Changes in Phytochemicals and Antioxidant Properties of Kaffir Lime Leaves under Chilling Storage

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ABSTRACT: Kaffir lime leaves (*Citrus hystrix* D.C.) is a herbalproduct which rapidly deteriorates after harvesting at ambient temperature and making it to have a short shelf life. Normally, low temperature storage is used to extend the shelf life of wide ranges of agricultural produce. The present study stored the kaffir lime leaves under cold storage with and without package and checked their phytochemical and antioxidant qualities every 3 days of interval and for a period of 21 days. Perforated polypropylene packages were used in this study. During the shelf life study, the data pattern showed a significant change in the kaffir lime leaves. A continuous decrease in the chlorophyll and ascorbic acid content was found in the kaffir lime leaveswhereas a gradual increase in the total phenolic content and total flavonoid content was observed. Furthermore, a steady increment in the antioxidant (Ferric reducing antioxidant power assay (FRAP)) and radical scavenging (2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)) activitieswas observed in the kaffir lime leaves. Overall, this study observed that leaves stored under packaging were slightly better in qualities as compared to control samples.
Keywords: Kaffir lime leaves, Cold Storage, Package, Phytochemical, Antioxidant activities

Introduction

Kaffir lime (*Citrus hystrix D.C.)* leaf is an aromatic herb that is used extensively to add a distinctive aroma and flavor to food. It is widely used in Thailand, and also it can be found in other countries in South East Asia especially Laos, Indonesia, Malaysia, and Vietnam. It is also served as a natural medicine to cure various diseases including heart disease, dizziness, and indigestion and it also applied topically to keep the skin nourishment (Raksakantong et al., 2016). Kaffir lime leaves have numerous phytochemicals and essential oils. The essential oils and volatile compounds play a vital role in the leaves for contributing it unique tangy flavor. α-pinene, camphene, β-pinene, limonene, copaene, linalool, β-cubebene, isopulegol, caryophyllene, citronellyl acetate, and citronellol are the major volatile compounds in the kaffir lime leaves (Kasuan et al., 2013). It is used either in the fresh or dry form and however, storing the fresh leaves under ambient conditions cause quality deterioration. A numerous processhas been applied to prolong the shelf life of various type of leaves.In Thailand, the kaffir lime leaves are mostly stored in the refrigerated conditions.

Kaffir lime leaves are grown widely in the tropical and subtropical regions of Thailand and one of the important drawbacks of horticulture

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produce in thoseregions is their sensitivity to cold climate. Prolonged storage might cause a chilling injury in the leaves and thus, accelerate the physiological and biochemical changes within the plants causing the loss of cellular integrity and leading to cell death. Kaffir lime leaves exposed to prolonged storage at 8°C cause chilling induced quality loss (Cozzolino et al., 2016). Packaging is one of the proven postharvest techniques that could increase the shelf life of perishable plant produce by limiting the gaseous environment and control the metabolic activity and water loss in plants under storage at various temperature conditions (Manolopoulou et al., 2010).The present study was aimed to examine the quality changes especially phytochemicals and antioxidant activities in the kaffir lime leaves during prolonged storage with and without packaging at 8°C.

Materials and Methods

Raw material and storage

Kaffir lime (*Citrus hystrix D.C.)* leaves were purchased from a local garden in Surat Thani province, Thailand. The matured leaves were carefully cut off from the stem end; the defected leaves were discarded. Then, the leaves were washed thoroughly in the tap water and then dipped them in the solution containing 100 mg/L sodium hypochlorite for 10 min and dried thoroughly of the moisture at ambient temperatures using an electric fan. After that, approximately 250 g of samples were put in the transparent perforated (4 holes/each side) polyethylene bags (30 cm x 20 cm). The bags were sealed with an electric impulse sealer. Samples put on a tray without packaging were used as a control. All the samples were stored for a period of 21 days at 8°C**,** and at every three days of intervals, the samples were measured for the following quality determinations.

Quality determinations

Chlorophyll content; the extraction and determination of chlorophyll content were carried out following the method of Bekhradi et al. (2015). The results were expressed as total chlorophyll content (mg per 100 g of fresh weight (FW)). Total phenolic content; the extraction and determination of total phenolic content in the leaves were measured in accordance with the method of Wongsheree et al. (2009). The results were expressed as mg gallic acid equivalent (GAE) per 100 g of FW. Total flavonoid content; the extraction and determination of ascorbic acid (AsA) content in the leaves were measured based on the method of Sulieman et al. (2015). The results were expressed as mg AsA per 100 g of FW. Antioxidant activities; the extraction and determination of FRAP, DPPH, and ABTS in the kaffir lime leaves were measured in accordance with the method of Venkatachalam et al. (2018). The results were expressed as percentages for DPPH and ABTS assay and mmol Fe^{2+} per 100 g of FW for FRAP assay.

Statistical analysis

The treatment and determination of samples were done in triplicate. The data were analyzed by one-way analysis of variance (ANOVA) with SPSS for Windows (V6). The significance of the difference among the treatments was measured using Duncan's Multiple Range Test (DMRT, with a level of significance of 0.05).

Results and Discussion

The change in total chlorophyll content in kaffir lime leaves stored under with and without packaging at low temperature is presented in Figure 1A. The prolonged storage conditions showed a decreased level of chlorophyll content in the kaffir lime leaves. However, the samples in the packages held up slightly more chlorophyll content in the kaffir lime leaves as compared to the control samples. Normally, the loss of chlorophyll content in the leaves isdue to chilling-induced dehydration of leaves. Yoon et al. (2011) also reported that the leaves stored under refrigerated condition could cause the dehydration and leads to the ceasing of photosynthesis. Artemio et al. (2002) reported that the cease of photosynthesis in the leaf is one of the sensitive response of plants to chilling temperature. Total phenolic content in the kaffir lime leaves tended to increase throughout the storage (Figure 1B). The results showed that leaves stored under packaging had a slightly lower level of total phenolic content as compared to the unpackaged samples. Normally, numerous factors can influence the total phenolic content in the plant produce, and the factors including temperature, water level, nutrient availability, cultivar, and maturity. Furthermore, the level of phenolics is also influenced by the phenylalanine ammonia lyase (PAL) enzymes, which is the precursor for phenolic synthesis in the plants. Several studies have reported that PAL is highly active under chilling conditions. This could be the reason that control samples which had direct exposure to chilling temperature could have accelerated the PAL enzyme and as a consequence, a slight increment in the phenolic level in the kaffir lime leaves. A similar trend was also observed in the total flavonoid contents (Figure 1C). Prolongedstorage gradually increased the flavonoid content in all the samples, and however, the level was observed slightly higher in the control samples compared to packaging. Junboon et al. (2013) reported low temperature storage could accelerate the phenolic metabolism in the plants and thus lead to higher accumulation of polyphenols. Figure 1D showed the changes in the AsA content in kaffir lime leaves during extended storage under with and without the package. The results found a continuous decrease in AsA throughout the storage. At the initial period, a decreasing level of AsA was minimal, and however, when the storage time had prolonged, the severity in the loss of AsA in leaves was observed. The sample in the package had kept the AsA level in leaves better than one in without package. Lee and Kader (2000) reported that the diminution of AsA occurs in plants when stored for a longer period under chilling condition. Furthermore, Zhang et al. (2013) reported that reducing AsA in leaves are the indication of the defense response of AsA against the chilling-induced diseases.

The antioxidant and radical scavenging activities of kaffir lime leaves with and without a package that stored under storage at low temperature are shown in Figure 2A-C. The radical scavenging capacity of the kaffir lime leaves was gradually increased throughout the storage in all the samples. The leaves showed a stronger scavenging activity against the ABTS radical than the DPPH radicals (Figure 2A-B). The results showed that prolonged storage period under low temperatures had significantly affected the quality of the kaffir lime leaves. During the prolonged storage period, the DPPH scavenging activity of the leaves was found

gradual increment and however when it reaches on the 18th days of storage, it started to decline. On the other hand, the ferric reducing power of kaffir lime leaves was also found a similar pattern with the leaves radical scavenging ability. FRAP activity was increased all over the storage, and however, when it reaches the end of storage, the activity was found low. The leaves that stored under package had shown a slightly lower antioxidant radical scavenging abilities as compared to control leaves. Junboon et al. (2013) reported that environmental or abiotic stresses such as storage temperature could increase higher phenolic metabolism. Furthermore, the AsA activity in this study had not influenced the antioxidant activity of kaffir lime leaves as compared to phenolics.

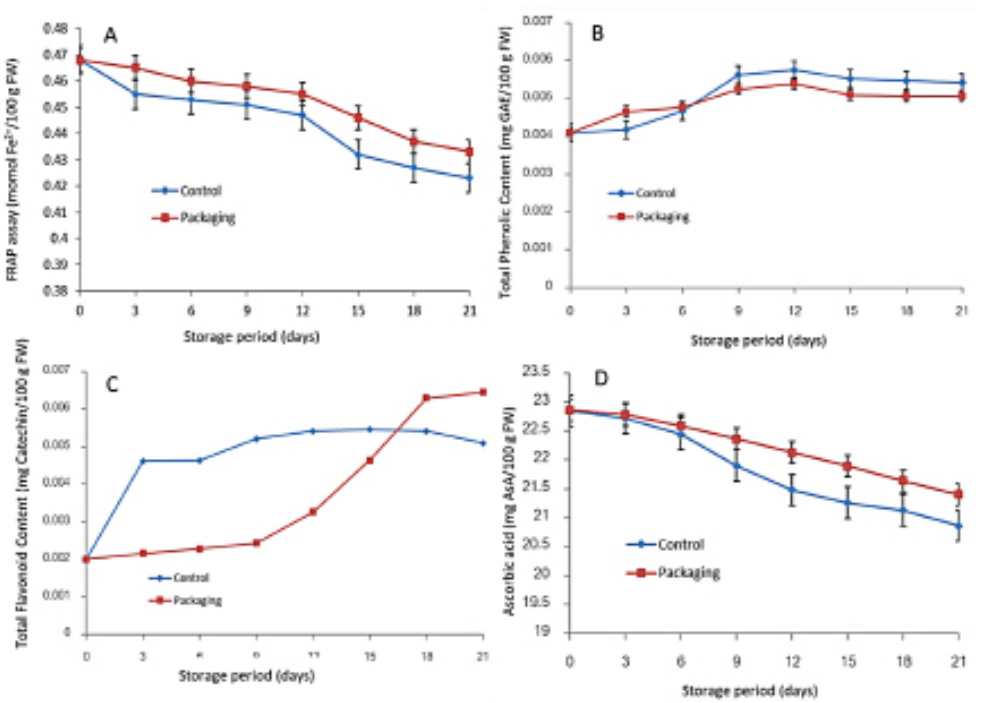

Figure 1 Changes in total chlorophyll content (A), total phenolic content (B), total flavonoid content (C) and ascorbic acid (D) in kaffir lime leaves with and without a package that stored under prolonged low temperature storage (8°C)

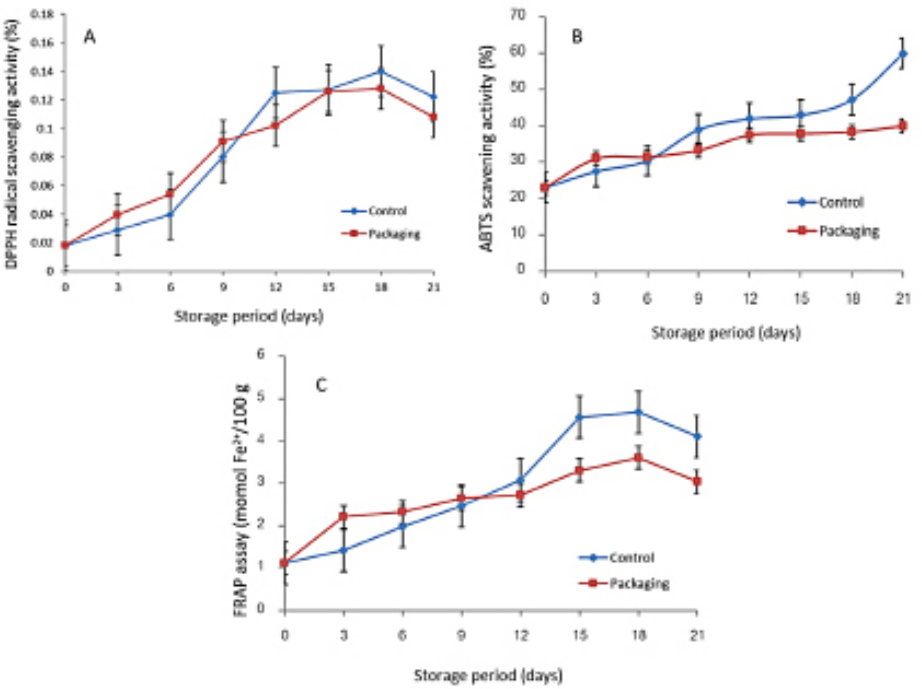

Figure 2 Changes in DPPH scavenging activity (A), ABTS scavenging activity (B) and FRAP assay (C) in kaffir lime leaves with and without a package that stored under prolonged low temperature storage (8°C)

Conclusions

The present study showed that kaffir lime leaves stored under prolonged storage at low temperature had significantly affected its overall quality. The package had retained the chlorophyll content, and ascorbic acid content in the kaffir lime leaves as compared to the control. However, the control group had retained the higher antioxidant and radical scavenging activities of kaffir lime leaves. Overall, low temperature storage could cause the quality changes in the kaffir lime leaves and however, this study recommends that if kaffir lime leaves treated with chemicals along with the packaging might able to retain the better quality.

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