

COMPARATIVE STUDY OF CHLOROPHYLL DEGRADATION FOR SPINACH, CORIANDER AND LEMONGRASS TEA LEAVES

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Abstract: Chlorophyll is a molecule produced by plant, algae and cyanobacteria and helps in conversion of light energy into chemical energy. Leaf chlorophyll content furnishes vital information and physiological status of plant. The green leaves changes color due to chlorophyll biodegradation. The chlorophyll a and chlorophyll b biodegradation pathways consist of large number of steps. The objective of present study is to compare degradation of chlorophyll content for three fresh leaves. Fresh leaves of spinach, coriander and lemongrass tea were used for the extraction of chlorophyll. The chlorophyll was extracted in 80% acetone and 20% distilled water. Filtered solution was used to detect chlorophyll-a, chlorophyll-b and total chlorophyll from visible spectrophotometer LI 722. Absorbance of chlorophyll contents was measured daily for twelve days continuously. Similarly filtered solution was kept in beaker and He-Ne laser light (5 mW, 632.8 nm) was irradiated on solution for 1 min to 10 min with 1 min of interval. The arrangement was done in dark room to avoid stray light contamination with chlorophyll. For every min absorption study was carried out and chlorophyll a, chlorophyll b and total chlorophyll content was estimated. It had been observed that degradation of chlorophyll b is more than that of chlorophyll a .in all cases. Day wise degradation study was carried for spinach and lemon grass tea leaves, temperature wise degradation was studied for coriander leaves and laser effect was studied for spinach, lemon grass tea and coriander leaves. In all measurements (day wise degradation, temperature wise degradation, irradiation using He-Ne laser degradation), it was observed that total chlorophyll content decreases with days, temperature and time of exposure respectively.

Keywords: Chlorophyll, Degradation, Spinach, Lemon grass, coriander, laser.

Introduction

The amount of chlorophyll in the leaf gives vital information about physiological status of plant. The green colour of leaves influences consumers acceptability in the market as their colour is taken as marker of freshness, ripeness, sweetness and dietetic values [1]. Customers generally buy fresh agricultural foodstuffs determined by their visual look, while additional quality attributes such as texture and aroma affect customers alternative to re-purchase the similar product. The coriander leaves (*Coriandrum sativum L.*) are most common in the food preparation all over the world as leafy vegetables. It is known for its stomachic, antibilious, diuretic, carminative, stimulant, and aphrodisiac properties in Ayurveda [2]. The coriander plant yields both the fresh green herb and the spice seed. The leaves are not appropriate to be dried for cooking use. Degree of greenness is vital in determining the ultimate quality of thermally processed vegetables, which acquire their green color as a outcome of chlorophyll. Researchers have informed that the color transforms (from bright green to olive brown) taking place during processing could be accredited mostly to conversion of chlorophyll a and b [4].

Spinach (*Spinacia olearacea L*) is annual plant and widely used in the diet and green salads as it contains functional food components by contributing vitamins, minerals and active component for digestion. Investigators showed that chlorophylls plays a minor role in degradation of chlorophyll in spinach leaves stored with or without ethylene and that most of the chlorophyll appears to be degraded by the peroxidase pathway, where the popyrin ring is opened and the resulting compound is colorless [5]. Lemon grass (*Cymbopogon Citrates*) are commonly cultivated as culinary and medicinal herb. Due to their scent and medical application and commonly leaves are used in the tea. Studies showed that the chlorophyll content was reduced in dehydrated lemongrass while maximum stability obtained in shade drying. The carotene content was augmented considerably after drying of leaves and maximum content found in cabinet dried lemongrass powder. The lightness, greenness and blueness were also being greater than before due to drying

procedures; it is different due to drying temperature and time of exposure. Hence, lemongrass can be conserved in powder form to keep its colour characteristics.

The main significant biochemical process in the plant is the photosynthesis in which ending product is chemical energy as light is absorbed by chlorophyll by the plant [6]. The main cause of discolouration in stored green vegetable is chlorophyll break down which is first visual symptom of senescence. Though chlorophyll degradation in senescence tissue is started by various factors such as water shortage, insufficient sunlight, temperature dissimilarity, humidity, plant hormone, but temperature variations affect significantly [7,8]. Considering all this it is essential to study the chlorophyll content in such leaves and effect of temperature and laser on chlorophyll of these leaves.

Methodology

Part A: Extraction of chlorophyll

About 250 g of freshly detached mature leaves of spinach, coriander and lemongrass tea leaves each, free from defects or injuries, were stored at 25 °C in a covered container under a stream of humidified air. Out of it, fresh leaves (>10 g) of each sample were removed and leaves without midribs were used for the extraction of chlorophyll. Pigments were extracted by grinding 10 g leaves in 80 ml of cold acetone and 20 ml of distilled water with a mortar and pestle. The solution was filtered with whatman filter paper and taken in test tube covered with aluminum foil. About 10 ml of chlorophyll of each sample (which were kept in darkness) were used for spectrophotometric analysis every day [5]. The absorption spectra were taken with UV visible spectrometer LI 722 in the 400 to 700 nm range. From absorption spectra, absorbance values at 645 nm and 663 nm were noted. Same procedure were used for successive ten days. The chlorophyll contents were calculated using Arnon's formulae [9].

$$\text{Chlorophyll a (mg/ml)} = 0.0127 \times A_{663} - 0.00269 \times A_{645}$$

$$\text{Chlorophyll b} = 0.0229 \times A_{645} - 0.00468 \times A_{663}$$

$$\text{Total Chlorophyll} = 0.0202 \times A_{645} - 0.00802 \times A_{663}$$

Where, A_{663} is absorbance at 663 nm wavelength

A_{645} is absorbance at 645 nm wavelength

Part B: Recording of absorption spectra for different leaves

1. For spinach and lemon Grass
Chlorophyll was extracted as discussed in part A. Chlorophyll a, chlorophyll b and total chlorophyll values were calculated from absorbance noted on absorption spectra values using Arnon's formula for successive 12 days.
2. For Coriander:
Chlorophyll was extracted as discussed in part A. Chlorophyll a, chlorophyll b and total chlorophyll values were calculated from absorbance values using Arnon's formula for different temperatures ranging from 80° C to 125° C with 5° C step.

Part C: Effect of He-Ne laser irradiation (5 mW) on chlorophyll

Chlorophyll was extracted as discussed in part A and filtered chlorophyll solution was kept in beaker and He-Ne laser light (5 mW, 632.8 nm) was irradiated on solution for 1 min to 10 min with 1 min of interval. The arrangement was done in dark room to avoid any stray light contamination with chlorophyll. For irradiation of laser for every minute, absorption study was carried out and chlorophyll a, chlorophyll b and total chlorophyll content was estimated.

Results and discussion

From absorption spectra amount of chlorophyll a, chlorophyll b and total chlorophyll were estimated. Fig. 1 shows Chlorophyll degradation of spinach leaves and lemon grass leaves recorded and estimated for

successive days for 12 days . Fig. 2 indicates Chlorophyll degradation of coriander leaves for different temperatures.

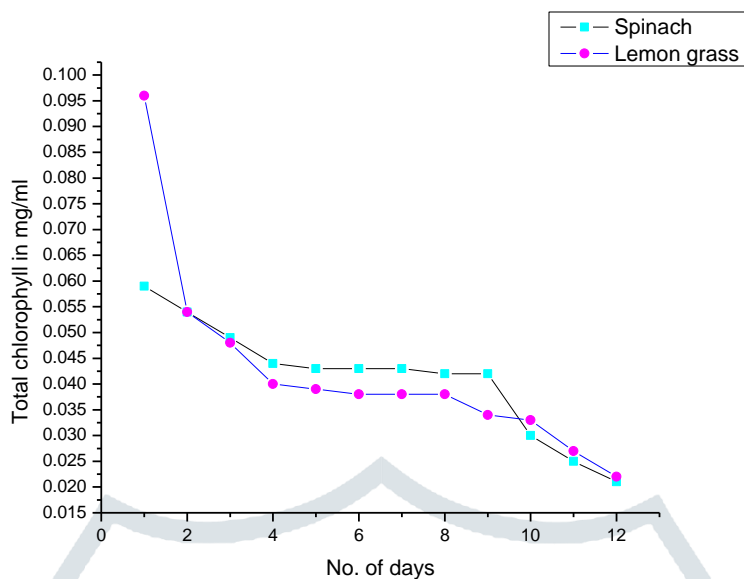


Fig. 1 Chlorophyll degradation of spinach leaves and lemon grass leaves recorded and estimated for successive days

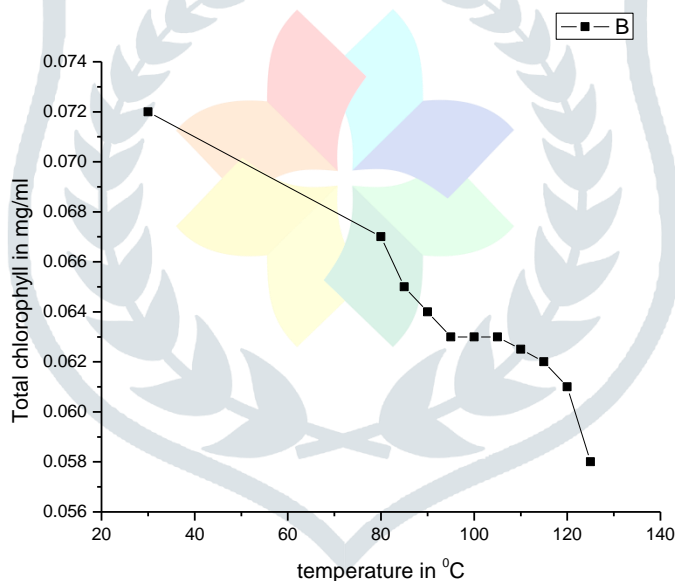


Fig. 2 Chlorophyll degradation of coriander leaves for different temperatures

Fig. 3 illustrates Chlorophyll degradation of spinach leaves for laser treatment for continuous 10 minutes

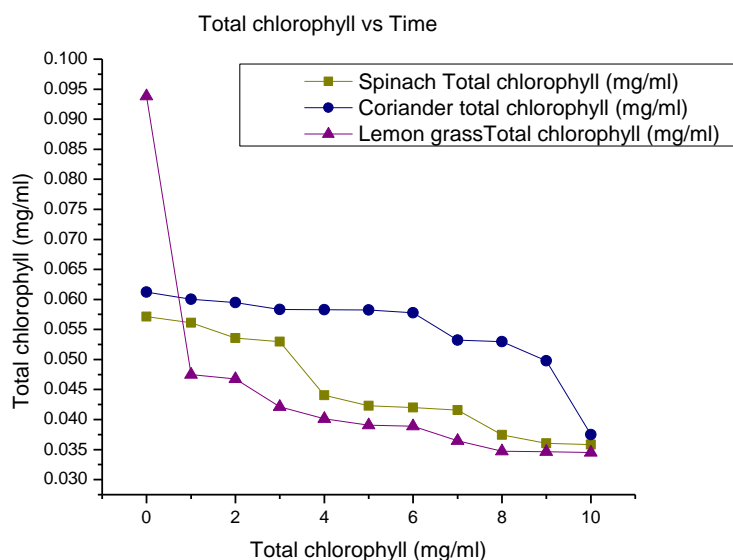


Fig. 3 Chlorophyll degradation of spinach leaves for laser irradiation for continuous 10 minutes

Conclusion:

In case of spinach the degradation of chlorophyll a is 48.79% chlorophyll b is 71.20 % and total chlorophyll is 62.95 % after 12 days. Similarly for lemon grass the chlorophyll a is 68.96% chlorophyll b is 81.21 % and total chlorophyll is 76.35 % after 12 days. In coriander leaves study chlorophyll a is 12.79 % chlorophyll b is 39.06 % and total chlorophyll is 30.55 % at 125 °C. Using laser radiation, in case of spinach the degradation of chlorophyll a is 19.75% chlorophyll b is 48.98% and total chlorophyll is 37.26% also in case of coriander leaves degradation of chlorophyll a is 11.59% chlorophyll b is 22.12% and total chlorophyll is 38.73% similarly in case of lemon grass tea leaves degradation of chlorophyll a is 49.76% chlorophyll b is 71.61% and total chlorophyll is 63.23%. It had been observed that degradation of chlorophyll b is more than that of chlorophyll a in all cases.

In laser irradiation study, it was observed that chlorophyll contents decreases with time of exposure as shown in following table 1:

Table 1: Comparative study of degradation by laser radiation, day wise and temperature wise

Degradation under condition	Degradation in	Ca	Cb	Tc
Laser	Spinach	19.75%	48.98%	37.26%
	Coriander	11.59%	22.12%	38.73%
	Lemon grass tea	49.76%	71.61%	63.23%
Day	Spinach	48.79%	71.20%	62.95%
	Lemon grass tea	68.96%	81.21%	76.35%
Temperature	Coriander	12.79%	39.06%	30.55%

In Laser study it has been noted that, Lemon grass tea leaves degradation is more than Spinach and Coriander after 10 minutes of low power of radiation. During leaf senescence, removal of Mg in chlorophyll bimolecular to form pheophytin is likely the first step, followed by removal of the phytol tail, catalyzed by enzyme is a pheophytinase (PPH). PPH specifically dephytylates the Mg-free chlorophyll pigment pheophytin and does not act on chlorophyll Chlorophyllide, which is the last precursor of chlorophyll biosynthesis. Hence, chlorophyll synthesis and breakdown are metabolically separated during leaf senescence. Based on study it is concluded that chlorophyllase may play a role in chlorophyll breakdown during fruit ripening and response to pathogens and wounding [10].

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