

THE EFFECT OF HEAT TREATMENTS ON THE CHLOROPHYLL IN GREEN PEPPER

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INTRODUCTION

The change in color of vegetables containing chlorophyll from a bright green to an olive green on cooking has been of concern to food scientists. The fact that magnesium is rather easily displaced from the molecule of chlorophyll when it is heated in the presence of organic acids has been reported (1, 2). These acids are exuded out from the partially destructed cell on cooking. Hydrogen replaces the magnesium and a pale greenish-gray compound known as "pheophytin a", or an olive green "pheophytin b" results.

According to A. O. A. C. method chlorophyll a and b can be determined simultaneously by spectrophotometer but pheophytin a and b have to be separated by column, partition paper, and thin-layer chromatography. Vernon (5) reported the equations for the analytical method that can be used for both chlorophyll and pheophytin by using maximum spectra.

This experiment was designed to determine the effect of different heating methods and time on the chlorophyll of green pepper.

MATERIAL AND METHODS

Green peppers were purchased from the local wholesale market and cut into small pieces after removing seeds. Peppers were divided into four groups and each group was heated at 100 C for 5, 10, and 15 min in boiling water, steam, and hot air. One group was heated in microwave oven for 1, 2, and 3 min. Each experiment was replicated three times.

Determination of Chlorophyll

The procedure outlined by Vernon (5) was employed in this experiment. One hundred twenty five milliliter of acetone were added to 35 g of diced sample to give a final solution which was 80 % in acetone and homogenized for 3 min in homogenizer. The resulting slurry was filtered through a fine glass filter with light suction and the filter cake residues were washed with 80 % acetone. The filtrates were brought to a final volume of 250 ml with 80 % acetone. The control sample were prepared by adding 3 ml of 80 % acetone to a 100 ml vol. flask and diluted to 100 ml with the filtered extract (used for the determination of chlorophyll). The conversion samples were prepared by adding 3 ml of sat.-oxalic acid in 80 % acetone in

a ol. flask and diluted to 100 ml with the same filtered extract (used for the determination of pheophytin). Both the control and converted sample were stoppered and kept in the dark at room temperature for 3 hrs, after which time the absorbances of both samples were determined at 649, 655, 665, 666 and 700 nm.

Equations for the calculation of chlorophyll and pheophytin

Total chlorophyll (mg/l) = 6.45 (A 665) + 17.72 (A 649)

Chlorophyll a (mg/l) = 11.63 (A 665) - 2.39 (A 649)

Chlorophyll b (mg/l) = 20.01 (A 649) - 5.18 (A 665)

Total pheophytin (mg/l) = 6.75 (A 666) + 26.03 (A 655)

Pheophytin a (mg/l) = 20.15 (A 666) - 5.87 (A 655)

Pheophytin b (mg/l) = 31.90 (A 655) - 13.40 (A 666)

Statistical method

Microwave-treated data were not used for statistical analysis since heating time was different. $3 \times 4 \times 3$ (Heat \times Time \times Replicates) factorial design was used to determine the significance of heating methods and time on the chlorophyll.

RESULTS AND DISCUSSION

Chlorophyll content in green pepper was ranged from 112 to 122 ug per g of fresh weight. Table 1 shows the results of different heat treatments on the chlorophylls and pheophytins. According to statistical analysis (Table 2) heating method and time had a significant effect ($P < 0.05$) on the chlorophylls of green pepper. Interaction between heating methods and time also showed a significant effect. Steaming had the greatest effect while hot air showed the least. Boiling water and steam treated sample retained about 65% and 55% of total chlorophyll after 15 min, respectively. Hot air treated sample retained most of chlorophyll. Chlorophyll a and b decreased as same trend as total chlorophyll and it decreased drastically at first five minutes. The ratio of chlorophyll a and b was about 3:1. However, at first 5 min heating chlorophyll b was lost more in boiling water-treated sample than in steam-treated sample, but steaming had more effect at 15 min than boiling water.

In case of total pheophytin, it also increased with increasing heating time which agree with the result of chlorophyll. As heating continues, organic acids diffuse from the vacuoles throughout the cell and into the cooking water. As the acids contact the chlorophylls they are converted to their respective pheophytins (3, 4). However, total pheophytin was produced more in boiling water-treated sample than in steam-treated one at 5 and 10 min heating. It was supposed that pheophytin produced in steam-treated sample could be converted to other products. Same results were found in pheophytin a but pheophytin b showed more in steam-treated sample than in boiling-water treated one. This was not unexpected, since data showed a small amount of pheophytin in control sample. Whether this pheophytin is present in the intact cell or is produced during the homogenization and extraction procedure cannot be answered by this experiment.

It was found that microwave energy did not have effect on the chlorophylls (Table 3). Only about 4% loss of total chlorophyll was found after 3 min. Chlorophyll a showed about 4% loss as found in total chlorophyll, but chlorophyll b nearly did not degrade by microwave energy.

Table 1. Chlorophyll and pheophytin content of green pepper treated with different heating methods 1, 2

Time (min)	0	5	10	15
Boiling water				
Total Ch.	112.07	87.43	77.57	72.71
Ch. a	89.43	60.21	57.86	53.50
Ch. b	34.71	25.93	23.43	18.79
Total Pheo.	2.71	28.79	41.86	43.00
Pheo. a	3.21	19.93	24.14	23.36
Pheo. b	1.00	9.93	13.79	15.79
Steaming				
Total Ch.	122.86	90.93	71.36	67.21
Ch. a	92.07	64.07	52.43	50.57
Ch. b	30.64	27.71	18.93	16.36
Total Pheo.	2.43	26.50	39.29	45.43
Pheo. a	2.14	15.36	21.71	26.00
Pheo. b	1.14	9.29	17.07	20.36
Hot air				
Total Ch.	118.36	111.50	106.64	109.07
Ch. a	89.86	84.86	75.07	75.36
Ch. b	30.86	30.36	32.57	31.94
Total Pheo.	3.93	5.14	6.14	7.14
Pheo. a	3.14	3.79	5.21	5.50
Pheo. b	1.14	2.29	2.93	3.93

1/ Means of six observations

2/ ug/g. F. W.

Table 2. Analysis of variance for the effect of chlorophyll content of green pepper treated with different heating methods and time.

Source	D. F.	S. S.	M. S.	F.
Heat	2	4,504.37	2,252.18	70.89**
Time	3	6,831.94	2,277.31	71.68**
Replicates	2	4.84	2.42	0.08
HxT	5	1,944.27	388.85	12.24**
HxR	8	28.10	3.51	0.11
TxR	11	123.60	11.24	0.35
Error	1	127.60	31.77	
Total	35	13,564.72		

** $p < 0.05$

Table 3. Chlorophyll and pheophytin contents of green pepper treated with microwave energy 1, 2

Time (min)	0	1	2	3
Total Ch.	116.71	113.64	105.50	109.71
Ch. a	78.00	71.50	73.05	74.71
Ch. b	33.57	33.46	33.14	33.00
Total Pheo.	3.64	10.57	11.14	12.36
Pheo. a	2.36	6.43	7.21	8.29
Pheo. b	1.36	3.03	5.57	6.00

1/ Means of six observations

2/ ug/g, F. W.

SUMMARY

The change in color of vegetables containing chlorophyll on cooking has been of concern to food scientists. This experiment was initiated to determine the effect of different heating methods and time on the chlorophyll of green pepper. Peppers were heated at 100 C for 5, 10, and 15 min by using boiling water, steam and hot air and for 1, 2 and 3 min in microwave oven. The results showed that chlorophyll content decreased with increasing heating time. Among the heat treatments employed steaming gave the greatest effect and boiling water was the next. Hot air and microwave treated samples changed only small amount of chlorophyll. Generally pheophytin formation was increased with decreasing chlorophyll content.

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要 約

Chlorophyll을 함유하고 있는 녹색 植物의 調理에 依한 색깔 變化는 대단히 重要하므로, 本實驗은 各各 다른 熱處理의 時間이 풋고추의 chlorophyll 含量에 미치는 影響을 조사하였다. 풋고추는 boiling water·steam 그리고 hot air 를 使用하여, 100°C에서 5·10·15 分間 加熱했고, microwave oven 에서는 1·2·3 分間 처리했다. 結果에 依하면 chlorophyll 含量은 一般적으로 加熱時間이 길어짐에 따라 감소하는 경향이였으며, 熱處理中 steam이 가장 큰 影響을 주었고 boiling water 가 그 다음이었다. Hot air 와 microwave 처리한 풋고추는 아주 적은 양의 chlorophyll 이 變化하였다. 일반적으로 pheophytin 生成은 chlorophyll 含量이 감소함에 따라 增加하였다.