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Anti-radical and microbial analysis of MAP stored Bitter gourd chips
Short-running title: MAP storage study of Bitter gourd chips

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A B S T R A C T

Bitter gourd (*Momordica charantia* L.) has been used since long time as food and medicinal plant. This research study was performed to determine the antioxidant activity of the Bitter gourd chips by DPPH radial-scavenging activity and ABTS assay. Total phenolic content was measured by Folin-Ciocalteu reagent. The different MAP gases concentrations used for the packaging of chips were 100% N_2: 0% CO_2, 70% N_2: 30 % CO_2 and 50 % N_2: 50 % CO_2. The temperatures used for storage were at room temperature, 37^{o}C and 45^{o}C. The best result was shown by 100% N_2: 0% CO_2 package stored at room temperature.

Introduction

India ranks second as the producer of vegetables in the world next to China. 5 to 6% of the vegetables produced belong to *Cucurbitaceae* family, commonly called as gourd family (Palamthodi and Lele, 2014). All parts of the plant impart medicinal values and have been used as traditional medicine for the treatment of wound healing, inflammation, hypertension, jaundice, kidney stone, microbial infections, chickenpox, etc. Bitter gourd is a rich source of phenolic compounds. It also possesses high anti-mutagenic and antioxidant activities. These natural phenolic compounds can be incorporated in many functional food items. Bitter gourd is rich in medicinal value, but due to its bitter taste very few people like to consume it. Today bitter gourd chips have a high selling value. It may be fried or baked with low oil absorption activity with full of health benefits.

The antioxidant activities in fresh fruits and vegetables and their products are being determined by ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1999; Guo *et al.*, 2003; Jimenez-Escrig *et al.*, 2001) oxygen radical absorption capacity (ORAC) (Cao *et al.*, 1993; Ou *et al.*, 2001; Prior *et al.*, 2003), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) (Leong and Shui, 2002; Miller and Rice-Evans, 1997) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams *et al.*, 1995; Gil *et al.*, 2002).

Today food packaging has emerged as an important criteria regarding food product's sale. The consumer demand for high food quality has increased in the past few years (Callegarin *et al.,* 1997). Modified Atmosphere Packaging has gained a lot of interest with respect to food packaging. It refers as a method where normal gas atmosphere is changed by a package headspace to optimal conditions to extend the shelf-life of packed products. The gases used mainly for MAP technology are carbon dioxide (CO_2) and nitrogen (N_2) (Sharma *et al.,* 2016).The objective of this research was to evaluate the anti-radical activity of bitter gourd chips by DPPH and ABTS methods at different MAP gases concentration. In respect of these, total phenolic content and microbial count was also estimated.

Materials and Methods

Bitter Gourd chips were prepared according to the method explained by Singh *et al.* (2014). The prepared chips were analyzed for their anti-radical activity by two methods, i.e. DPPH and ABTS at three different concentrations of gases using MAP technology. Further the microbial analysis of chips was done with respect to total bacterial count, total fungal count and coliform count.

Modified atmosphere packaging of chips

The prepared chips were packaged using MAP at different temperature i.e. room temperature, 37°C and 45°C in incubators. Different gas concentrations were used i.e. (a) 100% N_2: 0% CO_2 (b) 70% N_2: 30 % CO_2 (c) 50 % N_2: 50 % CO_2.

Anti-radical Activity Determination

DPPH radical determination

The DPPH radical scavenging activity of bitter gourd chips was determined according to the methods of Brand-William *et al.* (1995) with some modifications. Two hundred mg of sample was taken in centrifuge tube (in triplicate). Two hundred microlitre distilled water was taken in blank instead of the sample. Then 1 ml of DPPH (8 mg/100 ml of ethanol) solution was added to the sample and the blank. This set up was left at room temperature for 30 min (vortexed in between). Tubes were then centrifuged at 4000 rpm for 10 min. After that, 0.5 ml supernatant was poured in fresh tubes containing 1 ml of ethanol and the absorbance was taken at 517 nm against the ethanol by using UV-1800spectrophotometer (Shimadzu, Japan). Each crude extract was analyzed in triplicate. The percentage of inhibition was calculated against blank.

ABTS radial activity determination

The ABTS radical scavenging activity of bitter gourd chips was determined according to the methods of Miller *et al.* (1996) with some modifications. 19.2 mg of ABTS (2, 2–azino-bis-3-ethylbenzothialine-6- sulphonic acid) was dissolved in 5 ml of distilled water.1.892 g of ammonium persulphate was weighed and dissolved in double distilled water and made to 100 ml. 8 g of NaCl, 0.2 g KCl, 1.99 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 were dissolved in 800 ml of double distilled water and pH was adjusted to 7.2 and final volume was made 1 litre. ABTS working solution was prepared by using 5 ml of 7 mM ABTS stock solution, 88 µl of 190 mM ammonium persulphate added in the ratio of 1:0.35 and kept for 12 hour in dark in amber color glass bottle and then diluted (1:70) with buffer till it gives an absorbance of 0.70 ± 0.02 at 734 nm. 3 ml of ABTS working solution was taken out and mixed with 30 µl bitter gourd extracts in 3 ml cuvette. The contents were mixed for 10 seconds. 30 µl distilled water was used as blank. Phosphate

saline buffer was used as reference. After that the absorbance was measured at 734 nm by using UV-1800spectrophotometer (Shimadzu, Japan) and noted the decrease in absorbance at 10 seconds interval. The percentage of inhibition was calculated against blank.

Total phenolic content

The total phenolic content of the bitter gourd was determined according to the Folin-Ciocalteau method as described by Cliffe *et al.* (1994) with some modifications. Five hundred microlitre of the sample extracts were well mixed with 2.5 ml distilled water, 0.5 ml of 0.2 N Folin-Ciocalteau reagents and placed for 5 minutes. 1 ml of 75 g/l of Na_2CO_3 was then added. The above solution was then kept for incubation at room temperature for 30 minutes. Absorbance was measured at 760 nm using 1 cm cuvette UV-1800spectrophotometer (Shimadzu, Japan). Gallic acid (0 - 800 mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalents (GAE) per gram of extract.

Determination of microbial population

The chips were MAP packed by using low density polyethylene bag (LDPE) and stored at room temperature, 37°C and 45°C for a period of one month (Fig.4). The chips stored at 37°C were taken for optimization as they gave best results of storage with less number of microbial counts as compared with chips stored at room temperature and 45°C.

Statistical analysis

Significant test and evaluation of MAP stored Bitter gourd chips was done by using Statistical Package for Social Sciences (SPSS).

Results and Discussion

DPPH Inhibition Activity

The DPPH inhibition activity of MAP stored bitter gourd chips is presented in (Fig.1) indicated that there was enough difference in the DPPH inhibition activity of bitter gourd chips in all three different concentrations. The antioxidant activity by DPPH inhibition method was higher in 100% N_2 containing bitter gourd than 70:30 and 50:50 samples. The higher antioxidant activity for chips stored at100% N_2 indicated that the samples may retain good medicinal properties when compared to remaining both samples.

Chu *et al.* (2000) have reported that scavenging activities against DPPH of green leaves of potatoes blanched for 2 min at 100°C remained the same as for fresh ones.

ABTS Inhibition Activity

The ABTS inhibition activity of prepared bitter gourd chips is presented in Fig.2.The ABTS inhibition activity of chips stored at 100% N_2 concentration was found to show more inhibition than the chips stored at other concentrations of MAP gases. The ABTS inhibition activity was found to be lower than DPPH inhibition activity at same conditions. The analysis indicated that there was a significant difference in the ABTS inhibition activity among bitter gourd chips at all three concentrations of MAP gases.

Total phenolic content

Bitter gourd chips stored at 100% N_2 concentration showed less loss of total phenolic content when compared to chips stored at other concentration of gases (Fig.3).

Table.1 Microbial counts (total bacterial count, total fungal count and coliform count ($cfug^{-1}$) of modified atmosphere packed bitter gourd chips during storage

Storage Time (Days)	Total bacterial Count (10^{-2}×cfu/g)	Total Fungal Count (10^{-2}×cfu/g)	Coliform count
0	0.5	2	Nil
7	1.3	3	Nil
21	1.8	6	Nil
30	2.5	7	Nil

Fig.1 DPPH Inhibition Activity (%)

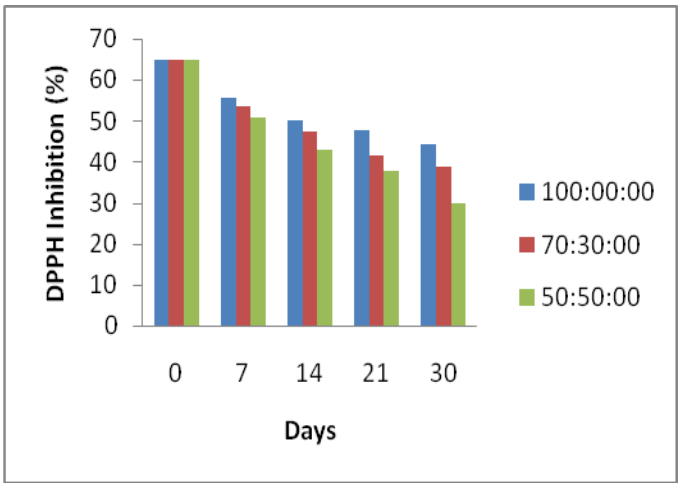

Fig.2 ABTS Inhibition Activity (%)

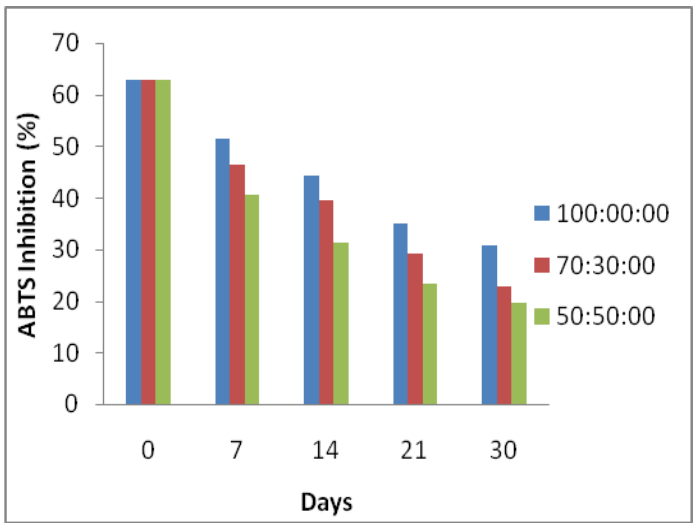

Fig.3 Total phenolic content (mg/g)

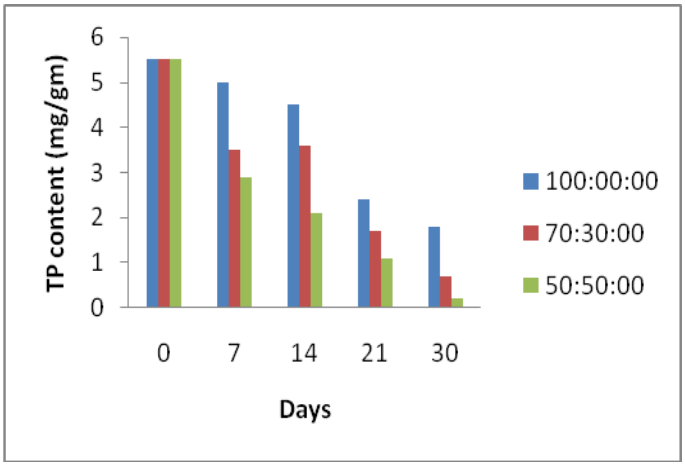

Fig.4 MAP stored Bitter gourd Chips

Decreasing temperature of processing was also found to preserve 80-100% of phenolic content in some vegetables (Roy *et al.*, 2007). Semi drying of tomatoes was found to lower the phenolic content by 30%, but drying of pepper gave contradicting results (Toor and Savage, 2006).

Changes in microbial count of optimized product

All the samples were subjected to microbiological analysis for total plate count (TPC), yeast and mold count (YMC) and coliform count. The TPC was determined by surface spreading the homogenate with appropriate dilutions (10^{-2}) on plate count agar (PCA) and incubated at 37 °C for 24-48 h. For yeast and mold detection, appropriate dilutions (10^{-2}) of sample was spread on potato dextrose agar (PDA) and incubated at 25 °C for 24-48 h. Coliforms in the samples were estimated by plating appropriate dilutions (10^{-2}) on Violet Red Bile Agar (VRBA) before being incubated at 37 °C for 24-48 h (AOAC, 2000). The mean total

bacterial count (TBC) of optimized product increased from 0.5 to 2.5×10^{-2} cfu/g during storage. It can be observed that the viable count for MAP packed bitter gourd chips increased from 2×10^{-2} to 7×10^{-2} cfu/g. The mean *coliform* count of optimized product was found to be nil during storage (Table 1).

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