by Plummer (1971) [23]. Four ml of freshly prepared 0.2 anthrone reagent was added to 1 ml of protein free carbohydrate solution and mixed rapidly. The mixture was heated in a boiling water bath for 10 min. The intensity of green colour thus developed was measured at 620 nm. Amount of reducing sugars was calculated from standard curve prepared for glucose and expressed in mg of glucose/ gm of fungal associated dried chilies material. Total sugars in chilli samples were assessed by phenol-sulfuric acid method suggested by Dubois *et al.* (1956) [9].

Phenols: The total phenol content of the chilli sample was determined as suggested by Bray and Thorpe (1954) [7]. The protein free extracts prepared with TCA were taken as test solution. One ml of test solution was taken in 25 ml standard volumetric flask and 1 ml of FC reagent was added followed by 2 ml of 20% sodium carbonate solution. Flask was thoroughly shaken and heated in a boiling water bath for exactly 1 min and cooled under running tap water and made up to 25 ml with distilled water. Absorbency of blue colour thus developed was measured at 650 nm in a spectrophotometer and the amount of phenols was calculated from standard curve prepared for catechol.

Ascorbic acid: Ascorbic acid content was analyzed according to the indophenol method (Harris and Ray, 1935) [13]. Ground chilli seed powder (5.0 g) was homogenized with acid mixture (15 g metaphosphoric acid in 40 ml glacial acetic acid and 450 ml water), and filtered through Whatman No.1 filter paper. Known volume of filtrate (25 ml) was titrated against indophenols (42 mg sodium bicarbonate was dissolved into a small volume of water followed by the addition of 52 mg 2,6-dichloro phenol indophenols and the volume was made up to 200 ml with distilled water). The standard graph was developed by using standard ascorbic acid solutions.

Capsaicin content: The capsaicin content was estimated as per suggested by Palacio (1977) [22]. About 2.0 grams of ground-dried chilli was passed through No.40 sieve (0.42 mm) and was placed in the 100 ml volumetric flask. The capsaicin was extracted by diluting with ethyl acetate up to 100 ml and allowed to stand for 24 hrs. One ml of the extract was taken and diluted with 5 ml of ethyl acetate just before reading, and then 0.5 ml of vanadium oxytrichloride ($VoCl_3$) solution (0.5% $VoCl_3$ in ethyl acetate) was added and shaken thoroughly. The absorbance of the solution was measured at 720 nm in UV–VIS dual beam spectrophotometer. Then reading was subtracted from the value obtained with 0.5 ml $VoCl_3$ added to 5 ml ethyl acetate (blank) and the reading was compared with the standard curve prepared for capsaicin. The amount of capsaicin in the samples were analysed and expressed in percentage.

Carotenoids content: Carotenoid content was analyzed according to the method of Wong (1928) [25]. Wherein, 0.2 g of chilli seeds was converted into ash in muffle furnace at 600 ^{0}C for 3 h. The ash was dissolved in 10 ml of 0.1 M HCl and the volume was made up to 50 ml with distilled water. 10 ml of solution was taken in a test tube, to which 1.0 ml of

potassium per sulphate (7%) and 4.0 ml of potassium thiocyanate were added, mixed thoroughly and the absorbance of the solution was measured in spectrophotometer at 540 nm. The carotenoid content of the sample was determined by using standard curve.

Results and Discussion

Changes in the Biochemical Constituents stored in Cold Storages.

Data on the biochemical constituents of chilli samples stored in cold storage are presented in (Table 2). The results clearly revealed that there was a gradual decrease in the levels of proteins, total sugars, reducing sugars, ascorbic acid and total phenol, Ash content, crude fiber, total carotenoids and total capsaicin with increasing storage period.

From the present study, it was seen that there are appreciable losses in the important biochemical constituents of chilli upon mould growth. Red pepper proved to be an ideal substrate for growth of many species of *Aspergillus*and *Penicillium*. High moisture content helps in survival and growth of the mould. Dense mycelial mat and sporulation (conidia) were prominent.

The nutritional information about the changes in important biochemical constituents of red chillies powder during mould infection and storage; most of these parameters studied are also major quality criteria's for pepper export and consumption. It is evident from the Table 2 that except total proteins all other constituents showed remarkable losses due to fungal growth. Total protein content increased from 162 mg/g to185mg/g in *Aspergillusflavus* and 154mg/g to 188mg/g in *Penicillium citrinum*. Onifade and Agboola (2003) [21] also observed a similar trend in the increase in protein content due to fungal infection in coconut and postulated that proliferation of microorganism synthesize several enzyme proteins and sometimes cause rearrangement of the nutritional composition of the substrate due to the formation of several degradation products thereby increasing its protein content. From Table (2) we can see that maximum nutritional losses were encountered for total carotenoids, sugars and fat. In all the biochemical constituent studied at initial incubation days there was no significant drop in the values, but after 20 days of infection due to increase in fungal biomass, there is a considerable drop in all the constituents. The growing fungus utilizes the constituents of the substrate for its growth may be the reason for same.

Moulds acquire nutrients by producing extracellular enzymes to break down organic material or the complex food sources. The resulting small molecules are then absorbed by the mycelium to fuel additional fungal growth. *Aspergillus sp.* are known to produce pectinase, xylanases, etc. which break down the hemicellulose, cellulose, lignin or all the insoluble carbohydrates (crude fiber) of the plant material into simple sugars. The soluble sugars are a good source of food and carbon.

There was decrease in Ascorbic acid content from 48mg/g to 41mg/g present in *A. flavus* (7mg/g Loss); The decrease in Phenols and ash content; which is an indicator of total mineral content of powder also was on similar trends as observed by Aziz *et al*. (2000) [5]; who reported that *Aspergillusflavus* depleted the zinc, copper and iron from the corn. Thus there is a direct correlation between metal uptake and mould growth. There was only (548.80 – 621.38mg/g) 72.58mg/g gain in capsaicin content (Table (2), the recorded loss in capsaicin content might be due to fungal enzymatic (peroxidases) oxidation of the vinyl moiety into other secondary substances.

Carotenes are largely responsible for the color of the red peppers. Initially, the color of the powdered red pepper was brilliant red, but after 30 days of infection, the powder was clearly looking moldy and dull colored. The carotenoids are very sensitive to oxidation and degradation in response to environmental stress. They are highly unstable and high relative humidity leads to enzymatic hydrolysis of the structure and in turn, makes it susceptible to the oxidation and loss (Desobry *et al.*, 1998) [8]. It can also be hypothesized that during microbial growth there is an increase in respiration rate which generates some amount of heat in the micro-atmosphere which may have an effect on the stability of carotenes. There are also reports on cleavage of β-carotenes into some flavor compounds by fungi (Zorn *et al.* 2003) [26]. The loss in ascorbic acid content can be attributed to its vulnerability to light, oxidation and heat. The level reduced from 7mg/g in the presence of *Aspergillusflavus*. 3mg/g in control sample. *Aspergillusflavus* secretes various phenolases in the substratum (Medina *et al.*, 2005) [19] and it is reported that crude fiber percentage is decreased from 15.46 to 7.78.

Very few reports are available on the changes in the biochemical constituents of chillies in response to infection. Bhardwaj *et al.*, (1985) [6] reported the biochemical changes occurring in bell pepper (*Capsicum annuum*) in response to infection by *Phytophthora nicotianae*. They found a significant decrease in total phenols and sugar content of fruits in response to infection. Azad (1991) [4] reported that there was a reduction in the content of sugar, ascorbic acid, nitrogen and sulfur in chilli fruits infected with *Colletotrichum capsici*. The decrease in the biochemical constituents in chillies, as reported in the present study, might be due to the utilization of these components by fungi associated with the pods.

The growth of molds and consequent mycotoxin production in spices was reported to be dependent upon a number of factors such as temperature, humidity, handling during harvest and storage (Atanda *et al.*, 1990; Garrido *et al.*, 1992) [3, 10]. Adebanjo and Shopeju (1993) analyzed the sources of molds associated with sun-dried vegetables in storage. The airspora contaminating the chilli samples during sun drying might be the source of mold infection during storage. Lee and Yu (1995) [16] reported the high incidence of *Alternaria alternata* and *Alternariasolani* on the red pepper.

Table 1: Nutritional value of green and dry chilli pepper.

Nutrients	Values (per 100 gms)	
	Green	Dry
Primary metabolites		
carbohydrates	3.0 gm	31.6 gm
Proteins	2.9 gm	15.0 gm
Fats	0.6 gm	6.2 gm
Minerals		
Calcium	30.0 mg	160.0 mg

Phosphorus	80.0 mg	370.0 mg
Iron	4.4 mg	2.3 mg
Magnesium	272.0 mg	---
Copper	1.4 mg	---
Manganese	1.38 mg	---
Molybdenum	0.07 mg	---
Zinc	1.78 mg	---
Phytonutrients		
Carotene	175.0 µg	345.0 µg
Cryptoxanthin - β	---	6252.0 µg
Luetin - Zeaxanthin	---	13157.0 µg
Phytin Phosphorus	7.000 mg	71.000 mg
Vitamins		
Vitamin C	111.0 mg	50.0 mg
Thiamine	0.19 mg	0.93 mg
Riboflavin	0.39 mg	0.43 mg
Niacin	0.90 mg	9.5 mg
Electrolytes		
Sodium	---	14.0 mg
Potassium	---	530.0mg
Magnesium	272.0 mg	---
Copper	1.4 mg	---
Manganese	1.38 mg	---
Molybdenum	0.07	---
Zinc	1.78 mg	---
Secondary metabolites		
Capsaicin		
Moisture	85.700 gm	10.000 gm
Dietary Fiber	6.800 gm	30.200 gm
Caloric Value	229	297

Table 2: Biochemical changes in infected dried chilli during storage

Bio Chemical Component	Sample stored in Cold Storage			Control		
	10th day	20th day	30th day	10th day	20th day	30th day
Protein (mg/g)	162	175	185	170	178	171
Total Sugar (mg/g)	250	240	232	290	292	298
Reducing Sugars (mg/g)	35	33	30	41	42	43
Ascorbic acid (mg/100g)	48	45	41	50	52	53
Total Phenols (mg/g)	0.42	0.38	0.36	0.48	0.50	0.52
Ash content (%)	8.42	3.42	2.88	9.75	9.45	8.42
Crude fibre (%)	15.46	8.60	7.78	18.98	17.75	15.46
Total Carotenoids (mg/100g)	112	23.35	15.29	133.65	111.28	112.00
Total capsiacin (µg/100g)	548.80	620.15	621.38	995.39	978.88	548.80

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