

Effect of storage on physiochemical parameters and aflatoxin production in non-roasted chilli powder in local markets

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Abstract

Chilli (*Capsicum annum*) is used worldwide in seasoning, coloring and flavoring food. Chilli powder tends to degrade its physio-chemical composition considerably with storage. This study considered changes of physio chemical parameters during storage of dried *Capsicum annum* powder. The objective was to find the storage conditions which the deterioration of physio chemical parameters can be slackened. The parameters considered were moisture content, pH, color, ascorbic acids, total phenolics, antioxidant activity and total ash content of non-roasted chilli powder. Aflatoxins presence was also monitored qualitatively. Room temperature and average supermarket temperature were provided with three light settings; normal, direct and without light condition, providing six combined storage conditions for four months. The results depicted that favorable physio-chemical parameters given which were kept under different environmental condition such as moisture content of 7.52% gives in storage, ash contents around 6.8%, pH of 4.6, ascorbic acid amount of 41.48 mg 100 g⁻¹ and total phenolics of 48.02 mg 100 g⁻¹ (DW). The ASTA color value was 252.8 units. The mean IC50 value was found to be 2.28 µg 100 g⁻¹. The significant different is visible among treatment combinations of light condition, temperature and also between chilli powder packets from large scale producers (LS₁, LS₂ and LS₃) and small scale producers (SS₁, SS₂ and SS₃). Accordingly, the storage with no light condition at temperature of 22 – 24 °C was concluded as the best storage condition which preserves the most quality attributes such as color, moisture content, ascorbic acid content, ash content, pH and gives required

acidic range. Aflatoxin generation has not accelerated with given storage conditions during the study period.

Key words: Chilli powder, Mycotoxin, Physio-chemical parameters, Storage

Introduction

Chilli (*Capsicum annum*) is an important cash crop (Family Solanaeaceae), where the pods of the plant are used most prominently as a spice in both raw and dried forms processed as pieces or in powdered form according to different regional food cultures. Chilli is valued mostly for its pungency and intense color associated as a spice. Also the dried pepper in the red, ripen stage is globally commercialized and valued for its health and sensory attributes. In traditional Sri Lankan cuisine-dried chilli powder plays a crucial role as a spice and that is prepared by drying ripened red *C. annum* pods and then by grinding and pulverizing them using roller grinders. Very little information is available about the stability and conservation of physio-chemical features of chili during the post-harvest storage and after processing them as red chilli powder. Hence, assessing the processed products during their storage time and environment in order to identify the consequences related with the nutritional value of red chilli powder is important. Chilli powder is very sensitive to light, heat and oxygen level fluctuations of the storage environment (Iqbal *et al.*, 2015). Also Iqbal *et al.* (2015) has clearly stated that storage conditions significantly affect and deteriorate overall physio-chemical properties of dry chilli under cold stores and ambient room temperature conditions along with time. in SLS 1563:2017, SLS 186:part 5, the generally accepted moisture level for chilli powder should is 10-11% moisture contents lower than 8% should accelerate pigment destruction (Singh and Ojha, 1974).When the value exceeds 11%, it might favor the potential fungal growth and even Aflatoxin generation (Hammami *et al.*, 2014). Hence, chili powder should maintain a favorable moisture level lesser than 13% and should be stored in cool conditions avoiding intense light (Mamun *et al.*, 2016). Similarly, part 4 in SLS: 186 indicate that maximum ash percentage by mass on dry basis is 8.0 along with acid insoluble ash limit of 1.3% by mass. During the chilli drying procedure, the pH values reduces compared to that in fresh chilli and the total acidity increases (Manjula and Ramachandra, 2014). With the time, due to microbial contamination a significant

reduction of pH could occur. Normally pH value of all dried chilli varied between 3.21 and 4.84 (Manjula and Ramachandra, 2014) and the accepted range should be 4.97 to 6.17 for fresh chilli peppers.

Chilli pepper are well known due to their richness in vitamin A, D, E and mostly of vitamin C/ Ascorbic acid (Chigoziri and Ekefan, 2013; Igwemmar *et al.*, 2013). Fresh green chilli pepper contains more vitamin C than citrus fruits and fresh red chillies are said to have more vitamin A than carrots (Chigoziri and Ekefan, 2013). Ascorbic acid is instrumental in neutralizing free radicals as well as done by antioxidants and phenolics. When the dried and processed chili powder stored during a study conducted for four months period, Ascorbic acid levels have shown a significant deduction from 35% by its original level (Daood *et al.*, 1996). Also the rate of reduction has increased during storage (Iqbal *et al.*, 2015). This is similar in the case for antioxidants and total phenolics (Iqbal *et al.*, 2015) as these groups are very sensitive to temperature during storage.

Aflatoxins are toxic metabolites produced as mycotoxins by different toxigenic fungi of genus *Aspergillus*. Aflatoxin contamination in chilli powder is especially highlighted in most cases, but studies on physio-chemical features along with qualitative analysis on aflatoxin generation has not been widely carried out. Ayidin *et al.* (2007) has shown that fungal contamination of chillies begins to occur during the harvesting phase, and poor post-harvest handling and storage promotes Aflatoxin formation. Out of the main crops which subjected to *Aspergillus* fungal attack, red chilli pepper takes the lead with contaminations of *Aspergillus* and *Alternaria* species, which generates Aflatoxin.

Hence, the present study was designed to fill the gap in the chilli powder industry regarding the effect of various storage conditions and the retention of its original physio chemical characteristics. Thus, the study investigated how the bio-active compounds and physio-chemical constitution of chilli powder vary during storage under different storage conditions. Generation of Aflatoxin due to fungal contamination was also evaluated during the storage.

Materials and methods

Sample selections

The samples of non-roasted chilli powder were obtained from local retail shops in Kandy district. Accordingly, 50 g packets with original packaging bearing the same batch number were collected from three large scale producers (standard production procedures and machineries are used for dehydration chilli pods and for grinding) each representing LS_1 , LS_2 , and LS_3 others SS_1 , SS_2 and SS_3 from small scale producers (sun dried chilli pods are used for making chilli powder). For storage study a total of 6 packets were separately selected; *i.e.* Freshly arrived samples to the market of each producers in triplicate. Again another sample of freshly produced ones from each producer were collected in triplicate to get the initial readings of each parameter going to be tested.

Arrangement of storage conditions

Six controlled storage conditions were created involving three different light settings.

1. Direct light OSRUM LUMILUX Warm white 15W/830 - L_1
2. Normal day light condition and no any artificial lighting bulb used - L_2
3. No light and maintain dark condition inside the box - L_3

And two different temperature settings

1. 22 °C - 24 °C - supermarket conditions - T_1 and
2. 27 °C - 29 °C ambient room temperature conditions in Kandy district - T_2

Table 1 depicts the storage conditions provided for chilli powder storage in four months period.

Table 1. Storage conditions provided to create the light and temperature combined effect

Lighting Temperature	Direct light L_1	Normal day light L_2	No light maintain dark L_3
Room Temperature (T_1)	Direct lighting and room temperature L_1T_1	Normal day lighting and room temperature L_2T_1	No light and room temperature L_3T_1
Supermarket temperature (T_2)	Direct lighting and supermarket temperature L_1T_2	Normal day lighting and supermarket temperature L_2T_2	No lighting and supermarket temperature L_3T_2

The moisture and ash contents, extractable color changes, ascorbic acid content total phenolic content, antioxidant activity, pH values and the aflatoxin presence was observed using following methods.

Extractable color determination

The extractable color was measured according to ASTA 20 method using 1 mg of chilli powder mixed with 10 mL of Acetone, shaken and kept in the dark for 4 hours. An aliquot of the transparent decanted extract was taken. The absorbance of the solution was measured using a JENWAY 6300 UV/VIS spectrophotometer, set at 460 nm and calibrated with an acetone blank. ASTA 20 color units were calculated from the readings obtained.

pH values - Chilli powder samples, 5 g diluted with 10 mL of distilled water was used for measuring the pH value at ambient temperature with the pH meter (PHS-3C, London, UK) which was calibrated with pH 4 and 7 (AOAC, 2000).

Determination of ascorbic acid content- From the freshly prepared standard ascorbic acid solution, 5 mL was taken and to that, 5 mL of 3% HPO_3 was added and titrated with the 2,6-dichlorophenol-indophenol dye until a persistant pink color was observed for 15 seconds. Titre obtained here was used to determine the dye factor. Ascorbic acid was quantitatively determined according to the 2,6-dichlorophenol-indophenol AOAC official method 967.21. From the chilli powder samples, 10 g was measured and was blended with 2.5 mL of 3% metaphosphoric acid, and distilled water was then added up to the 100 mL mark. The blend was filtered with whatman no: 01 filter paper to remove the powder residue and 10 mL of the filtrate was titrated with freshly-prepared standard of 2,6-dichlorophenolindophenol dye (DCPIP) until a light, but a recognizable distinct rose pink color persisted for 15 sec., then the titre value was used in the final calculation.

Determination of total phenolic contents - The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure. From the samples, 1.5 g was measured and introduced into test tubes. Then, 12.5 mL of 80% methanol was added and kept in the shaker for 90 minutes before 100 μL of supernatant was extracted

carefully using a micro pipette in to a separate test tube. Then 150 µl of Folin-Ciocalteu's reagent along with 2.5 mL distilled water and 300 µl of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min.s in a dark place. Finally, absorption at 765 nm was measured (Jenway 6300, UV-vis spectrophotometer). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 1 g of dry material.

Moisture determination - The AOAC 2000 method was used for determining the moisture content using the hot air oven at a temperature of 105 °C for duration of 6 hours. Accordingly, 5 g of the sample was measured to a pre cleaned and dried glass petri dish and then was carefully placed inside the oven. After 06 hours, when the samples have reached a constant weight ± 0.01 g, they were again carefully placed inside the desiccator to cool and the equilibrated weights were taken immediately.

Ash content determination - Ash on dry basis was determined following the method described in AOAC 2000 where 5 g of samples was measured accurately in to pre weighted, clean and dried crucibles and were carefully placed in the muffle furnace at 550 °C. Then it was incinerated for 6-8 hours until it is free of black organic particles and was consist of white/grey ash. Then the crucibles were carefully removed from muffle furnace and allowed to cool in the desiccator. After cooling, the weights were taken and the process is repeated untill there is no further loss of weight is indicated.

Determination of the antioxidant activity - To 1 g of chilli powder, 10 mL of 80% methanol was added, and was allowed in the shaker for 90 minutes. From the supernatant, 1 mL was taken and mixed with 1 mL 80% methanol. Then, a five-fold serial dilution was carried out using 1mL of the preparation. Then for each test tube, 2.5 mL of a 0.004% methanolic solution of DPPH was added. After an incubation period of 30 minutes in darkness at room temperature (27 ± 2 °C), the absorbance was recorded against a blank at 517 nm (UV-6300, Jenway spectrophotometer). Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the control. Ascorbic acid was used as a positive control.

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For each set of five values obtained from the serial dilution of a single sample, percentage inhibition activity of DPPH was found using the following formulae;

$$\% \text{ DPPH inhibition activity} = \left[A_0 - \frac{A_1}{A_0} \times 100 \right]$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Using the trend line of dilution series, the IC₅₀ value which is the amount of antioxidant needed to inhibit 50 % of the DPPH amount was calculated under the prevailing antioxidant capacity.

Determination of Aflatoxin - Five grams of sample was spread evenly on a petri-dish, kept directly under Black light of UV lamp and observed for any fluorescence occurred. As the positive control, a peanut sample infected with *Aspergillus* spp. was used.

The experiment was conducted as a Complete Randomized Design. All the quality attributes were measured in triplicates for each treatment. Collected data were statistically analyzed by one way ANOVA using Minitab® 19.1 (64-bit) statistical software.

Results and discussion

Results and discussion In Table 2 to 7, data is given to express the variation pattern of means of each parameter in storage study of chilli powder under different light and temperature conditions with comparing the fresh chilli powder sample (FM).

Color

The mean ASTA color value ranged between 218.35- 252.8 of stored samples and 199.57-270.92 of each producers samples (Table 2). Mean color value was significantly ($p < 0.05$) high in each large scale producers and low in small scale producers (Table 3). This is because of application of standard production methods such as dehydration of raw materials under specific light temperature conditions. Normal day light, direct light with room temperature and even under supermarket temperature with direct light combinations were recorded significantly higher loss of color with storage. This is because light and

temperature has a combined effect on the color value of chili powder during storage. Color value of samples stored at supermarket temperature without light condition was significantly ($p < 0.05$) higher than all the other storage conditions. In addition, no light preserved the color more than other two light conditions. This has to be thoroughly considered as color loss due to partial bleaching in storage in the chilli powder industry, because other than flavoring, chilli powder is majorly used as a colorant in cookery. This observation confirms the findings of Castaneda (2016) who reported that red spices tend to bleach faster under direct light exposure of fluorescent bulbs.

pH

As depicted in Tables 2 and 3 the pH values were ranged from 4.6-4.9 where there is no significant effect with storage and producer. It has been reported previously when conventional solar drying condition pH values are increased (Mangaraj *et al.*, 2001). Room temperature under normal day light condition facilitated increased pH values. This happens due to reduction of ascorbic acids and phenolic contents in chilli powder during storage. Similar effect could be seen with direct light exposure in the two temperature conditions tested. Under the supermarket conditions, even inside no light (dark) storage, pH values tend to remain at high levels compared to those recorded in normal room temperature under no light (dark) condition storage. Light has a major effect on deterioration of acidic components as ascorbic acid and phenolic acids in chili powder which explains the changes observed.

Ascorbic acid content

Amounts of ascorbic acids tend to decrease by extending storage durations under any storage conditions which was confirmed by occurring the maximum value of 41.48 mg 100 g⁻¹ in fresh sample and the minimum value of 7.88 mg 100 g⁻¹, in stored sample exposed to direct light under super market temperature (Table 2). Significantly ($p < 0.05$) the highest retention of Ascorbic acid was recorded in the samples from supermarket temperature without light (dark) storage. This verifies the acceleration of degradation of ascorbic acid due to increase of light condition. Daood *et al.* (1996) have also reported a significant reduction of Ascorbic acid during storage of chilli. Also samples from large scale producers (LS) are shown the less reduction of ascorbic acid than small scale producers (SS) samples (Table 3) due to the usage of machineries for dehydration of raw

material enhance the retention of color during storage.

Table 2. Color, pH and Ascorbic acid content of fresh and stored chilli powder under different light and temperature conditions

No.	Treatment	Color	pH	Ascorbic acid (%)
1	FM	242.84 ^b	4.81 ^a	41.48 ^a
2	L ₁ T ₁	218.35 ^d	4.90 ^a	22.05 ^d
3	L ₁ T ₂	224.14 ^c	4.85 ^a	7.88 ^c
4	L ₂ T ₁	220.66 ^{cd}	4.69 ^a	25.66 ^c
5	L ₂ T ₂	238.73 ^b	4.77 ^a	21.25 ^d
6	L ₃ T ₁	242.29 ^b	4.60 ^a	16.56 ^d
7	L ₃ T ₂	252.8 ^a	4.77 ^a	33.86 ^b

Means with the same letters within a row are not significantly different ($p < 0.05$).

Abbreviations: FM-Fresh market sample, L₁T₁-direct light and room temperature, L₂T₁-normal day light and room temperature, L₃T₁- no light and room temperature, L₁T₂-direct light and supermarket temperature, L₂T₂-normal day light and supermarket temperature, L₃T₂-no light and supermarket temperature.

Table 3. Color, pH and Ascorbic acid content from different large scale (LS) and small scale producers

No	Producers	Color	pH	Ascorbic acid (%)
1	LS ₁	240.97 ^c	4.80 ^a	24.82 ^b
2	LS ₂	251.28 ^b	4.66 ^a	23.92 ^b
3	LS ₃	270.92 ^a	4.67 ^a	30.96 ^a
4	SS ₁	238.07 ^c	4.81 ^a	22.29 ^c
5	SS ₂	199.57 ^d	4.79 ^a	24.71 ^b
6	SS ₃	204.74 ^d	4.90 ^a	20.51 ^d

Abbreviations: LS₁, LS₂ and LS₃ are samples from large scale producers and. SS₁, SS₂ and SS₃ are small scale producers

Phenolic content

According to the table 4, when the phenolic contents were considered, the mean value of market sample was 30.44 mg g⁻¹. However, the value significantly ($p < 0.05$) increased with storage conditions. Thus, the minimum value of 32.74 mg g⁻¹ was

recorded in sample obtained under normal light with supermarket temperature where the maximum value of 48.02 mg g⁻¹ was recorded in a sample without light (dark) condition under room temperature condition. Significantly second and third highest phenolic contents were recorded the samples with supermarket temperature and normal day light and direct light condition, respectively. The combined effect of storage temperature and light condition on total phenolic content has been retained after the experiment period. Accordingly, the supermarket temperature showed a higher retention of phenolics in the samples than the room temperature, suggesting that phenolic contents directly affected by the temperature. However, even under the cooler temperatures in supermarket condition, with no light condition tends to have lesser phenolics retained than in normal light condition. Irrespective of the temperature, darkened conditions tend to retained lesser phenolic contents sometimes. Chilli powder from small scale producers (SS) has retained significantly lesser phenolic content than that of large scale producers (LS) (Table 5). This is because production methodologies and storage types.

Moisture content

The mean moisture content of fresh market sample is 8.36 %. This is also verified by the significant ($P < 0.05$) minimum value in this study which is 7.52 % which is from a sample obtained under no (dark) light condition in super market storage. Significant ($P < 0.05$) maximum value is recorded as 11.92 % from the sample which has exposed to normal light under room temperature (Table 4). Super market temperatures and no light (dark) conditions are shown significant reduction of moisture content. The moisture retention is very essential within the standard limits in order to avoid partial bleaching and microbial growth accelerations. When the value exceeds 11%, it might favor the potential fungal growth and even Aflatoxin generation (Hammami *et al.*, 2014). No light (dark) conditions tend to maintain uniform temperature inside them for hot as well as for colder conditions. Table 5 shows that although there is a significant different among producers, moisture content align under $< 11\%$ category except one producer. For both temperature conditions where significant lower amount is seen under supermarket temperature than room temperature. Govindarajan *et al.* (1987) have stated that Chilli peppers and powders are well known as hygroscopic and thus, show constant fluctuations in moisture level with respect to the environment available. It has also been reported that

equilibrium moisture content depends on the material and air temperature (Singh and Ojha, 1974).

Table 4. phenolic content and moisture content of Fresh and stored chilli powder under different light and temperature conditions

No.	Treatment	Phenolic content (mg g ⁻¹)	Moisture Content (%)
1	FM	30.44 ^c	8.36 ^b
2	L ₁ T ₁	33.1 ^d	11.74 ^e
3	L ₁ T ₂	34.84 ^c	10.56 ^d
4	L ₂ T ₁	32.74 ^d	11.92 ^e
5	L ₂ T ₂	36.97 ^b	10.26 ^d
6	L ₃ T ₁	48.02 ^a	9.11 ^c
7	L ₃ T ₂	33.26 ^d	7.57 ^a

Means with the same letters within a row are not significantly different (p<0.05) Abbreviations are referred under footnote of table 3

Table 5. Phenolic content and Moisture content of different producers

No	Producers	Phenolic content (mg g ⁻¹)	Moisture Content (%)
1	LS ₁	41.02 ^b	9.69 ^b
2	LS ₂	34.94 ^c	9.61 ^b
3	LS ₃	49.32 ^a	11.17 ^d
4	SS ₁	31.39 ^d	9.15 ^a
5	SS ₂	27.75 ^f	9.95 ^c
6	SS ₃	29.33 ^e	10.01 ^c

*Means with the same letters within a row are not significantly different (p<0.05)
Abbreviations are referred under footnote of table 4*

IC 50 value

The majority samples in the storage have shown IC 50 value ranging within 1.49-5.77 µg 100 g⁻¹ in table 6. The minimum IC 50 value, hence having the significant (P<0.05) maximum antioxidant capacity of 2.28 µg 100 g⁻¹ recorded in the samples from normal light condition under room temperature. Second significant (P<0.05) higher

value goes with direct light and room temperature condition again. Therefore, there is a significant effect in deduction of the antioxidant capacity of these samples along with the storage duration, light and temperature. The lowest IC 50 value or the highest antioxidant capacity was seen in the sample from direct light exposure under room temperature. The light condition and IC 50 inhibition significantly depends on temperature and producer of chili powder (Table 6, 7). This indicates that the room temperature storage and the normal lighting conditions allow more retention of antioxidant capacity than the supermarket storage temperature at the same light exposures.

Heat and oxygen concentration factors have affected clearly on the producer relevance in retaining the antioxidant capacity of chili powder during storage. The results agrees with Iqbal *et al.* (2015) that the reduction of ascorbic acid, antioxidants and total phenolics has increased during storage, as these groups are very sensitive to temperature. Normal and direct light exposures show interesting variations in IC 50 value whereas direct light recorded a high range of IC 50 values, indicating the retarded antioxidant capacity during storage. Large scale producers maintained almost lower IC 50 values in storage condition (Table 7).

Ash content

Regarding the Ash amounts of the given sample under the storage study only, the mean Ash value is 6.49% and the samples have amounts of Ash values ranging from 5.91%-6.8%, and the ash contents did not change significantly among fresh and old samples of no light and supermarket storage and normal light and room temperature samples also those are significantly higher than other storage conditions (Table 6). As the Ash content in a given sample shows the amount of inorganic matter available along with sandy matter, the present results has shown that without light storage condition has favored retaining of Ash without binding them in to more organic chelators, such as the polyphenolic contents. Hence, significantly minimal ash values are observed under direct light and normal lighting conditions. Also significantly different is shown among ash contents in producers (Table 7).

Table 6. IC 50 and Ash content of Fresh and stored chilli powder under different light and temperature conditions

No.	Treatment	IC 50 ($\mu\text{g g}^{-1}$)	Ash Content (%)
1	FM	1.49 ^f	6.49 ^a
2	L ₁ T ₁	2.61 ^d	5.98 ^b
3	L ₁ T ₂	5.77 ^a	5.91 ^c
4	L ₂ T ₁	2.28 ^e	6.53 ^a
5	L ₂ T ₂	5.23 ^b	5.94 ^b
6	L ₃ T ₁	5.58 ^a	6.15 ^b
7	L ₃ T ₂	4.05 ^c	6.80 ^a

Table 7. Ash content and IC 50 value of different producers

No	Producers	IC 50 ($\mu\text{g g}^{-1}$)	Ash Content (%)
1	LS ₁	3.62 ^c	5.93 ^c
2	LS ₂	4.34 ^a	6.17 ^c
3	LS ₃	3.14 ^d	6.90 ^a
4	SS ₁	4.53 ^a	5.79 ^c
5	SS ₂	4.02 ^b	6.26 ^b
6	SS ₃	3.5 ^c	6.49 ^b

Means with the same letters within a row are not significantly different ($p < 0.05$).

Abbreviations are referred under footnote of table 4

Aflotoxin

No quantifiable levels of aflatoxin were formed during the storage time of 04 months. The short study period is not enough duration to produce a detectable proliferation of the fungal toxin within the samples under the controlled storage.

Conclusions

According to the obtained results, the storage conditions without light under supermarket temperature range, that is within 22 °C to 24 °C can be concluded as the best storage condition which preserves most of the analyzed parameters which represent

both consumer acceptability and the quality of chilli powder as mainly the Color, pH values within the required acidic range, moisture content, ash content and the ascorbic acid contents. The Antioxidant activity has shown preferably within a higher level when stored in normal light under room temperature. Therefore it can be concluded as from the above summarized deductions, Chilli powder has to be stored in opaque containers *i.e.* without light under 3 °C - 5 °C lesser temperature than the ambient temperature. Storage conditions studied has not enhanced the proliferation of *Aspergillus* spp. growth to produce detectable amounts of Aflatoxin during the study period. Chilli powders from large scale producers were given higher standard than small scale ones.

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