Influence of vacuum packaging on seed quality and mineral contents in chilli (*Capsicum annuum* L.)

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Revised: 11 January 2011 / Accepted: 13 January 2011 / Published online: 10 February 2011
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Abstract Studies were carried out to find out the influence of vacuum packaging on physical parameters of whole chilli and biochemical constituents in chilli seeds. Chilli fruits were stored in vacuum packed and jute bags stored at room temperature (25 ± 2 °C), cold storage (4 ± 1 °C) under both light and dark conditions for a period of 24 months. At the end of the storage period, seeds were separated from fruits and various parameters *viz.*, moisture content, capsaicin content, ascorbic acid, carbohydrates, protein and mineral elements like Fe, P, Na and K were analyzed. It was observed that the samples stored in vacuum packed bags maintained the quality with least deterioration in all the quality parameters compared to samples stored in jute bags.

Keywords Chilli seeds · Vacuum packaging · Minerals · Capsaicin · Protein · Ascorbic acid content

Introduction

Chilli [*Capsicum annuum* L.] is an important spice crop in India and is grown in the tropical, subtropical and temperate regions of the world. The superior intrinsic quality (attractive red colour and pungency) of Indian chillies accounts for its global demand (Rithesh et al. 2000). The consumption of chilli, apart from its taste and colour, has immense medicinal value due to the presence of

capsaicin (Lee et al. 2007), oleorecin, carotenoids and minerals like iron, phosphorus, sodium and potassium (Kang et al. 1995; Rithesh et al. 2000). Mineral elements are essential for proper composition of body fluid, maintenance of cellular water balance, acid base balance and nerve transmission (Wardlaw 1999). Deficiency of these minerals leads to muscle cramps, mental confusion, loss of appetite and irregular cardiac rhythm. Like vitamins, minerals function as co-enzymes and enzyme activators which are essential for proper functioning of living cells. The fruits are one of richest known sources of vitamin C, as noted, and important source of vitamin A (Howard 2000).

There are many factors that can narrow down the gap between potential and farm level yield. Among them, use of quality seeds is the most important one (Ahmad 2001). Although seed quality is governed by genetic makeup, seed storage and retention of viability are important which greatly affect seed vigor. Most of the post harvest losses are occurring while threshing, transportation and storage. These losses should be reduced to make use of maximum produce. Post harvest losses are huge at farmer's level. Maintenance of seed quality during the storage period is important not only for successful crop production but also for maintaining the quality and integrity of the seeds because of their constant threat and of genetic erosion (Barua et al. 2009).

In general, fruits stored under modified or controlled atmospheres maintain higher soluble sugars and organic acids, while vegetables show lower values for these compounds (Bognar et al. 1990). Arpaia et al. (1987) showed that controlled atmosphere maintains higher levels of uronic acids, galactose, arabinose, rhaminose, cellulose and starch in kiwis. Extending shelf life of either fresh or dried fruits and vegetables has been the major concern in the recent past and several techniques have been evolved, of which, vacuum packaging of processed foods is emerging as one

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of the important tools considering its advantages in maintaining the shelf life. Vacuum packaging is the procedure that results in a reduced oxygen level in a sealed package. The anaerobic environment of vacuum packaging prevents the growth of spoilage microorganisms especially aerobic ones which are responsible for off odor, slime and texture changes (Nunez et al. 1986). There are not many studies evaluating micronutrients and biochemical activities in controlled atmospheric storage and specially with vacuum packaging.

The present research is conducted with the specific objective to find out the influence of vacuum packaging on quality parameters like mineral composition, capsaicin, protein, total carbohydrates and ascorbic acid content in chilli seeds.

Materials and methods

Sample collection, vacuum treatment and storage

Chilli samples collected at uniform crop maturity were dried to constant moisture content (10%) in mild hot sun during April 2006. Chillies used for vacuum packaging were destemmed to remove stalk. Dried chillies were vacuum packed (OLPACK 510, Interprise Breussel S.A., Belgium) in multi layer polythene bags of 350×180×150 mm dimension. For comparison, they were also stored in jute bags (farmer's practice of storage). Both vacuum packed bags and jute bags were stored at room temperature (25±2 °C) and cold storage (4±1 °C) under normal light and dark. The dark room facility was created with thick black cloth to avoid the build up of temperature. The material was stored for 24 months and at the end of storage period, seeds were drawn from chilli fruits of all the treatments for analyzing various parameters, the details of which are mentioned below.

Biochemical analysis

Capsaicin content

Capsaicin content was analyzed as per the method 21.3 of ASTA (Anonymous 2004). For the extraction of capsaicinoids, 25 g of ground chilli seed sample was weighed into a 500 ml soxhlet flask together with 200 ml of rectified spirit and several glass beads was added to avoid bumping. This was refluxed gently for 5 h by heating in Soxhlet apparatus. The extract was cooled and filtered into a stoppered test tube through Whatman No.1 filter paper pre-wetted with rectified spirit. This sample was then analyzed by HPLC [Waters 2487, dual wavelength detector, 515 HPLC pump, IN-LINE degasser, pump control module 2.], the symmetry C18 column [250 mm length, 0.25 mm i.d, 250 µ particle size] was used, with the combination of acetonitrile and 1% acetic acid in water (60:40 v/v) as a mobile phase, flow rate

of the mobile phase was kept at 0.8 ml/min. The HPLC was monitored at 280 nm and the injection volume was 20 µl. For peak identification, solution of reference standards were analyzed under similar conditions and their retention time were compared to those of samples. 0.1 g of standard N-vanillyl-n-nonamide (Sigma 97%), was made up to 100 ml with rectified spirit and was kept as a stock solution (1,000 ppm) in amber colored standard flask. The working standard was prepared by adding 10 ml of stock and was made up to 100 ml with rectified spirit and was stored in refrigerator. The contribution of each identified compound was expressed as the percentage of its peak area. The samples were analyzed by Empower-2 software. The capsaicin in the seed sample was calculated in Scoville Heat Units (SHU) (Scoville 1912) as follows,

$$\text{Capsaicin (SHU)} = (C/A) \times (C_S/W_X) \times (H_C/R_C)$$

where,

C Average peak area of capsaicin

A Average peak area of standard

C_S Concentration of standard (mg/ml)

W_X Weight of sample (mg/ml)

H_C Heat factor for capsaicin

R_C Response factor for capsaicin relative to standard.

Capsaicin content in per cent was calculated as follows,

$$\text{Capsaicin content(\%)} = \frac{\text{Total SHU}}{16 \times 1000}$$

Protein content

Protein content was estimated as per the method of Lowry et al. (1951). Seed powder (500 mg) was macerated thoroughly in 10 ml of phosphate buffer (pH 6.6). The contents were centrifuged at 4000 × g for 30 min at 4 °C in refrigerated centrifuge (Sigma 3K30, 230 V, 50 Hz). One ml of supernatant was taken in a volumetric flask and made up to 100 ml with distilled water and used for protein estimation. From this, 1.0 ml of sample was taken into a test tube, to which 5.0 ml of alkaline copper sulphate reagent (AR grade) was added and shaken well. The solution was incubated for 10 min at room temperature. After that, 0.5 ml of Folin-Ciocalteu reagent was added with proper mixing and allowed for 10 min for color development. The absorbance of the solution was measured at 650 nm on UV–VIS dual beam spectrophotometer [Labomed, Inc., USA]. Bovine Serum Albumin was used as standard.

Carbohydrate content

This was determined by phenol sulphuric acid method (Dubois et al. 1956). Ground chilli seeds (100 mg) were

hydrolyzed by keeping in boiling water bath for 3 h with 5.0 ml of HCl (2.5 N) and cooled to room temperature. The solution was neutralized with solid Na_2CO_3 until the effervescence ceases and the volume was made up to 100 ml with distilled water. The contents were then centrifuged at $8000 \times g$ for 30 min at 27 °C. One ml of supernatant was diluted to 100 ml with distilled water. Diluted (1.0 ml) sample was transferred into test tube and 1.0 ml of phenol (5%), 5.0 ml of conc. H_2SO_4 were added, shaken well and kept in water bath maintained at 30 °C for 20 min. The absorbance of the solution was measured at 490 nm in UV–VIS dual beam spectrophotometer [Labomed, Inc., USA]. Carbohydrate concentration in test sample was calculated using glucose standard.

Ascorbic acid content

Ascorbic acid content was analyzed according to the indophenol method (Harris and Ray 1935). Ground chilli seed powder (4.0 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 indophenol. The ascorbic acid content was calculated by using standard (ascorbic acid).

Mineral contents

Iron content was analyzed according to the method of Wong (1928). Wherein, 0.2 g of chilli seeds were converted into ash in muffle furnace at 600 °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. Ten ml of solution was taken in a test tube, to which 1.0 ml of potassium persulphate (7%) and 4.0 ml of potassium thiocyanate were added, mixed thoroughly and the absorbance of the solution was measured in UV–VIS dual beam spectrophotometer (Labomed, Inc., USA) at 540 nm. The iron content of the sample was determined by using standard curve.

Phosphorus, potassium and sodium contents in seed samples were extracted by wet ash method (Jackson 1962). Phosphorus was determined by vanadomolybdate method (Jackson 1962) using spectrophotometer (Labomed, Inc., USA). Potassium and sodium contents were estimated using flame photometer (Elico, CLU 354).

Statistical analysis

The interpretation of analytical data was performed by the application of analysis of variance (ANOVA). The experimental design was a factorial type with Completely Randomized Design (CRD) with four replications to test the significance of various variables. Angular transforma-

tion is a technique used to convert data made up of percentage or frequencies (because percentage data will not follow normal distribution) into a form that can be analyzed by ANOVA.

Results and discussion

Physical parameters

The samples stored in vacuum packed bags had maroon red colour fruits, characteristic flavor of capsaicin with no extraneous matter thus indicating no deterioration. On the contrary, the samples stored in jute bags were infested with mold and characteristic white patches on the entire surface coupled with odd odor. This is mainly attributed to the variations in the moisture content of the fruits which was constant for vacuum packed bags. While, it fluctuated in jute bags, depending on the relative humidity of the atmosphere. Changes in the moisture content of dried and frozen food can affect nutritional quality. Increase in moisture content of dried food will promote microbial deterioration and accelerate rancidity and oxidation of vitamins. While, the loss of moisture will cause fat oxidation and browning reactions to occur resulting in off-flavor and off-colour. Reduction in rehydrating capacity of the samples increased with increase in storage period (Satish Naik et al. 2005). However, reduction was less for those packed in vacuum package as compared to conventional package. This might be attributed to the reduction of water binding sites due to chemical and structural changes in cells of the seed samples. Similar results were observed in mushroom by Kumar and Sreenarayanam (2000) and Deshpande and Tamhane (1981). Temperature fluctuation can increase the rate of deterioration of foods, contribute to colour fading of highly coloured products. Prolonged exposure to heat, light and oxygen resulted in 20–53% loss of the initial carotenoids (De Guevara et al. 2002; Minguez-Mosquera and Hornero-Mendez 1993). Kader et al. (1989), Wright and Kader (1997a) reported that low levels of O_2 and high CO_2 influence both the reduction of carotenoid loss by oxidation, and the inhibition of its biosynthetic pathway.

The ash content constitutes inorganic part of the solid matter. It was lowest in the jute bags stored under room temperature followed by cold storage. While, no appreciable changes were observed in the samples stored under vacuum packed bags, irrespective of storage temperature. It could be mainly attributed to increased respiration as a result of higher moisture content in the jute bags, while, there was no exchange of gases in vacuum packed bags. Similar result was observed in cheese by Abdulla and Nusr (2009).

Biochemical parameters

Capsaicin content

The capsaicin content varied considerably between the treatments with jute bag stored samples showing nearly 76.5% reduction in capsaicin content compared to original value. Significant differences were evident between the vacuum packed and jute bags. However, vacuum packed bags didn't show significant differences between the storage conditions viz., room temperature, cold storage and dark. A similar influence of storage conditions has been reported by Ayub Hossain and Gottschalk (2009) and Zanoni et al. (2000) in dried tomato halves wherein, a significant loss of color and other quality parameters were found at room temperature compared to cool chamber. The deterioration of capsaicin content in jute bags was probably because of higher oxidation and absorption of moisture from the ambient atmosphere. The samples held under vacuum packaging are unlikely to suffer from oxidation and maintain the original moisture content because of the property of the films used for packaging. Storage of bunched onions under modified atmospheric packaging and moderate vacuum packaging also indicated superiority in maintaining various quality parameters over conventional storage (Hong and Kim 2004).

Nutritional quality

Apart from pungency and attractive color, dried chillies also contain considerable amounts of protein, ascorbic acid and

carbohydrate contents. The protein content ranged from 42.4 mg/g (jute bags) to 71.2 mg/g (vacuum packed bags stored under dark). While, the freshly harvested seeds had the protein content of 75.2 mg/g. This indicates that the protein content in the seeds is also subjected for deterioration depending on the storage conditions indicating its sensitivity to moisture, light, temperature and oxygen (Vertucci and Roos 1990). Loss of protein content was maximum in jute bags stored at room temperature which could be mainly attributed to property of protein chains forming loose mesh when the moisture content is more. With loss in moisture content, they become disorganized resulting in nutrients loss. In addition, the reaction between simple sugars and amino acids leads to breakdown of protein molecules (Ben-Vehoshua 1985; Sandhya and Singh 2004). The reduction of protein, rehydration ratio and increase in moisture content was comparatively higher in ordinary heat sealed storage against vacuum packaging which was attributed to the lower activity of proteinase (Kumar and Sreenarayanam 2000).

Ascorbic acid is a sugar acid with antioxidant properties. It is observed that the ascorbic acid content declined during storage period for all the samples (Table 1). It is also observed that ascorbic acid content degraded rapidly in room environment than that of cold storage. In addition, dark stored seeds showed least reduction over other storage conditions indicating the sensitivity of ascorbic acid to both light and temperature. Significant loss in ascorbic acid content of seeds stored in jute bags could also be attributed to increase in certain cellular enzymes which promote the oxidation of ascorbic acid (Yeh et al. 2005). Sanchez-Mata et

Table 1 Influence of vacuum packaging on biochemical parameters in chilli seeds

Treatments	Storage period (months)	Moisture content (%) $\bar{X} \pm SD$	Ash content (%) $\bar{X} \pm SD$	Capsaicin content (%) $\bar{X} \pm SD$	Protein content (mg/g) $\bar{X} \pm SD$	Carbohydrate content (mg/g) $\bar{X} \pm SD$	Ascorbic acid content(mg/g) $\bar{X} \pm SD$	Iron (mg/g) $\bar{X} \pm SD$	Phosphorus (%) $\bar{X} \pm SD$	Potassium (%) $\bar{X} \pm SD$	Sodium (%) $\bar{X} \pm SD$
T ₁	0	10.0±0.05 (18.39)	3.1±0.09 (10.22)	0.08±0.02 (1.62)	75.2±2.34	400.0±15.81	0.11±0.00	1.5±0.05	0.6±0.22 (4.25)	0.5±0.24 (3.84)	1.0±0.17 (5.59)
T ₂	24	9.9±0.05 (18.32)	3.0±0.08 (9.93)	0.06±0.03 (1.40)	69.6±1.25	260.0±5.47	0.08±0.00	0.9±0.02	0.5±0.24 (3.84)	0.5±0.21 (3.95)	1.0±0.15 (5.67)
T ₃	24	9.8±0.07 (18.24)	2.9±0.00 (9.80)	0.07±0.03 (1.52)	70.2±1.64	370.0±3.91	0.08±0.00	1.2±0.04	0.5±0.21 (3.95)	0.5±0.24 (3.84)	1.0±0.17 (5.59)
T ₄	24	9.6±0.08 (18.05)	2.9±0.00 (9.80)	0.07±0.02 (1.52)	71.2±2.13	360.0±3.36	0.10±0.00	1.1±0.02	0.5±0.21 (3.95)	0.5±0.21 (3.95)	1.0±0.17 (5.59)
T ₅	24	14.6±0.04 (22.48)	2.2±0.11 (8.43)	0.02±0.01 (0.81)	42.4±1.88	120.0±2.94	0.06±0.00	0.5±0.02	0.3±0.33 (2.85)	0.0±0.00 (0.00)	1.0±0.17 (5.59)
T ₆	24	14.1±0.27 (22.08)	2.5±0.09 (9.05)	0.03±0.01 (0.99)	60.8±1.74	140.0±2.94	0.08±0.00	0.8±0.03	0.5±0.24 (3.84)	0.5±0.24 (3.84)	1.0±0.17 (5.59)
SEM		0.0155	0.0099	0.0034	0.2334	0.9185	0.0004	0.0046	0.0309	0.2625	0.0211
CD (5%)		0.0437	0.0280	0.0096	0.6581	2.5895	0.0012	0.0129	0.0873	0.0740	0.0597

The values in the parenthesis are treatment mean of angular transferred values

T₁=Control (freshly harvested chilli seeds), T₂=Vacuum packed chilli seeds at room temperature, T₃=Vacuum packed chilli seeds at cold storage, T₄=Vacuum packed chilli seeds at dark, T₅=Jute bag packed chilli seeds at room temperature, T₆=Jute bag packed chilli seeds at cold storage
 $\bar{X}$ mean value ($n=4$), SD Standard Deviation

al. (2003a, b) observed that the samples stored at reduced O₂ levels had higher vitamin C content due to lower oxidation process. Vitamin C degradation is due to both autoxidation and enzymatic degradation viz., ascorbate–oxidase, polyphenol–oxidase, cytochrome–oxidase and peroxidase (Barath et al. 1993; Erdman and Klein 1982). Hossain and Gottschalk (2009) reported that ascorbic acid degraded linearly with storage time both in room and cool chamber environments. The degradation was also related to the moisture content of the samples during storage. Samples with higher levels of moisture content degrade more rapidly than the lower levels of moisture content due to oxidation (Chiu et al. 2003).

Carbohydrate content decreased significantly (70%) in jute bags under ambient condition at the end of the storage period compare to initial value. While, vacuum packed bags stored under cold recorded only 7.5% reduction. It clearly indicating the influence of storage conditions on the stability of the sugars. These changes could be attributed to the balance between anabolic and catabolic processes. Inter–conversions between the carbohydrates during the storage are greatly influenced by O₂, temperature, CO₂ and light. Sanchez-Mata et al. (2003a, b) opined that respiration involves a high consumption of simple sugars and both respiration and degradation processes are intensified during later stages of storage. Arpaia et al. (1987) and Larson et al. (1997) reported that vegetables storage under atmospheres with low levels of oxygen maintain higher levels of starch, reduced respiration and carbohydrate consumption.

Pattee et al. (1981) reported significant changes in the levels of fructose, glucose, inositol, raffinose, ribose and sucrose during storage periods. Thus rapid depletion of the carbohydrates in seeds might be responsible for loss of vigor.

Mineral contents

Considerable influence of packaging material and storage conditions in the mineral contents of seeds was observed (Table 1). It was further observed that decrease in mineral content was less in vacuum packed bags compare to jute bags. Variation in the mineral contents between the packaging materials could be attributed to redistribution of mineral elements in seeds and possible microbial contamination (Bognar et al. 1990; Perring and Pearson 1987). The iron content was drastically reduced in the jute bags irrespective of the storage conditions. While, the iron content in vacuum packed bags was much higher compared to jute bags though it recorded a reduction of nearly 20% over the original value. The higher reduction of iron content in jute bags may be attributed to its sensitivity to temperature. Kayisoglu and Demirci (2006) reported that iron content in both conventional and vacuum packed bags was affected significantly only up to 6 months of period.

The phosphorus content indicated a decline of 16.7% in vacuum packed seeds, while the decline was 50% in the jute bag stored seeds. While, there were no changes in both potassium and sodium contents of the seeds during the storage period between the treatments, except that no potassium was detected in jute bags stored under room temperature. It clearly emphasizes the role of temperature for maintaining the potassium content in seeds. Immunity of sodium and potassium for deterioration during the storage irrespective of storage conditions and packaging material could be mainly attributed to the nature of the existence of these elements, both of which are monovalent cations which are strongly bound to the cell wall. Higher moisture of stored seeds in jute bags may be the main reason of quick quality deterioration. Similar observation has also been made by Ayhan Topuz et al. (2009).

Conclusion

Seed deterioration is an inexorable and an irreversible process. The quality and viability of chilli seeds are subjected to variations during storage conditions and it has been found that the life span of seeds depends on moisture content of the seeds, relative humidity, temperature, light and oxygen content under which the seeds are stored. It has been found in the present study that it is possible to extend the shelf life of chilli seeds up to 24 months without deterioration in quality parameters and mineral contents by storing them under vacuum package. Since seed is an important input in agriculture which determines not only the production but also the productivity, it is essential to maintain the quality as well as seed vigor.

Acknowledgements This work was funded by Interprise Brussels, Bruxtainer Division, Belgium and University of Agricultural Sciences, Dharwad. The authors greatly acknowledge the support of Jean-Marie GILLE, Paul Graindorge, Mohan Bajikar and the Vice-Chancellor, UAS, Dharwad.

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