

CHANGE OF CHLOROPHYLL AND VITAMIN C IN GREEN PEAS (*Pisum sativum*) DURING THERMAL PROCESSING

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ABSTRACT

Fresh green peas are rich in vitamin C, a powerful natural water-soluble anti-oxidant. Green peas are normally consumed after thermal processing, mostly canning or cooking, which causes vitamin C degradation and chlorophyll breakdown. This study aimed to determine the changes of vitamin C and chlorophyll during thermal processing through analyzing their contents in peas during typical thermal processing stages, including blanching, boiling, and pasteurizing. The results revealed that the vitamin C content was reduced significantly when the blanching temperature increased by 10°C. The vitamin C in green peas blanched at 70°C, 80°C, and 90°C for 3 minutes were 32.68, 12.60 and 13.02 mg%, respectively. A high loss of chlorophyll was recorded when the peas were blanched at 90°C. The experiment's results also showed that the vitamin C and chlorophyll losses were higher in the peas put in water of 22°C than those put in water of 100°C prior to boiling. Pasteurizing at 95°C for 8 or 11 minutes gave lower vitamin C content in the peas than at 100°C for 5 minutes. For retention of vitamin C and chlorophyll, it is recommended that green peas be put in initial water of 100°C during boiling, blanching, and pasteurization as fast as possible in the canning process.

Keywords: Chlorophyll, green pea, temperature, vitamin C.

Ảnh hưởng của các quá trình chế biến nhiệt đến hàm lượng chlorophyll và vitamin C trong đậu Hà Lan (*Pisum sativum*)

TÓM TẮT

Đậu Hà Lan là một nguồn vitamin C rất dồi dào nhưng hầu như chỉ được sử dụng sau khi đã qua các quá trình chế biến nhiệt. Các quá trình này sẽ dẫn đến sự tổn thất rất lớn hàm lượng vitamin C. Mặt khác, xử lý nhiệt trong quá trình chế biến cũng ảnh hưởng đến hàm lượng chlorophyll, một sắc tố quan trọng quyết định màu xanh của sản phẩm. Nghiên cứu này xác định hàm lượng vitamin C và chlorophyll trong đậu Hà Lan được chần, luộc và thanh trùng ở các nhiệt độ khác nhau. Kết quả cho thấy hàm lượng vitamin C bị giảm xuống rõ rệt khi nhiệt độ chần tăng thêm 10°C. Hàm lượng vitamin C trong đậu Hà Lan được chần ở 70°C, 80°C và 90°C trong 3 phút lần lượt là 32.68, 12.6 and 13.02 mg%. Sự tổn thất chlorophyll xảy ra rõ nhất khi chần đậu Hà Lan ở 90°C. Kết quả nghiên cứu cũng chỉ ra rằng sự tổn thất hàm lượng vitamin C và chlorophyll cao hơn ở đậu Hà Lan được đưa vào nước nguội rồi nâng đến nhiệt độ sôi so với đậu Hà Lan được đưa ngay vào nhiệt độ 100°C trong quá trình luộc. Đậu Hà Lan đóng lọ được thanh trùng ở 95°C trong 8 hoặc 11 phút có hàm lượng vitamin C thấp hơn so với sản phẩm được thanh trùng ở 100°C trong 5 phút. Để giữ được hàm lượng vitamin C và chlorophyll, đậu Hà Lan nên được đưa ngay vào nhiệt độ sôi trong quá trình luộc, chần và thanh trùng nhanh nhất có thể trong quá trình đóng lọ.

Từ khóa: Chlorophyll, đậu Hà Lan, nhiệt độ, vitamin C.

1. INTRODUCTION

Green peas are one of the most nutritious leguminous vegetables that are rich in phyto-

nutrients, minerals, vitamins, and antioxidants. Fresh green pea is a good source of ascorbic acid (vitamin C). A serving of 100 g of fresh green peas contain about 40 mg or 67% of daily

requirement of vitamin C (USDA National Nutrient Data Base). Vitamin C is a powerful natural water-soluble anti-oxidant. Green peas are normally used after one or more steps of thermal processing, including canning or cooking, that may result in changes of color, texture, flavor, and nutritional quality. The water-soluble nutrients, such as vitamins C and B, and phenolic compounds are susceptible to degradation during thermal processing and can leach into the cooking water (Rickman *et al.*, 2007). Not only the nutritive qualities, but the sensory qualities are also vital factors that are difficult to control during processing, especially the color. This is a major sensory characteristic in determining product acceptability. Chlorophylls are the most widely distributed plant pigments responsible for the characteristic green color of vegetables. Chlorophylls are known to be easily degraded by conditions such as dilute acids, heat, light, and oxygen (Erge *et al.*, 2008). It is important to prevent or at least minimize chlorophyll degradation during thermal processing in cooking and the food industry. This study was conducted to determine the effect of temperature during thermal processing on the changes in vitamin C and chlorophyll content during the processing of green peas. The results of the study will contribute to building the scientific data on the variation of vitamin C and chlorophylls during processing, and will reflect the relationship between these components with the sensory quality of the products. These will be the basis for the experimental design in the field of research, as well as a basis for minimizing nutrition loss in cooking and the food industry.

2. MATERIALS AND METHODOLOGY

2.1. Materials

Fresh green peas (*Pisum sativum*) were collected from a wholesale market and stored at 0°C under 95% relative humidity in polyethylene bags until the analyses. All analyses were completed within 3 days.

2.2. Chemicals

Chemicals used in this study included 2,6-Dichlorophenolindophenol, acetic acid, standardization ascorbic acid solution, sodium acetate solution, acetone 100%, and oxalic acid.

2.3. Thermal processing experiments

2.3.1. Blanching experiment

Cleaned green peas were subjected to heat treatment at 70°C (for 1, 2, 3 minutes), 80°C (for 1, 2, 3 minutes), and 90°C (for 1, 2, and 3 minutes) in a water bath (GFL - Germany). At the end of the heating periods, samples were taken and immediately cooled under tap water at 22°C. Blanched green peas were left to drain before conducting the following analyses. Fresh green peas were used as the control sample.

2.3.2. Boiling experiment

Cleaned green peas were divided into two parts. The first part of green peas was put into an initial water bath of 100°C and boiled for either 5, 10, or 15 minutes. The second part was put into an initial water bath of 22°C, then heated to 100°C and boiled for 5, 10, or 15 minutes. Boiled green peas were allowed to drain before conducting the following methods of analyses. Fresh green peas were used as the control sample.

2.3.3. Pasteurization experiment

Green peas were canned with the processing as follows: (1) cleaned green peas, (2) soaked peas in warm water (45-50°C, 5-6 hours), (3) soaked peas in cool water (10-12 hours), (4) blanched peas (90°C, 3-4 minutes), (5) put peas into jars (the proportion of green peas to net weigh was 50.5%), (6) filled jars with solution (salt 1%, sugar 2%), (7) capped, seamed, and sealed jars, (8) pasteurized jars, and (9) cooled jars for the final products.

Canned green peas were pasteurized at 85°C (5, 8, 11 minutes), 90°C (5, 8, 11 minutes), 95°C (5, 8, 11 minutes), 100°C (5, 8, 11 minutes).

The experiments were carried out in 3 replications. There were 5 samples for each

replication. The total number of samples for each experiment was 15.

2.4. Methods of analysis

2.4.1. Determination of chlorophyll

Chlorophyll was determined according to Lichtenthaler *et al.* (1987). Chlorophyll a+b contents were estimated by extraction of the green peas in 100% acetone. The optical density was measured by the absorption (A) at 661.6, 644.8, and 470 nm, and then calculated with the equations of the pigment amount in per mg per ml extract solution.

Concentration of chlorophyll a: C_a ($\mu\text{g}/\text{ml}$)=
 $11.24 \times A_{661.6} - 2.04 \times A_{664.8}$

Concentration of chlorophyll b: C_b ($\mu\text{g}/\text{ml}$)=
 $20.13 \times A_{644.8} - 4.19 \times A_{661.6}$

Concentration of $C_{a+b} = 4.0 \times A_{661.6} + 18.09$
 $\times A_{644.8}$

Chlorophyll content (mg/g) = $(C_{a+b} \times V) /$
 (1000 x m)

V: volume of extracted solution

m: weight of sample (g)

2.4.2. Determination of vitamin C

Vitamin C was determined with the specific titrant 2,6-Dichlorophenolindophenol according to AOAC 967.21.

Principle: 2,6-Dichlorophenolindophenol (DCPIP) is reduced to a colorless form by ascorbic acid. The dye is blue in alkaline solution and red in acid.

Preparation of reagents: 2,6-Dichlorophenolindophenol solution (0,001 mol/L): 0.05 g of DCPIP was dissolved in distilled water, diluted to 100 ml, and filtered. The DCPIP solution was kept in a refrigerator.

Ascorbic acid solution: 0.05 g of pure ascorbic acid was dissolved in 20 ml of 10% oxalic acid and diluted with distilled water to exactly 250 ml in a volumetric flask.

Standardization of the 2,6-Dichlorophenolindophenol: 10 ml of standard ascorbic acid solution was pipetted into a small

flask and titrated with 2,6-Dichlorophenolindophenol solution until a faint pink color persisted for 15 seconds. The concentration was expressed as mg ascorbic acid equivalent to 1 ml of DCPIP solution.

Titration: 5 g of green peas were weighed exactly into a breaker. 40 ml of oxalic acid was added and stirred for 5 minutes, then filtrated into a 100 ml volumetric flask and diluted to volume with distilled water. 10 ml was pipetted into a small flask and 2.5 ml acetone added. The solution was titrated with DCPIP until a faint pink color persisted for 15 seconds. This was repeated with the blank sample.

Calculation:

$$\text{Vitamin C (mg\%)} = \frac{(a-b) \times f \times V1 \times 100}{W \times V2}$$

a: ml for test solution titration

b: ml for test blank titration

f: mg ascorbic acid equivalent to one ml DPIP standard solution

V1: volume initial test solution

V2: volume test solution titrated

W: weight of sample

2.4.3. Analysis of experiments data

The experiments data were analyzed by Excel 2007 and Minitab16 software

3. RESULTS AND DISCUSSION

3.1. Effect of blanching on the changes in chlorophyll and vitamin C in green peas

Blanching is one of the most important stages in green pea processing. Blanching aims to inhibit the afterward biochemical actions, and reduce the microorganisms on the surface of materials. However, this technique also makes unexpected changes to the quality of materials. Vitamin C content of green peas is affected by blanching's temperatures and times as shown in Figure 1. The results showed that the vitamin C content of the green peas was reduced markedly as the blanching temperature

increased from 70°C to 90°C. There was no significant change in vitamin C content of fresh green peas and the samples blanched at temperatures up to 80°C for 1 minute. The loss of vitamin C was not considerable when blanching at 70°C for 1 minute; the loss reached 62.5% at a blanching temperature of 90°C for 1 minute. Green peas blanched at 80°C for 2 minutes had their vitamin C content reduced sharply and showed a significant difference with the previous samples. The loss observed in green peas during blanching was in agreement

with the work by Igwemmar *et al.* (2013) who reported average losses of 10.6% of vitamin C in green peas through heating at 60°C for 5 minutes, and 58.3% at 60°C for 30 minutes.

The results in Figure 2 show that chlorophyll content reduced gradually as the blanching temperature and time increased. Chlorophyll content in green peas blanched from 70°C in all tested times to 90°C for 1 minute were not distinctly different. However, a high loss of chlorophyll content can be seen when blanching green peas at 90°C from 2 minutes.

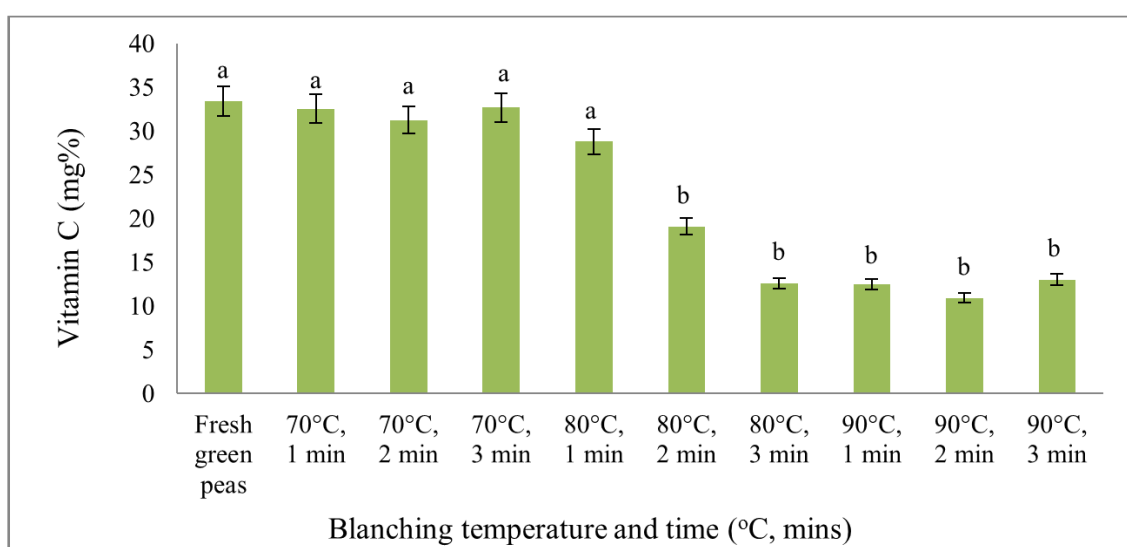


Figure 1. Effect of blanching on the changes in vitamin C content of green peas

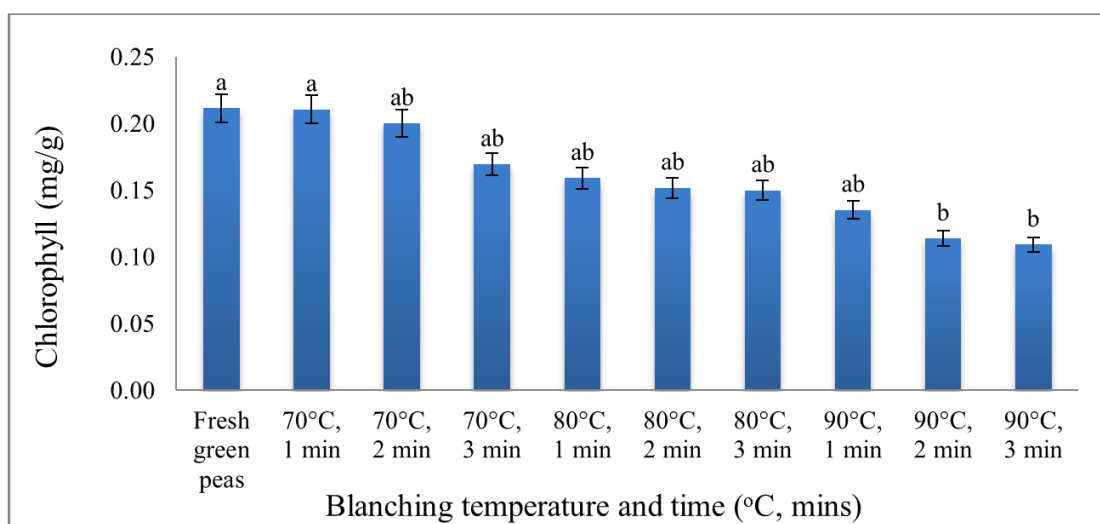


Figure 2. Effect of blanching on the changes in chlorophyll content of green peas

According to Kidmose (2002), the degradation of chlorophyll to pheophytins, in which the magnesium ion is displaced with two hydrogens, makes the plant material change color from green to dull olive-green. This alteration is most widespread in green vegetables and takes place during thermal processing. At 60°C and above, chlorophyll is progressively converted to pheophytin, and the rate increases rapidly as the temperature rises (Ryan-Stoneham and Tong, 2000). Erge *et al.* (2008) investigated the thermal degradation kinetics of chlorophyll and visual green color in green peas at 70°, 80°, 90°, and 100°C. The results indicated that the degradation of chlorophyll a at 100°C had the fastest rate with a half-life value of 10 minutes, followed by 90°, 80°, and 70°C, which had half-life values of 15.68, 31.08, and 35.91 minutes, respectively. It was concluded that the degradation of chlorophyll a and chlorophyll b followed a first-order kinetic model.

3.2. Effect of boiling on the changes in chlorophyll and vitamin C content of green peas

Chlorophyll and vitamin C content of green peas boiled in different methods and times are shown in Table 1.

The results showed that the vitamin C contents of fresh green peas are generally higher when compared with those of the boiled peas. However, green peas put in an initial water bath of 100°C and boiled for 5 minutes gave no significant difference in vitamin C content compared to the fresh green peas, while a high loss of vitamin C (52.3%) was found at 15 minutes of boiling time. The highest loss of vitamin C (69.5%) was seen in green peas put in an initial water bath of 22°C, then heated to 100°C, and boiled for 15 minutes. The results also pointed out the fact that the loss of vitamin C is higher in green peas put in an initial water bath of 22°C than those put in an initial water bath of 100°C prior to boiling. Zaman *et al.* (2012) reported that green peas contained a greater amount of ascorbic acid in their fresh

state (28.5 mg%) as compared to a boiled state (23.0 mg%).

It can also be observed in Table 1 that the reduction of chlorophyll content of green peas boiled for 5 or 10 minutes was not remarkable while the chlorophyll content of green peas boiled for 15 minutes decreased considerably. Loss of chlorophyll was lower in green peas put in an initial water bath of 100°C and boiled than those put in an initial water bath of 22°C, then heated to 100°C, and boiled. The lowest content of chlorophyll (0.16 mg/g) given was by green peas put in an initial water bath of 22°C, then heated to 100°C, and boiled in 15 minutes. Turkmen *et al.* (2006) observed in green peas that cooking resulted in a loss of chlorophyll a and b and the loss was lower than in spinach, broccoli, leek, squash, or green beans. According to their report, green peas had a retention of 91% in chlorophyll a and 86% in chlorophyll b after being boiled for 5 minutes.

3.3. Effect of pasteurization on the change in chlorophyll and vitamin C content of green peas

Pasteurization is the vital period which determines hygiene quality of the products during canning of fruits and vegetables. Many studies have examined the effects of thermal processing during canning on ascorbic acid for various commodities. Murcia *et al.* (2000) reported that vitamin C in fresh and canned broccoli were 112 and 18 mg%, respectively (a loss of 84%). Loss of vitamin C during canning were 88% in carrots and 73% in green peas as reported by Howard *et al.* (1999) and Weits *et al.* (1970), respectively. The results of the present study showed that vitamin C content in canned green peas reduced sharply compared to fresh green peas. Green peas pasteurized at 85 to 90°C for 5 to 11 minutes gave no significant difference at $\alpha = 0.05$ in vitamin C content. Pasteurizing at 95°C for 11 minutes, and at 100°C for 8 minutes or 11 minutes gave the lowest vitamin C content in green peas with the

loss of 64.2, 57.5 and 58.5% respectively. Pasteurizing of 95°C for 8 or 11 minutes gave a lower vitamin C content in green peas than 100°C for 5 minutes.

The color of green vegetables after canning usually changes from green (chlorophyll) to olive-green (pheophytin) due to the serve heat treatments for the long time. Lo pez-Ayerra *et al.* (1998) found that chlorophyll content of spinach was lost about 99.9% after canning as a consequence of the heating. In contrast, only 16% of the chlorophyll was lost during frozen storage. Chlorophyll in green peas pasteurized at 85°C for 11 min or at a higher temperature and longer time dramatically reduced losses which ranged from 43.2% to 64.2%. Significant differences in chlorophyll content were not seen among these samples of green peas. Green peas

pasteurized at 85°C for 5 min gave the highest retention of chlorophyll, a loss of only 23.7%.

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Table 1. Effect of boiling on the changes in chlorophyll and vitamin C content of green peas

Samples	Chlorophyll (mg/g)	Vitamin C (mg%)
Fresh green peas	0.21 ^a	33.40 ^a
Initial water of 22°C, then heated to 100°C, boiled in 5 minutes	0.18 ^{ab}	25.85 ^{ab}
Initial water of 22°C, then heated to 100°C, boiled in 10 minutes	0.17 ^{ab}	20.15 ^{bc}
Initial water of 22°C, then heated to 100°C, boiled in 15 minutes	0.16 ^b	10.19 ^d
Initial water of 100°C, boiled in 5 minutes	0.20 ^{ab}	31.69 ^a
Initial water of 100°C, boiled in 10 minutes	0.20 ^{ab}	27.07 ^{ab}
Initial water of 100°C, boiled in 15 minutes	0.17 ^{ab}	15.92 ^{cd}

Note: The experimental values within columns that have no common superscript are significantly different ($p < 0.05$)

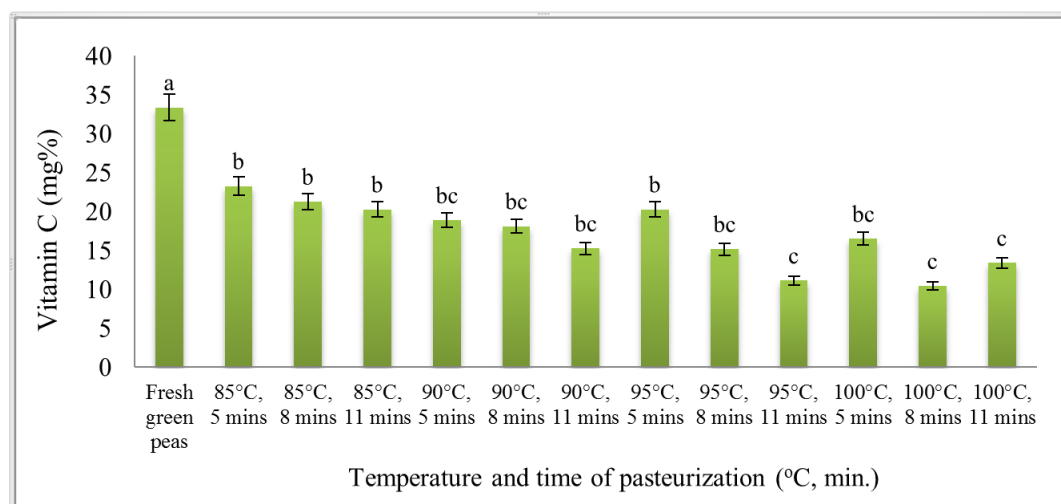


Figure 3. Effect of pasteurization on the change in vitamin C content of green peas

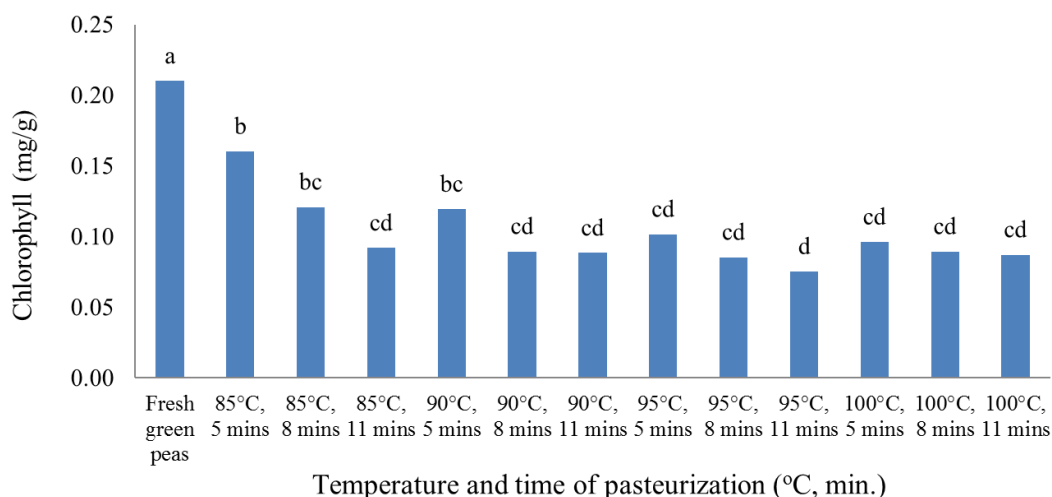


Figure 4. Effect of pasteurization on the change in chlorophyll content of green peas

4. CONCLUSIONS

Thermal processing significantly affects the contents of chlorophyll and vitamin C in green peas. Vitamin C content in green peas blanched at 80°C for 3 minutes decreased sharply, while the loss of chlorophyll was seen when blanching the green peas at 90°C for 2 minutes. Pasteurizing at 95°C for 11 minutes and at 100°C for 8 minutes or 11 minutes gave the lowest vitamin C content in green peas. Green peas pasteurized at 85°C for 5 min gave the highest retention of chlorophyll. The loss of vitamin C and chlorophyll is higher in green peas put in an initial water bath of 22°C than those put in an initial water bath of 100°C prior to boiling. In order to retain the vitamin C and chlorophyll contents, it is recommended that green peas should be placed immediately into boiling water during boiling, blanching, and pasteurization as part of the canning process.

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