# Nutritional Composition and Antimicrobial Properties of Chili Pepper (Capsicum sp.) Leaves

**Riza Abilgos-Ramos, Amelia Morales, and Henry Mamucod**
*Rice Chemistry and Food Science Division*
*Philippine Rice Research Institute, Maligaya*
*Science City of Muñoz, Nueva Ecija,*
*Philippines*

Nutrient content and antimicrobial properties of fresh and powdered chili pepper (Capsicum sp.) leaves were evaluated. Collected leaf samples were washed, oven-dried, and crushed to a fine powder. Nutritional analysis of fresh chili pepper leaves revealed high -carotene (243.0 ± 21.2 µg/100 g), iron (6.9 ± 0.0 mg/100 g), calcium (550.0 ± 1.4 mg/100 g), and dietary fiber (6.30 ± 0.0 g/100 g) contents. Dried and powdered sample resulted in concentration of -carotene (32,151.0 ± 1067.7 µg/100 g), iron (34.4 ± 1.2 mg/100 g), calcium (2270.5 ± 6.3 mg/100 g), and dietary fiber (27.3 ± 1.4 g/100 g). The water activity and moisture content of packed and stored for 10 dried and powdered leaves were <0.60 and 7.1 ± 0.0%, respectively. Chili pepper leaves showed good antimicrobial property against some pathogenic bacteria and fungi. Zone of inhibition was produced by ethanolic extract of chili pepper leaves against Staphylococcus aureus, Salmonella sp., Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Penicillium chrysogenum, and Fusarium oxysporum. Results showed that chili pepper leaves are a significant source of -carotene, iron, calcium, and dietary fiber that can be incorporated as a supplementing ingredient in different food products or preparations. Chili pepper leaves as a food ingredient are a substantial source of micronutrients. Dried and powdered form can be incorporated in many food preparations.

**Keywords:** antimicrobial properties, -carotene, calcium, chili pepper leaves, dietary fiber, iron

## INTRODUCTION

Plants are the main dietary sources of iron, vitamin A (in the form of β-carotene), and folate for people in the rural areas of developing countries. Being micronutrient-dense, incorporating them in food or dishes as ingredients is a way to ensure supply of essential nutrients in the diet. Green leafy vegetables are rich in micronutrients such as β-carotene, folate, and iron (Ng, Chye, & Ismail, 2012; Gupta & Prakash, 2011). By consuming these indigenous vegetables, nutritional security can be cheaply achieved (Gupta, Jyothi Lakshmi, Manjunath, & Prakash, 2005; Afolayan & Jimoh, 2009) and sustained.

Chili pepper (Capsicum sp.) is widely cultivated in the Philippines. With 787 hectares harvested to chili pepper in 2011, the average volume of production in the country is 1698 metric tons (BAS, 2012). As a popular ingredient in Asian cuisine, its leaves are usually added in a chicken ginger stew with unripe papaya. The green grassy herby aroma of chili pepper leaves (CPLs) gives the dish its distinct taste. Chili pepper leaves impart milder heat and pungency in foods in comparison with that of the fruit. A CPL contains a significant amount of β-carotene and iron even in cooked form (FNRI, 1997) and capsaicin ($C_{18}H_{27}NO_3$), which has therapeutic properties (Yaldiz, Ozguven, & Sekeroglu, 2010). A substantial amount of lutein and chlorophyll is also found in the leaf (Kim, Ahn, Lee, Moon, Ha, & Kim, 2011). With its good nutritional profile, a CPL is a good candidate for ingredients in different food preparations. However, because of the lack of information and appropriate technology, the utilization of CPLs is not fully maximized. This underutilized vegetable is highly perishable because of high moisture content. Without refrigeration, it gets easily damaged, wilted, or rotten right after harvest. The need to process this agricultural crop into a more stable and practical form is therefore necessary. This study was conducted to process CPLs into crushed and powdered forms using previously optimized oven-drying method and evaluate its nutritional profile in fresh and powdered forms, shelf life, and antimicrobial property.

## MATERIALS AND METHODS

### Collection and processing of chili pepper leaves into crushed and powdered forms

Fresh CPLs (var. Taiwan hybrid) were collected from Sto. Domingo, Nueva

Ecija, Philippines. A total of 11 kg of fresh leaves were thoroughly washed under running water in the laboratory. Unhealthy leaves were discarded. The leaves were rinsed twice with distilled water, laid in trays, and air-dried for several minutes. After which, the leaves were oven-dried at 40 °C for 12 h. One part of the dried leaves was crushed manually to produce crushed form. The other part was crushed and passed through a 425-μm mesh sieve to obtain powdered form.

## Analysis of nutrients in fresh and powdered CPL

Nutritional composition was determined by measuring the moisture content (MC), dietary fiber (DF), β-carotene, vitamin C, calcium, and iron content of fresh and powdered chili pepper leaves. Calcium and iron were determined by dry ashing and measured by inductively coupled plasma spectrometry (AOAC 2005 method 927.02 and 999.11). β-Carotene was extracted through saponification and quantified by high performance liquid chromatography (AOAC 2005 method 941.15). Total dietary fiber was measured by enzymatic-gravimetric (AOAC 2005 method 992.16) and vitamin C by Luff-Schoorltitrimetry (AOAC 2005 method 967.21).

## Moisture content determination

The MC of the sample was determined by oven-drying method. Approximately 1.0 g of sample was weighed in tared aluminum pan. The pan was placed in an oven at a temperature of 105 °C for 5 h. After drying, the samples were removed from the oven and placed into a dessicator for 30 min. After which, the samples were weighed and placed back again into the oven until constant values were obtained. Percent moisture was calculated as:

$$\% \text{ Moisture} = \frac{\text{wt. of sample before drying} - \text{wt. of sample after drying}}{\text{wt. of sample before drying}} \times 100$$

Determination of physicochemical properties and microbial load

## $A_w$ measurement

The $A_w$ of the samples was determined using a barium chloride-calibrated LufftDurotherm Wert-Messer No. 5803 Aw meter. Around 5.0 g of sample was placed and spread evenly to cover the bottom of the sample container.

The sensor head was secured tightly and was left for 2.5 h at 24 ± 1 °C. Reading was done by viewing the meter of the sensor head.

### Microbial load evaluation

Total plate and mold counts were monitored monthly by pour plate technique (Fernandez, Dalmacio, Raymundo, Zamora, & Mendoza, 2008). One gram of the sample was mixed with 9 mL of 0.1% peptone solution. Successive dilutions were made by transferring 1 mL of the suspension medium to 9 mL of 0.1% peptone solution. One milliliter of the diluted samples was transferred in petri plates and poured with Standard Plate Count Agar for total plate count and Potato Dextrose Agar (PDA) for mold count determinations. Plated samples were incubated at 30 °C, and colonies were counted after 24 to 48 h.

### Shelf life determination

The crushed and powdered chili pepper leaves were stored in sealed 0.07-mm PE and 0.07-mm aluminum-coated bags and kept at ambient temperature (26–28 °C). The moisture content, water activity, and microbial load were monitored every other month up to 10 months of storage.

## ANTIMICROBIAL PROPERTY

### Plant extraction

About 33.31 g of powdered leaves were soaked with 500 mL of 80% ethanol for 48 h. The sample was filtered under suction through ordinary filter paper using a Buchner funnel. The supernatant was concentrated through rotary vacuum evaporator at 60 °C and 70 cm Hg to remove the ethanol. The concentrated extract was stored in amber bottle at 4 °C until analyzed.

### Test microorganisms

Foodborne and/or human pathogens such as Escherichia coli, Pseudomonas aeruginosa, Salmonella spp., Bacillus subtilis, and Staphylococcus aureus were used as bacterial test microorganisms and maintained in Nutrient Agar (NA) slants. Whereas, Aspergillus niger, Fusarium oxysporum, and Penicillium

chrysogenum were used as fungal test microorganisms and maintained on PDA slants. The cultures were obtained from the National Collection of Microorganisms of the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna, Philippines.

## Antibacterial activity

Antibacterial activity of the CPL extract was evaluated by paper disc diffusion method (Doughari & Pukumams, 2007). Filter paper discs (6 mm) were dipped into CPL extract for about 10 sec, and excess extract was drained off. A disc soaked in 80% ethanol was also prepared for the negative control. The discs were then oven-dried at 30 °C for about an hour. For the meantime, 24-h bacterial cultures were adjusted to 0.5% McFarland standard and inoculated onto NA plates. The disc which approximately contained 10 to 15 µL of extract was placed and gently pressed down onto the surface of the inoculated NA plates. Paper discs of standard antibiotics namely: 10-µg ampicillin, gentamycin, and streptomycin and 30-µg tetracycline were used as the positive control. The plates were then incubated upside down at 37 °C, and the zone of inhibition around each paper disc was measured with a ruler after 24 h.

## Antifungal activity

Antifungal property of CPL extract was evaluated following the method of Ferdes, Ungureanu, Radu, and Chirvase (2009) with some modifications. A loopful of 48-h fungi was inoculated at the center of the acidified PDA plate and then incubated upside down at 30 °C for 48 h. For the meantime, 1.0% of CPL extract was mixed into acidified PDA (experimental), then distributed in plates and was allowed to solidify. The PDA plate without extract was served as the control. A piece of peripheral fungal growth from the acidified PDA plate was obtained and transferred in the experimental and control plates. The plates were then incubated at 30 °C, and the colony diameter was measured using a ruler after 5 days. Percent inhibition ratio was then calculated as:

$$\text{Inhibition ratio (\%)} = \text{colony diameter in control plate} - \text{colony diameter in experimental plate} \times 100 \text{ colony diameter in control plate}$$

## STATISTICAL ANALYSIS

All analyses were conducted in duplicates. The ANOVA and subsequent comparison of means using Tukey's HSD (honestly significant difference) were determined using SAS statistical software v. 9.1 (SAS Institute, Cary, NC, USA) at $p < 0.05$.

## RESULTS AND DISCUSSION

Percent recovery of powdered and crushed CPL from fresh sample is presented in Table 1. The high MC (75.41%) of the fresh leaves resulted in considerably low recovery of dried leaves. Only 24.81% of crushed and 21.15% of powdered sample were produced from fresh leaves.

Table 2 presents the baseline nutritional profile of fresh and powdered CPL. The drying process resulted in concentration of nutrients of CPL. β-Carotene content was 32,151.0 µg/100 g which will have beneficial effect on vitamin A status if fat (such as cooking oil) will be used in food preparation as fat enhances carotenoid absorption for bioconversion to vitamin A (Jayarajan, Reddy, & Mohanram, 1980). Addition of 0.5% powdered chili pepper leaves in salt bread showed significant increase in folate, iron, and β-carotene (Abilgos-Ramos, Manaois, Morales, & Mamucod, 2015). High beta-carotene from green leafy vegetables (drumstick leaves) was observed to reduce total cholesterol and control triglyceride levels in rats (Oinam, Urooj, Phillips, & Niranjan, 2012). Vitamin C in powdered CPL was 28 mg/100 g, calcium was 2,270.5 mg/100 g, and iron was 34.4 mg/100 g. Dietary fiber content (27.3 g/100 g) was also considerably high. The high amount of DF is helpful in lowering low-density lipoprotein and total cholesterol levels, prevention of cardiovascular diseases, and improvement in digestive health. From these results, a CPL as dietary source of minerals and vitamins is of particular importance. However, the high fiber content may affect the bioavailability of the iron unfavorably (van Jaarsveld, Faber, van Heerden, Wenhold, van Rensburg, & van Averbeke, 2014). Nevertheless, chili pepper leaves in powdered form can be used as supplementing ingredient in food products or preparations with little nutritional value (e.g., rice crackers).

Initial MC of crushed CPL was significantly higher than that of powdered form. However, on the 8th until 10th month of storage, the MCs of the samples were comparable regardless of form and packaging material

(Table 3). Surprisingly, increase in moisture content from 3rd-to-8th-month storage was noted in all samples which can be explained by permeability of PE material used but needs further investigation for samples packed and stored in aluminum-coated material. The Aw of all samples generally remained stable throughout the storage time with only slight changes on the 2nd until 6th month of storage. This indicates good product storability (Table 4) as dried products with MC ranging from 5 to 15% and Aw below 0.6 have good storage stability and are less susceptible to microbial spoilage (deMan, 1999).

In terms of microbial load, powdered samples had significantly lower initial total plate and mold counts compared with crushed samples. As the storage time progressed, no significant trend was observed between the treatments. However, the microbial counts of all samples significantly dropped after two months of storage (Figures 1 and 2). Based on the standards of the World Food Program of the United Nations (UN, 2009), the values were within acceptable limits (<10 × 104 cfu/g for TPC and bacterial count and <1000 cfu/g for mold count). Low MC and Aw might have been responsible for the low microbial growth. Also, Fernando, Ligia, Mayra, and Ignacio (2011) reported that CPLs contain antimicrobial peptides (AMPs) that might have inhibited microbial growth during storage. The AMPs are small rich cysteine peptides with biological activities of inhibiting bacterial and fungal growth.

In order to evaluate the antimicrobial activity of CPL, ethanolic extract of the leaves were screened against common foodborne pathogenic bacteria and fungi. The highest inhibition (14.75 mm) produced by CPLs was observed against Staphylococcus aureus. On the other hand, the least inhibition (8.50 mm) was seen against Bacillus subtilis. The inhibitory property of CPLs was comparable with that of streptomycin against Pseudomonas aeruginosa (Table 5). Cichewicz and Thorpe (1996) found that the plain and heated extracts of Capsicum species were also found to exhibit varying degrees of inhibition against Bacillus cereus, B. subtilis, Clostridium sporogenes, Clostridium tetani, and Streptococcus pyogenes. In terms of fungal inhibition, CPLs had a significant effect against Penicillium chrysogenum (37.61%). The least inhibitory effect (1.62%) was observed against Aspergillus niger (Table 6).

## CONCLUSION

Processing of CPLs into dried and powdered supplement resulted in concentration of its nutrients and product stability. Results also showed that CPLs had comparable nutritional value (vitamin C, calcium, iron, dietary fiber, and β-carotene) with the more popular moringa leaves. Both aluminum-coated and polyethylene plastic pack containers provided good storability of crushed and powdered CPL in terms of MC, Aw, and microbial load for 10 months. However, CPLs showed moderate antimicrobial property against certain pathogenic bacteria. The use of other solvents for CPL extraction and increase in the concentration for antimicrobial property must be investigated. This study provides evidence that CPLs are a significant source of micronutrients such as iron, β-carotene, and calcium. With this information, chili pepper leaves can be used as a supplementing ingredient in food products or preparations. Development of nutritional information per serving size of the developed supplement is recommended. The market potential of the powdered product must also be considered to determine the consumer perception and acceptance of the product. The use of other nutrient-dense green and leafy vegetables such as alugbati (Basella alba) as supplement must also be explored. Increased utilization of CPLs can boost its agricultural production and may subsequently improve the economic status of farm families.

## ACKNOWLEDGMENT

The authors are grateful to Mr. Ramon Garcia for the chili pepper leaves.

## REFERENCES

Abilgos-Ramos, R.G., Manaois, R.V., Morales, A.V., & Mamucod H.F.. (2015). Quality characteristics and consumer acceptability of salt bread supplemented with chili pepper (Capsicum sp.) leaves. *Food Science and Technology Research 21*(1) 117–123.

Afolayan A.J. & Jimoh, O.F. (2009). Nutritional quality of some wild leafy vegetables in South Africa. *International Journal of Food Science and Nutrition 60*(5) 424–431.

[AOAC] Association of Official Analytical Chemists (2005). Official Methods of Analysis, 18th ed., Washington DC, USA: Method no. 927.02, 925.10, 967.21, 985.35, 985.29, 991.42, 992.16, 999.11.

Cichewicz R.H. & Thorpe P.A. (1996). The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. *Journal of Ethnopharmacology* 52: 61–70.

deMan, J.M. (1999). Principles of Food Chemistry. 3rd Edition. Aspen Publishers, Inc. Gaithersburg, Maryland, USA.

Doughari, J.H. & Pukumams, D.E.N. (2007). Antibacterial effects of Balanites aegyptiaca L. Drel. and Moringa oleifera Lam. on Salmonella typhi. African *Journal of Biotechnology 6*(19) 2212–2215.

Ferdes M., Ungureanu, C., Radu, N., & Chirvase, A.A. (2009). Antimicrobial effect of Monascus purpureus red rice against some bacterial and fungal strains. New Biotechnology 25 S 1.

Fernandez, W.L., Dalmacio, I.F., Raymundo, A.K., Zamora, A.F., & Mendoza, B.C. (2008). Laboratory Manual in General Microbiology. University of the Philippines Los Baños, Laguna 3rd ed.

Fernando, M.S., Ligia, B.A., Mayra, D.B., & Ignacio, I.F. (2011). A review of a promising therapeutic and agronomical alternative: Antimicrobial peptides from Capsicum sp. *African Journal of Biotechnology 10*(86) 19918–19928.

[FNRI] Food and Nutrition Research Institute (1997). The Philippine Food Composition Tables. Department of Science and Technology. Metro Manila, Philippines 4, 41p.

Gupta, S., Jyothi Lakshmi, A.J., Manjunath, M.N., & Prakash, J. (2005). Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT — Food Science and Technology 38339-345.

Gupta. S & Prakash, J. (2011). Nutritional and sensory quality of micronutrient-rich traditional products incorporated with green leafy vegetables. *International Food Research Journal 18* 667–675.

Jayarajan, P., Reddy, V, & Mohanram, M. (1980). Effect of dietary fat on absorption of β-carotene from green leafy vegetable in children. *Indian Journal of Medical Research 71*, 53–66.

Kim, J.S., Ahn, J., Lee, S.J., Moon, B., Ha, T.Y., & Kim, S., (2011). Phytochemicals and antioxidant activity of fruits and leaves of paprika (Capsicum Annuum L., var. Special) cultivated in Korea. *Journal of Food Science 76* (2).

Ng, X.N., Chye, F.Y., & Ismail, A.M. (2012). Nutritional profile and antioxidative properties of selected tropical wild vegetables. *International Food Research Journal 19*(4) 1487–1496.

Oinam, N., Urooj, A., Phillips, P.P., & Niranjan, N.P. (2012). Effect of dietary lipids and

drumstick leaves (Moringa oleifera) on lipid profile and antioxidant parameters in rats. Food and Nutrition Sciences 3: 141–145.

United Nation International Children Emergency Fund (UNICEF). 2003. Vitamin and mineral deficiency: A global damage assessment report.

van Jaarsveld, P., Faber, M., van Heerden, I., Wenhold, F., van RENSBURG, W.J., & van Averbeke, W. (2014). Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. *Journal of Food Composition and Analysis 33*, 77–84.

Yaldiz, G., Ozguven, M., & Sekeroglu, N. (2010). Variation in capsaicin contents of different Capsicum species and lines by varying drying parameters. Industrial Crops and Products 32: 434–438.

**Table 1.** Percent recovery of crushed and powdered chili pepper leaves.

| Form of Chili Pepper Leaves | Recovery after drying | |
|---|---|---|
| | Weight (g) | Percent (%) |
| Oven-dried | 57.97 | 28.99 |
| Crushed | 49.61 | 24.81 |
| Powdered | 42.30 | 21.15 |
| Fresh (initial weight) = 200 g | | |

**Table 2.** Nutritional composition of fresh and dried chili pepper leaves.

| Analytes | Form of Chili Pepper Leaves | |
|---|---|---|
| | Fresh | Dried/powdered |
| Moisture, % | 75.71 ± 0.24 | 7.14 ± 0.04 |
| Dietary fiber, g/100 g | 6.30 ± 0.04 | 27.27 ± 1.41 |
| Beta Carotene, µg/100 g | 243.00 ± 21.21 | 32,151 ± 1067.73 |
| Vitamin C, mg/100 g | 3.00 ± 0.01 | 27.62 ± 0.04 |
| Calcium, mg/100 g | 550.00 + 1.41 | 2270.50 ± 6.36 |
| Iron, mg/100 g | 6.87 ± 0.01 | 34.36 ± 1.22 |

**Table 3.** Moisture content (%) of crushed and powdered chili pepper leaves during storage.

| Form of CPL and Packaging Material | Month of Storage | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Crushed, Aluminum coated | 7.44a,C | 7.23ab,C | 8.62b,B | 8.81c,AB | 8.99a,A | 6.65a,D |
| Powdered, Aluminum coated | 7.12b,B | 6.85cb,B | 8.27c,A | 8.47d,A | 8.90a,A | 6.61a,B |

| | | | | | | |
|---|---|---|---|---|---|---|
| Crushed, PE | 7.44a,C | 7.51a,C | 9.04a,B | 9.35a,A | 9.37a,A | 7.10a,D |
| Powdered, PE | 7.12b,C | 7.10bcb,C | 8.69b,B | 9.06b,AAB | 9.14a,A | 6.91a,C |

Mean values with different small letters in the same column and capital letters in the same row are significantly different ($p < 0.05$). n = 2.

**Table 4.** Aw of crushed and powdered chili pepper leaves during storage.

| Form of CPL and Packaging Material | Month of Storage | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 |
| Crushed, Aluminum coated | 0.45a,AB | 0.51c,AAB | 0.56a,A | 0.55ab,A | 0.54a,A | 0.56a,A |
| Powdered, Aluminum coated | 0.42a,AB | 0.54b,A | 0.57a,A | 0.54ab,A | 0.54a,A | 0.54a,A |
| Crushed, PE | 0.45a,A | 0.50c,A | 0.56a,A | 0.57a,A | 0.56a,A | 0.56a,A |
| Powdered, PE | 0.42a,AB | 0.57a,A | 0.52a,A | 0.53b,A | 0.58a,A | 0.59a,A |

Mean values with different small letters in the same column and capital letters in the same row are significantly different ($p < 0.05$). n = 2.

**Table 5.** Zones of inhibition produced by chili pepper leaves and reference antibiotics against commonpathogenic bacteria.

| Test Bacteria | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | CPL | Tetracycline | Gentamycin | Streptomycin | Ampicillin |
| | extract | (30 ug) | (10 ug) | (10 ug) | (10 ug) |
| Bacillus subtilis | 8.50c,D | 39.75b,B | 32.00a,C | 28.75a,C | 46.75a,A |
| Escherichia coli | 12.75b,C | 46.25a,A | 30.00a,B | 31.25a,B | 44.00ab,A |
| Pseudomonas aeruginosa | 12.25b,C | 50.50a,A | 31.00a,B | 17.00c,C | 46.25a,A |
| Staphylococcus aureus | 14.75a,D | 36.75b,B | 30.00a,C | 30.00a,C | 49.00a,A |
| Salmonella sp. | 12.75b,D | 36.00b,A | 29.75a,B | 23.00b,C | 37.75b,A |

Mean values with different small letters in the same column and capital letters in the same row are significantly different ($p < 0.05$). n = 2.

**Table 6.** Percent inhibition of chili pepper leaves against common pathogenic fungi.

| Test Fungi | Average Colony Diameter (mm) | | Inhibition (%) |
|---|---|---|---|
| | control | experimental | |
| Aspergillus niger | 46.25 | 45.00 | 1.62b |
| Fusarium oxysporum | 21.00 | 16.50 | 21.50ab |
| Penicillium chrysogenum | 13.25 | 8.25 | 37.61a |

Mean values with different small letters in the same column and capital letters in the same row are significantly different ($p < 0.05$). n = 2.

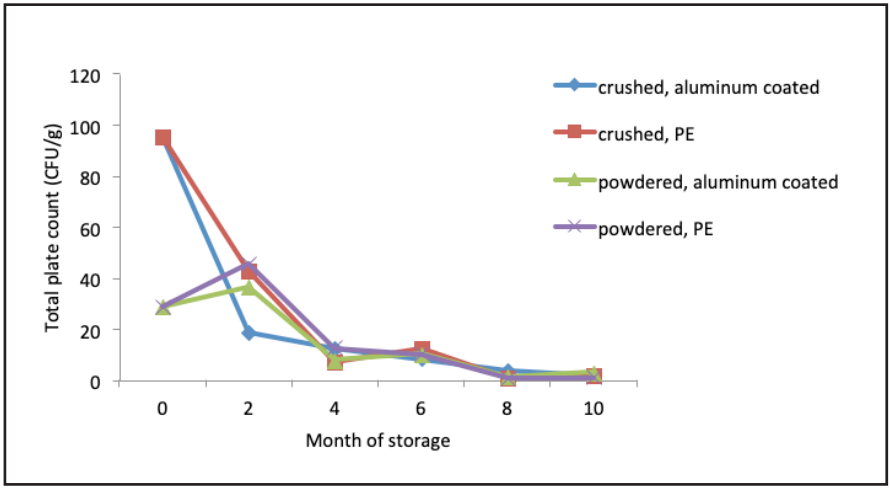


**Figure 1.** Total plate count of chili pepper leaves during storage (cfu/g).

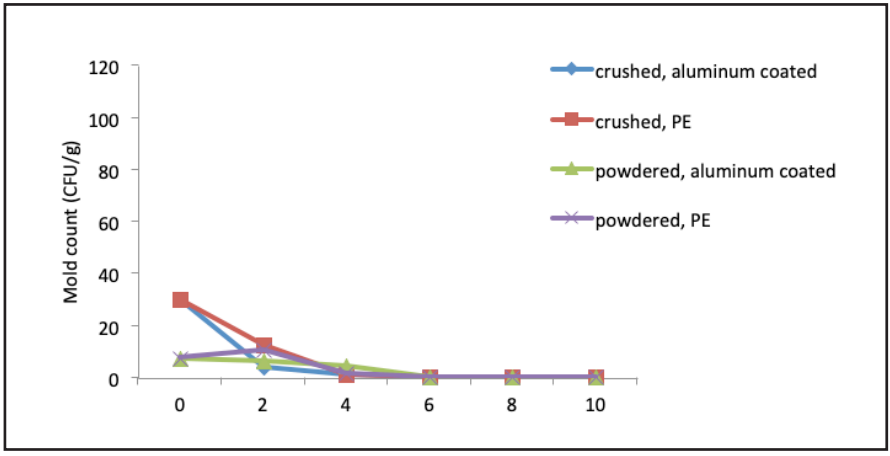


**Figure 2.** Mold count of chili pepper leaves during storage (cfu/g).