Pigment Stability of Lavers *Porphyra tenera* Kjellman during Processing and Storage

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김의 가공 저장중에 있어서의 색소의 안정도

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김(海苔)의 중요색소인 chlorophyll, carotenoid, phycobilin 색소의 가공 및 저장중의 안정도를 실험하였다. 생김의 색소함량, 생김의 저온저장, 抄製·乾燥김의 열 처리, 열 처리한 김의 실온저장시에 일어나는 이들 색소의 변화에 대한 실험 결과를 요약하면 다음과 같다.

1. 생김의 chlorophyll a, xanthophyll (lutein+zeaxanthin), 및 carotene (α-+β-carotene)의 함량은 각각 1,525, 627, 409 μg/g였고 β-carotene, lutein, zeaxanthin, 및 α-carotene의 비교 함량은 33.7, 35.9, 12.2, 5.8%의 순이었다.

2. 생김을 실온(20°-25°C), 2°~5°C, 및 −15°C에서 저장하였을 때색소의 감소는 대체로 2°~5°C 때가 다른 조건에서 보다 적었는데 carotene 만은 −15°C 때가 2°~5°C 때보다 다소 낮은 결과였다. phycocyanin은 phycoerythrin 보다 많은 감소를 보였다.

3. 초제·日乾한 김 또는 초제 직후의 김을 40°, 60°, 80°, 100°C에서 열 처리하였을 때 양자의 경우 모두 저온에서 처리할수록 색소감소가 적었고 carotenoid는 전자보다 후자의 경우 감소량이 적었다. Carotenoid 중 xanthophyll은 carotene 보다 열에 안정하였다. Phycoerythrin은 phycocyanin 보다 열에 불안정한 경향이 였는데 높은 온도일수록 현저하였다.

4. 열 처리한 김을 50일간 저장하였을 때, 색소의 변화는 열 처리 온도에 따라 다소의 차는 있었으나 60°C에서 처리한 것이 대체로 감소량이 적었다. 그러나 큰 차이는 없었다. Xanthophyll, carotene, phycobilin은 모두 고온에서 처리한 것이 저장중의 색소 감소율이 낮았고 따라서 장기저장이 가능한 것같은 경향을 나타내었다. Xanthophyll과 carotene은 일광건조하지 않고 열 처리한 것에서 감소가 적은 반면 chlorophyll a는 日乾 열 처리한 것에서 낮은 감소를 보였다.

5. 색소의 안정도와 분해반응의 억제를 감안한 열처리조건은 60°C에서 6.0% 정도의 수분량을 유지할 때까지 처리하거나 그 이상의 고온에서 처리하되 열에 의한 색소의 분해를 최소한으로 하는 시간으로 한정하는 것이 적당하다고 보았다.

6. 김초제액의 pH 조절은 저장중에 있어서의 chlorophyll a의 안정화에 효과가 있었으며 pH 7.8 일 때가 성적이 좋았다. 초제액에 한천, 알긴산등의 해조추출물을 첨가하였을 때 김의 균일한 성형과 윤택의 증진에 효과가 있었다.

Introduction

Color is a dominate factor in deciding the quality of the dried product of lavers *Porphyra tenera* Kjellm. a kind of marine red algae, which is made by the process of drying and heat treatment. The color of lavers appears to be a harmony of three different groups of pigment namely phycobilins, chlorophylls, and carotenoids representing red and blue, green, and yellow colors respectively. These pigments are unlike in physical and chemical properties. Phycobilins mainly consisting of phycoerythrin, phycocyanin, and allophycocyanin, are water soluble and quite heat unstable whereas chlorophylls and carotenoids are fat soluble. Chlorophylls and carotenoids are also rapidly degraded by the action of enzymes, acids, and the effect of temperature particularly in the presence of light and oxygen during processing and storage.

Some investigators (Fujikawa, 1936; Sano, 1955a, 1955b, 1956, 1957, 1960, Obata and Yamanishi, 1949; Yamakawa, 1953) have done research on the color of lavers. Most of the reports, however, were of color revelation, measured qualitatively with absorption data on crude extracts, in relation to the environmental conditions of cultivation fields or cultural media such as water current, salinity, mineral nutrition, and light intensity. Yamakawa (1953) measured seasonal color changes in fresh lavers and stated that the lavers harvested at the stage revealing a deep color had better flavor. Obata and Yamanishi (1949) experimented heat discoloration with dried lavers and pointed out that moisture content and oxygen most significantly affected the stability of pigments during the storage of the product. Reports on isolated pigments are rare but a report on the separation of carotenoids of lavers was written by Katayama (1964). He isolated carotenoids on celite-MgO columns and determined the relative contents of four identified carotenoids as α- and β-carotene, lutein, and zeaxanthin.

In this study, the results of three parts of experiment are combined. In Part I and II, chlorophyll a and carotenoids were isolated on sugar-starch columns with 0.5 % n-propanol petroleum ether solution as a developing solvent and their contents were analysed spectrophotometrically using Shimadzu Model QV-50. In Part III, the retention of biliproteins during processing and storage were measured with the changes in their optical densities at the absorption maxima. With the results of the pigment analysis at the steps of processing and storage the stability of the pigments is discussed. And the effects of temperature and pH on the pigment stability during heat treatment and storage are described. Another set of experiments was run to detect the effects of pigment fixing agents added and some aids for uniform spreading of chopped lavers in preparing laver sheets. Still another set of experiments was performed to improve the processing procedure from the view point of color retention. In this psocedure sun drying which has to be done before heat treatment in general procedures was eliminated and thin layers of wet lavers were directly heat treated. The results are shown in detail in the text.

I. Stability of Chlorophyll a in Lavers during Processing and Storage

Preparation of Dried Lavers

Fresh lavers *Porphyra tenera* Kjellm. were obtained from the Chang-Rim Laver Field located at the entrance of the Nak-Dong River in the outskirts of Pusan city. The lavers, having been eliminated spurious algae like green lavers and other defects, were cut into 10cm shreds, chopped by a 3mm plate in a chopper, and rinsed. Six hundred grams of drained lavers were dispersed in 4 *l* water and uniformly suspended as possible, and then a 300ml portion of the suspension was poured into a 191×209mm wooden frame under which a mat of thin bamboo strips, slightly larger than the frame, was placed in order to provide a thin layer or film of lavers after the draining off of excess water. This step of procedure requires skill for yielding a uniform thickness of the layer. The thin layer on the bamboo mat was air dried upside down in the shade or in the sun, until the film of lavers was separated itself from the mat. Finally the sheets of lavers were heat treated in a hot air dryer and packaged for storage.

For the heat treatment, packers usually apply a low temperature (usually 30 to 40°C) treating the lavers for two or three days until a moisture content low enough for a long term storage period is reached. To the experiment samples various temperatures were applied to determine the effect of heat on the stability of pigments during the treatment. The results are fully discussed in this text.

Analysis of Chlorophyll Pigments

Extraction of Chlorophylls It is generally more difficult to extract pigments from algae than from leaves of higher plants due to the presence of polysaccharides. As pointed out by Aronoff (1953), straight methanol was more satisfactory than 80% acetone, a favorite extractant for the latter. In the preliminary test, a mixture of methanol and petroleum ether (2:1 v/v) was also found to be most effective and preferred over straight methanol. Strain (1954) recommended the use of the mixed solvent for the reason that the pigments should be dissolved in petroleum ether solution for the pigment separation in column chromatography.

For the pigment extraction, 5g of fresh laver or 1.5g of dried laver, cut into 1 cm^2 pieces and thoroughly mixed, was weighed and blended for 5 minutes in a Waring blender with 100ml methanol-petroleum ether mixture, 5g sodium sulfate, and a small amount of magnessium carbonate, and then stored overnight in a freezer at −15°C. The extracts were filtered through a glass filter and the residue was blended for another 5 minutes with 80ml of the mixture solvent and left to stand for 6 to 8 hours in the freezer. The second extracts were filtered and the residue was re-extracted with 50ml methanol and then finally filtered. All the filtrates were combined to make up a 300ml pigment solution. The above procedure resulted in 95 to 98% pigment extraction.

A 50 ml portion of the 300ml pigment solution was taken in a 500ml separation funnel and the pigments were transferred to ether by adding an equal volume of ethyl ether and a 10%

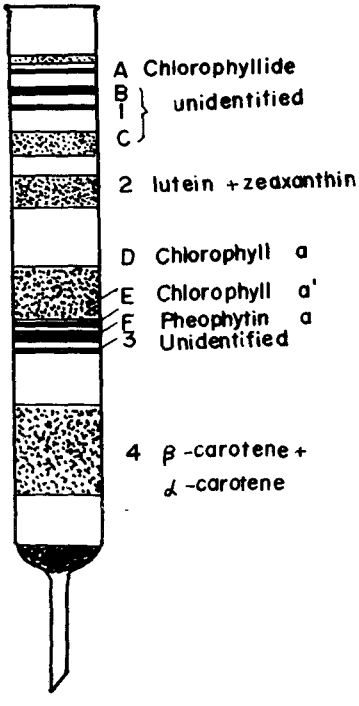
sodium chloride solution. The ether extracts were washed five times with 50ml of distilled water. The bubbling method of Association of Official Agricultural Chemists in 1955 helped to prevent emulsion formation while washing. The same effect resulted when water was added slowly along the funnel wall with gentle swirling. The ether extracts were dehydrated with powdered sodium sulfate and concentrated to a dry state in a rotary vacuum evaporator, and the pigments then were dissolved in redistilled petroleum ether making a sample up to 10ml in volume for pigment isolation.

Determination of the Contents of Chlorophyll a and Its Derivatives

Chlorophyll a and its degradation products were isolated on sugar-starch columns and their contents were determined with a spectrophotometer. Powdered sugar is the most commonly used adsorbent for the separation of chlorophylls. Strain(1958), Stoll and Wiedmann(1959), Sweeny and Martin(1958), and Perkins and Roberts(1962) used a benzene-light petroleum mixture or 0.5% n-propanol petroleum ether solution as developing solvents. Smith and Benitez (1955) used petroleum ether alone. Alternatively, the column was developed and eluted with solutions of acetone in light petroleum ether starting with 5% acetone and finishing with 25% (Sweeny and Martin, 1961). Tan and Francis (1962) used mixture columns consisting of 70% sugar and 30% corn starch for separating chlorophylls from pheophytins for quantitative determination in extracts of processed spinach. In this experiment, mixtures of confectionary sugar, produced by the Cheil Sugar Refinery Co., Pusan and corn starch (75:25 w/w) were used and developed with 0.5% n-propanol in petroleum ether. The results of preliminary tests showed that a 25% mixture of corn starch resulted in better separation than a 30% mixture. Besides, the use of 10% ethyl ether petroleum ether solution could aid in the separation of pheophytin from an unidentified xanthophyll which appeared just below the zone of pheophytin (Perkins and Roberts, 1962).

For preparing columns, two spoonfuls of the mixture adsorbent were tightly and uniformly pressed with a cork tamper into a vertically held glass tube (2.5×25cm) which was plugged with cotton wool at the tapered base, until the column was 20cm in length. In packing the adsorbent, the tube was attached to a suction flask while applying 10 lbs/in^2 of vacuum with a sucker or vacuum pump. The top surface of the column was covered with a layer of sodium sulfate 0.5cm thick which helped to maintain a smooth surface and remove traces of moisture in the solvent and pigment solution. Before adding 3ml of previously prepared pigment solution, the column was washed with 50ml of redistilled petroleum ether (b.p. 50-60°C). Pigments stained on the column were washed out with a small amount of petroleum ether and 10% ethyl ether petroleum ether solution was added little by little until a gray pheophytin band was separated approximately 2cm from the green band of chlorophyll a, and then developed with 0.5% n-propanol petroleum ether until the pigments were fairly well separated. An effective solvent flow for good separation seemed to be between a 50 and 60 drop elution per minute.

Figure 1 shows an example of chromatogram on which at least eight different zones of pigment were resolved. These zones were identified with the absorption data described in Table 1. Figure 2 shows a visible absorption spectra of chlorophyll a, pheophytin a, and two other



derivatives which were considered allomerized products of chlorophyll a. Chlorophyllide a often appeared in certain samples but so little in amount that it had only qualitative significance. On the other hand, Chlorophyll d was supposed to be distributed in red algae. However, since it was not only indistinguishable from a pigment concentration like our samples, but was also negligible regarding its role in color changes, the loss or retention of chlorophyll a was determined as a major factor for the fading of green color of lavers. Those identified pigments, chlorophyll a, chlorophyll a' and pheophytin a were scraped out of the column with a sharp edged spatula, eluted in a definite amount of redistilled ethyl ether and their optical densities were measured in a spectrophotometer at wavelengths 662, 662, and 667 mμ respectively. The contents of pigments were computed using extinction coefficients measured by Smith and Benitez (1955) and presented in μgs per g of dry sample.

Fig. 1. A chromatogram of pigments in lavers
Column: Sugar-starch (75:29 W/W)
Solvent: n-Propanol-petroleum ether (1:200 V/V)

Table 1. Identity of Pigments in Lavers

Zone	Color	Solvent	Absorption Maxima (mμ)		Pigment
			Observed	Reported(*)	
A	green	ethyl ether			chlorophyllide a
B	blue green	〃	652, 605, 413, (398)		unidentified
1	light yellow	n-hexane	477, 448, 420		〃
C	blue green	ethyl ether	670, 624, 595, 430, (410)		〃
2	yellow	acetone	480, 450, (371)		lutein+zeaxanthin
D	blue green	ethyl ether	662, 612, 576, 530, 429, 409,	662, 615, 578, 533.5 430, 410	chlorophyll a
E	blue green	〃	〃	〃	chlorophyll a'
F	gray	〃	667, 607, 568, 530, 506, 470, 408	667, 609.5 560, 534, 505, 471, 408.5	pheophytin a
3	yellow	—	—	—	unidentified
4	yellow	acetone	(480), 450, —		α−, +β-carotene

(*) Smith and Benitez, 1955.

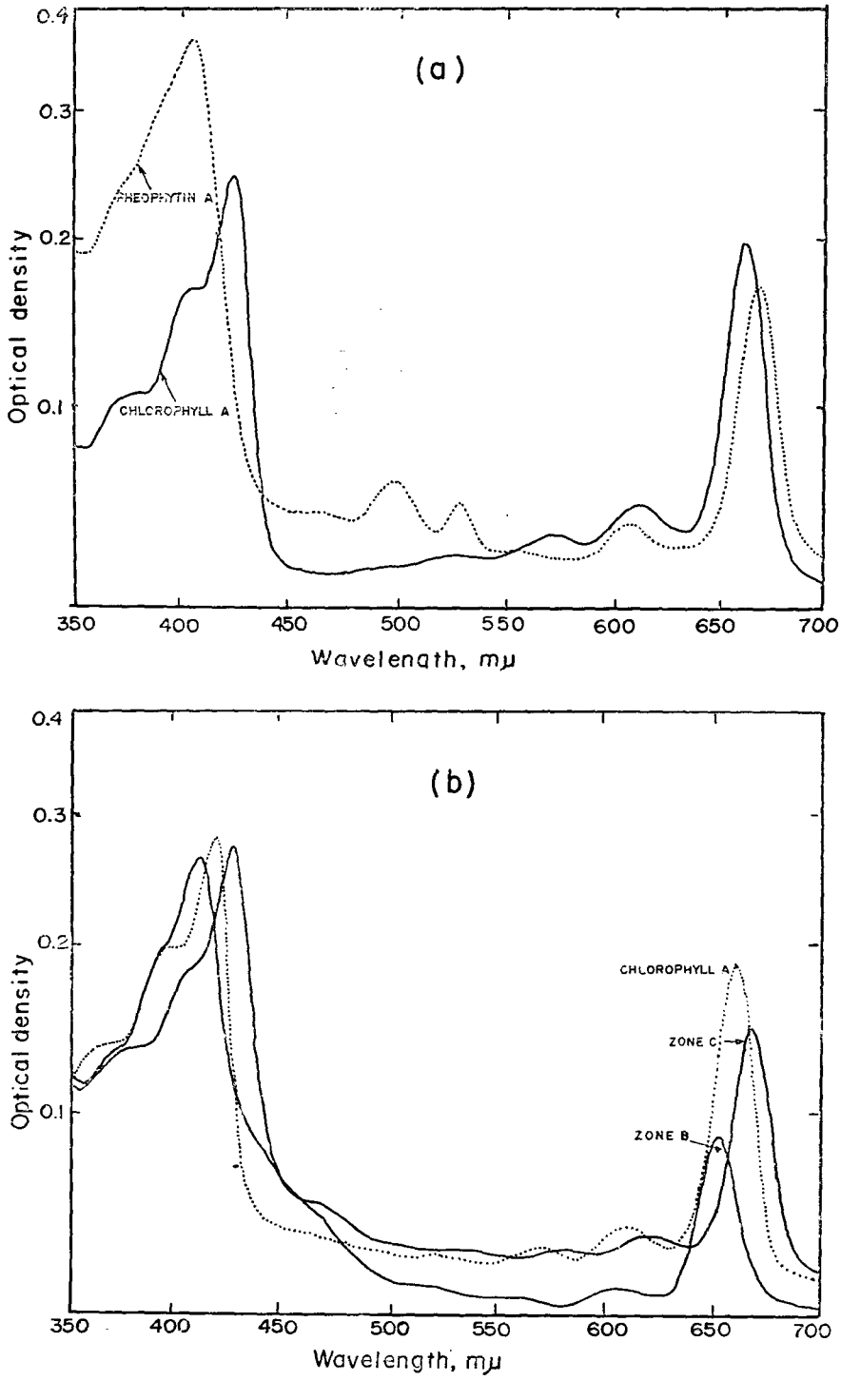


Fig. 2. Absorption spectra.

(a) Chlorophyll a and pheophytin a in ether.

(b) Two degradation products of chlorophyll a in ether.

Results and Discussion

Pigment Contents of Fresh Lavers

The chlorophyll a, carotene and xanthophyll contents of fresh lavers, as shown in Table 2, averaged 1,525, 627, and 409 μg/g of dry sample respectively. Actually the total carotene and xanthophyll covered the total amounts of α-carotene and β-carotene, and lutein plus zeaxanthin respectively.

Table 2. The Contents of Pigments in Fresh Lavers

Number of experiment	Chlorophyll a in μg/g	Xanthophylls in μg/g	Carotenes in μg/g
1	1,679	496	386
2	1,529	523	409
3	1,525	715	415
4	1,357	773	423
Average	1,525	627	409

Chlorophyll a Retention during Storage at Low Temperatures

It is a well known fact that pigments in plant tissues after harvest are rapidly degraded by the influence of temperature, pH, and the action of enzymes. The loss of pigments not only results from the shipping of fresh lavers from the culture field to the factories but also in storage prior to processing especially in the case of bad weather. As a matter of fact wet fresh lavers are often left in the corner of factories because drying is unavaliable under cloudy or rainy conditions. Consequently, the lavers become rotted and discarded at worse and at best the products are of very poor quality to include color quality. The first experiment, therefore, was attempted to determine the influence of coldness and freezing on the stability of pigments during storage at low temperatures. A batch of fresh lavers was stored in a refrigerator at 2 to 5°C while another batch was stored in a freezer at −15°C. The results of chlorophyll a retention in both cases are shown in Table 3, and compared with that of lavers stored at room temperature (20 to 25°C). The results showed that chlorophyll a was rapidly lost even at low temperatures. Approximately 20% of the pigment already disappeared during a 2 day storage period at room temperature. Freezing generally is more effective than cold storage in retarding enzymatic reactions and spoilage of food. The result, however, was contrary as far as the stability of the pigment was concerned. In a 2 week storage period at 2 to 5°C and −15°C, 37 and 40% chlorophyll a was lost respectively. It is assumed that a high rate of chlorophyll a degradation in frozen materials can be attributed to acid catalysed conversion of the pigment to pheophytin a, owing to mechanical damages of algal tissue by freezing. In the analysis of pigments on sugar columns more formation of pheophytin a was confirmed in frozen materials. In fact, it was also noticed that deterioration of freshness of materials partially occured after a 2 week storage period at 2 to 5°C, but a period of not more than one week would insure preservation of freshness and resistance to bad weather. Since pigment degradation appeared to be more serious than the deterioration of freshness, as an alternative method to preserve the quality of materials it was propcsed that lavers be stored in chilled sea water for several days as this would

Table 3. Chlorophyll a Retention in Fresh Laver during Storage

Storage Time (Days)	Chlorophyll retention		
	Room Temp. %	2 to 5°C %	−15°C %
0	100.0	100.0	100.0
1	87.2	—	—
2	82.7	—	—
7	—	73.8	65.4
15	—	63.1	59.8

result in both better quality preservation and better pigment stability.

Retention of Chlorophyll a during Heat Treatment

Heat treatment of lavers The effects of heat are expected to reduce the moisture level, inactivate enzymes, and retard other undesirable reactions during a long period storage of dried lavers as does blanching when applied to fruits and vegetables. However, the circumstances are slightly different from those in the blanching of fruits and vegetables. In the heat treatment of lavers, heat lability of algal pigments ought to be primarily considered because of their heat instability, particularly that of biliproteins. As a matter of fact, packers usually apply a low temperature long time treatment. A practical method is for lavers previously sun dried to be stored for 2 to 3 days in a large chamber with still air at 30 to 40°C until the moisture content decreases enough for long term storage. The final moisture content usually maintained is approximately 7.0%.

Lavers have to be sun dried before heat treatment in order to avoid shrinkage caused by rapid heating while materials are wet. But sun drying involves another problem since it takes a full day which is long enough to cause the loss of pigments by radiation. Aronoff and Mackinney (1943) early reported that in a system exposed to oxygen and red light, the destruction of chlorophylls was enhanced and proceeded in a straight forward manner. This is true even of drying in the shade. Therefore, two sets of experiments were attempted to compare the results of heat treatment when sun-dried and wet materials were treated. Sun drying is eliminated, when wet lavers are treated. Lavers, both sun-dried and wet, were treated for 2 hours in a hot air dryer with fast air circulation at temperatures of 40, 60, 80, and 100°C, respectively. The moisture content was determined imediately after the treatment and the pigments were analysed. Chlorophyll a and its degradation products were isolated on sugar-starch column. The chlorophyll a retained was quantitatively measured and others were qualitatively discussed according to the appearance on the zones of the sugar columns.

Table 4. Effects of Heat Treatment on Chlorophyll a Retention

Heat treatment (°C)	Sun dried materials		Wet materials	
	Moisture (%)	Chlorophyll a retention (%)	Moisture (%)	Chlorophyll a retention (%)
Control	11.7	100.0	15.2	100.0
40	7.7	98.4	10.8	98.3
60	6.5	97.6	9.6	96.3
80	5.0	94.3	6.8	86.9
100	3.4	81.8	4.0	94.6

Heat lability of chlorophyll a The results of Table 4 showed the decreases in moisture level and percent retention of chlorophyll a during heat treatment at each temperature applied. Sun dried lavers were 2 to 3 percent lower in moisture content than wet lavers in each case. As shown in Table 4, chlorophyll a tended to be more labile at high temperatures than at low, but chlorophyll a was comparatively more heat stable than carotenes and xanthophylls, particularly at a high temperature. The worst case was the approximately 20% loss of the pigment at

100°C. At other temperatures, the pigment was ratarded more than 90% except for one at 80°C in the case of wet lavers. Comparing the results of sun-dried materials with wet materials, chlorophyll a was retained slightly more in the latter than in the former. This might suggest that the pigment stability is influenced by the moisture level during the treatment. It was also noted that shrinkage of lavers did not significantly occur at temperatures lower than 80°C.

From the results of a qualitative analysis of pigments on sugar-starch columns, it seems that the major route of chlorophyll a loss during heat treatment can be attributed to the conversion of chlorophyll a to chlorophyll a′ and enzymatic degradation by chlorophyllase. As shown in Figure 1, chlorophyllide a was detected on the top zone of the column and chlorophyll a′ was clearly separated from chlorophyll a zone which, however, was spectrically identical with chlorophyll a as reported by Strain (1958), Strain and Manning (1942), and Freed et al. (1954). On the other hand, Freed et al. (1954) also reported the separation of chlorophyll a″ on the same column but it was not clearly detectable in our samples. The conversion of chlorophyll a to a′ was considered an isomerization by many investigators. Strain (1955), however, examined the reaction and reported that the converted pigments would be degradation products at the initial stage of oxidation, pointing out that the conversion reactions between chlorophyll a′ and a, and between a″ and a′ were irreversible. In this experiment the reaction was considered the same way so that the chlorophyll a′ zone was excluded to compute the retention of chlorophyll a.

The degree of conversion of chlorophyll a to a′ tended to increase depending on a rise in the heat treatment temperature. This isomeric or oxidative conversion can also be induced by treating materials with alcoholic solvents as indicated by Aronoff (1953), but a control sample showed traces of a slight but constant amount of chlorophyll a′ in comparision with a considerable amount in heat treated ones.

The activity of chlorophyllase was not prominent during heat treatment. An almost identical amount of chlorophyllide a was detected in some of the samples but undetectable in the rest. The case of wet lavers treated at 80°C showed a slightly increased amount but this was also insignificant. It was expected that a considerably large amount of chlorophyllide a should be detected at 70 to 80°C if the enzyme was active during heat treatment, since the temperature is the optimum for the enzyme (Willstäter, 1928; Weast and Mackinney, 1940). In addition, pheophorbide a was also not detected on pigment analysis of the materials during storage.

Chlorophyll a degradation caused by acid was not serious because a negligible amount of pheophytin a was detected in a few samples only. Perhaps two hours heat treatment was short enough to provide time for these acid catalysed reactions.

From these results, it was concluded that the main reaction for chlolophyll a degradation during heat treatment is the conversion of chlorophyll a to a′ or any other oxidative reactions whose products could not be detected on sugar columns. And the enzymatic degradation by chlorophyllase was another responsible reaction for the loss of pigment, particularly at 80°C although the activity of the enzyme was not dominant in the materials.

Chlorophyll a Retention during Storage

In sun dried materials Heat treated lavers as described on previous pages were wrapped with

waxed paper, packaged in a polyethylene bag to prevent moisture intake, and stored in a dark place for fifty days at room temperature (20 to 25°C). Moisture intake during the fifty day storage was not more than 2% greater than the initial moisture content. Pigments were analysed at five or ten day intervals in the storage period and percent retention of chlorophyll a was calculated toward the initial pigment content of the control sample. The changes in chlorophyll a and percent retention of sun dried materials are shown in Table 5 and Figure 3. Each sample's pigment content at the initial stage of storage was the same as the content immediately after heat treatment.

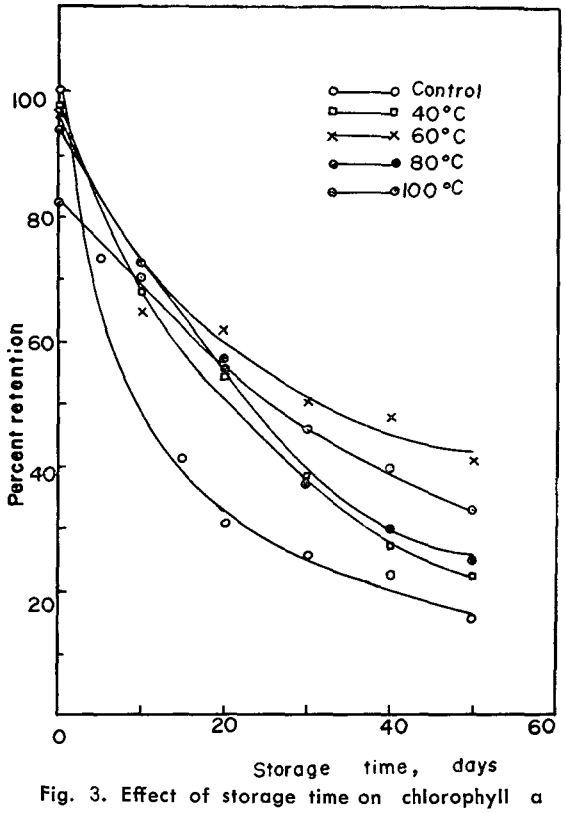


Fig. 3. Effect of storage time on chlorophyll a retention. Material: Sun dried lavers, Temp.: Room temp.

As shown in Figure 3, chlorophyll a tended to diminish very rapidly during storage. The rate of the pigment degradation, however, was dependent upon the conditions of previous heat treatment. The control without heat treatment showed the lowest pigment retention whereas the others showed a considerable degree of retention with variations depending on the heat treatment temperatures. The rate of the pigment degradation in the samples followed the first order reactions, except one which was treated at 100°C and was in a zero order reaction rate. The rate constant and the half life of the reaction at each temperature computed from the data in Table 5 are listed in Table 6. The control had a half life of 11 days and in both cases at 80 and 100°C showed a somewhat longer half life than the former. In heat lability of the pigment, 20% of chlorophyll a was lost during heat treatment at 100°C, but the pigment loss during storage appeared to be at a remarkably low rate. This effect might result from

Table 5. Effect of Storage Time on Chlorophyll a Retention

Storage time (Days)	Chlorophyll a retention, heat treated at;									
	Control		40°C		60°C		80°C		100°C	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0	615	100.0	605	98.4	600	97.6	580	94.3	503	81.8
5	451	73.3	455	74.0	—	—	—	—	—	—
10	253	41.1	407	67.8	400	65.0	446	72.5	430	85.4
20	183	29.8	334	54.3	381	62.0	350	56.9	336	54.6
30	152	24.7	232	37.7	306	50.0	230	37.4	285	46.3
40	140	22.8	167	27.3	293	47.6	184	30.0	247	40.2
50	94	15.3	143	23.3	246	40.0	152	24.7	203	33.0

Table 6. Rate Constant and Half Life for the Loss of Chlorophyll a

Heat treatment (°C)	K_1 value $K_1 \times 10^{-2}$	Half-life (days)
Control	6.14	11.2
40	3.39	20.4
60	1.91	36.1
80	2.56	27.0
*100	(K_0) 1.26	32.5

* At 100°C, the K was calculated as in zero order reactions.

sufficient heat inactivation of enzymes which are directly or indirectly involved in the pigment degradation reactions. In the case of an 80°C temperature the degradation rate was faster than in the case of 100°C. This might be due to the effect of chlorophyllase activation at its optimum temperature even though the enzyme reacted very slowly. Walker (1964) reported a similar result in stating that chlorophyll a retention is less in frozen beans blanched at 70–80°C than in that at 100°C.

Heat inactivation of enzymes has been studied by many investigators. Dietrich et al. (1959) suggested for the condition of inactivation of catalase and peroxidase that beans should be blanched for a short time at at least 93 to 100°C. Dietrich and Neumann (1965) mentioned that catalase was more rapidly inactivated than peroxidase at the same conditions, and survival of peroxidase played a great role in degradation of chlorophyll a during storage of beans. Eheart (1969) examined the effect of blanching on the stability of chlorophyll a in storage and reported that the pigment was retained more in hot water blanched broccoli than in steam blanched.

In this experiment, sufficient inactivation of enzymes by heat treatment at 100°C seemed to result in the lowest rate of pigment degradation during storage. On the other hand, the other temperatures were believed to be somewhat low for complete inactivation of the enzymes and consequently resulted in a faster rate of pigment degradation during storage following the first order reactions. In spite of a slight pigment loss during heat treatment at 40°C, the rate of pigment degradation was greater than in other cases. In contrast, the sample treated at 60°C showed a remarkable retention of chlorophyll a during storage although the temperature was still too low to inactivate enzymes. These results, therefore, suggest that there must be factors other than enzyme action involved in the pigment degradation.

In the results of column chromatographic analysis of pigment changes during storage a slight or negligible amount of chlorophyllide a and a large amount of pheophytin a were detected, and chlorophyll a′ was obviously fading or faded away. Besides, two other blue green zones often appeared in between chlorophyllide a and xantophyll zones (see Figure 1). The absorption spectra of these two zones shown in Figure 2 showed two peaks at the wavelengths 10 mμ shorter or longer than that of chlorophyll a (see Table 1). Coodwin (1958) reported that a chlorophyll a degradation product showed the maximum absorption of red light at 652 and 635mμ instead of the 662 and 644mμ for chlorophyll a. Strain (1958) also reported that allomerized derivative of chlorophyll a had an absorption maximum at the wavelength 20mμ shorter than that of chlorophyll a. From these results two chloroghyll a like zones identified on the sugar column were presumed to be non enzymatically degraded products of chlorophyll a. The appearance of the zones might postulate that chlorophyll a was degraded by oixidative reactions during storage even if the products appeared in small amounts and were sometimes undetectable. Afterwards, these products would be decomposed to certain lower molecular colorless compounds as mentioned

by Seybold (1943).

Mackinney and Weast (1940) early reported that the loss of chlorophyll a during prolonged processing was mainly due to the effects of plant acids. Dutton et al. (1943) also reported that, in unblanched dehydrated spinach, chlorophylls were completely converted to pheophytins in 16 week period storage although the rate was lowered when water content decreased. The main pathway of chlorophyll a degradation during the storage of lavers appeared to be the acid catalysed conversion of chlorophyll a to pheophytin a. As shown in Table 7, the formation of pheophytin a correlated closely with loss of chlorophyll a. The rate constant of pheophytin formation was

Table 7. Conversion of Chlorophyll a to Pheophytin a
Material: Heat treated sun dried lavers, Storage temp.: Room temp.

Storage time (Days)	Loss of chlorophyll a (Chl. in μg/g) and formation of pheophytin a (Pt. in μg/g) at various temperatures of heat treatment									
	Control		40°C		60°C		80°C		100°C	
	Chl.	Pt.	Chl.	Pt.	Chl.	Pt.	Chl.	Pt.	Chl.	Pt.
5	164	78	150	79	—	—	—	—	—	—
10	362	175	198	145	200	182	134	68	73	55
20	432	342	271	207	220	221	230	145	167	170
30	463	415	373	257	294	268	350	266	218	210
40	471	388	375	263	307	391	396	392	356	165

approximately 0.01 in all cases, and the conversion reaction roughly followed the first order reaction. It might, therefore, suggest that the conversion reaction is held in a state of equilibrium so that pheophytin a was gradually increased. The rate of the conversion reaction is also influenced by the process of self decomposition in pheophytin a. This possibly occurs during a long period of storage.

The above results suggest that the chlorophyll a degradation reactions during storage involved at least three different factors such as enzymatic, acid catalysed, and non enzymatic oxidation reactions. The two major aspects of the pigment loss, however, seem to be the conversion of the pigment to pheophytin a by acid and heat lability of pigment since enzymatic and oxidation reactions like allomerization did occur but they were minor during both heat treatment and storage of the materials. At high temperature enzymes were inactivated but the pigment was markedly degraded by the effects of heat. Moreover, oxidative degradation was possibly accelerated whereas a considerable degree of moisture level was retained and the conversion of the pigment by acid

Table 8. Effect of Storage Time on Chlorophyll a Retention
Material: Heat treated wet lavers, Storage temp.: Room temp.

Storage time (Days)	Chlorophyll a retention, heat treated at;									
	Control		40°C		60°C		80°C		100°C	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0	535	100.0	499	93.3	515	96.3	466	86.9	506	94.6
10	317	59.3	350	65.4	323	60.4	—	—	—	—
15	—	—	—	—	—	—	258	48.2	313	58.5
20	193	36.1	239	44.7	269	50.3	226	42.2	274	51.2
30	168	31.4	183	34.2	195	36.5	190	35.5	187	34.9
40	141	26.4	159	29.7	186	34.8	151	28.1	162	30.3
50	120	22.4	134	25.0	146	27.3	112	20.9	129	24.1

was dominant at a lower temperature. These two contrasting phenomena might be restrained at a certain intermediate temperature such as 60°C at which the influence of degradation reactions is less than at the high and low temperature extremes as was confirmed in the results of this experiment. As a matter of fact chlorophyll a was remarkably retained in the materials treated at 60°C. The half life was 35 days under the given conditions in which heat treatment was limited to 2 hours only and oxygen control was not directed during storage. Further extension of half life would be expected if heat treatment was prolonged beyond 2 hours and the atmosphere of containers was controlled.

In Wet Materials The results were quite different from the case of sun dried materials. As shown in Table 8 and Figure 4, chlorophyll a was rapidly lost regardless of the conditions of heat treatment. The final pigment retention was similar to that of sun dried materials but the rate of pigment degradation was much greater following the first order reactions. Even the one that indicated the best retention of pigment at 60°C maintained no more than a 20 day half life. The values of rate constant (K_1) and half life are described in Table 9. Another noticeable difference was that the sample treated at 100°C held a longer half life than the one treated at 60°C although th_e retention percentage of the pigment was lower. It was presumed from this result that either the heat durable enzymes survived or that because of a comparatively high moisture the acid conversion of chlorophyll a to pheophytin a was accelerated. However, a distinctive increase of chlorophyllide a was not detected in the pigment analysis on the columns, so that the enzymes other than chlorophyllase which had an indirect effect on the pigment loss were involved if enzymatic activity was an influence. Since the pigment conversion to pheophytin by acid was confirmed as the major pathway of the pigment degradation emphasis had to be put on the acid reactions. It is, therefore, supposed that the moisture level of lavers retained after heat treatment is the most important factor for the stability of the pigment in storage.

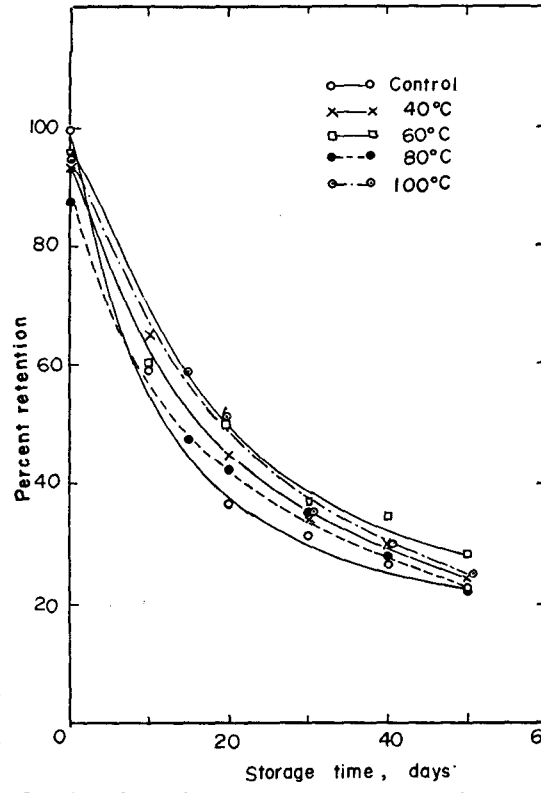


Fig 4. Effect of storage time on chlorophyll a retention. Material: Wet lavers, Storage temp.: Room temp.

Table 9. Rate Constant and Half Life for the Loss of Chlorophyll a Material: Wet lavers, Storage temp.: Room temp.

Heat treatment (°C)	K_1 value ($K \times 10^{-2}$)	Half life (Days)
Control	5.16	13.3
40	3.46	19.9
60	3.43	20.1
80	3.24	21.2
100	2.96	23.3

From observations of chlorophyll a stability during storage, it is concluded that the heat treatment conditions of a 2 to 3 day period treatment at 30 to 40°C in a large chamber as is presently applied by packers, must be improved. Our results suggest that better pigment retention can be obtained from materials treated at 60°C. Treatment at 60°C in a dryer with fast air circulation requires adequate time to retain less than a 6.5% moisture but not much lower, or treat ment at 100°C requires a short time to minimize the loss of pigment by heat. Alternately, acid control of algal tissues is necessary to retard the acid conversion of chlorophyll a to pheophytin a as far as the reaction is the major pathway of the pigment degradation.

Effect of pH on Chlorophyll a Conversion

In general, food color is sensitive to pH change and each pigment has a certain range of pH for its stability. Chlorophylls are said to be stable in the range of pH 7.0 to 8.0 and slightly acidic media (Kamada and Katayama, 1966). Sweeney and Martin (1961) also reported that chlorophylls in vegetable foods were quite unstable at pH 6.0 to 7.0 and become fairly stabilized when treated in a pH controlled solution at above 7.0. In this experiment, lavers were dispersed in the solution f pH maintained at 6.3, 7.0, and 7.8 by the addition of odium carbonate and phosphate buffer solutions, and the l ms or sheets of lavers were prepared as usual. Dried sheets f lavers in the shade were stored at room temperature for fifty days and the pigment was analysed at 10 day inter- va ls during the storage period. The results show as seen in Figure 5 that the pigment was retained more in the samples treated at pH 7.0 and 7.8 than in the case of pH 6.3. There was not so much difference between pH 7.0 and 7.8 but that the pigment could be stabilized by treating materials with weak alkaline solution was indicated as far as chlorophylls are concerned. Regarding the fact that chlorophyll a conversion by acid produced the most significant reaction of pigment loss during storage, the control of pH of the materials is essential for stabilization of chlorophyll a in this respect.

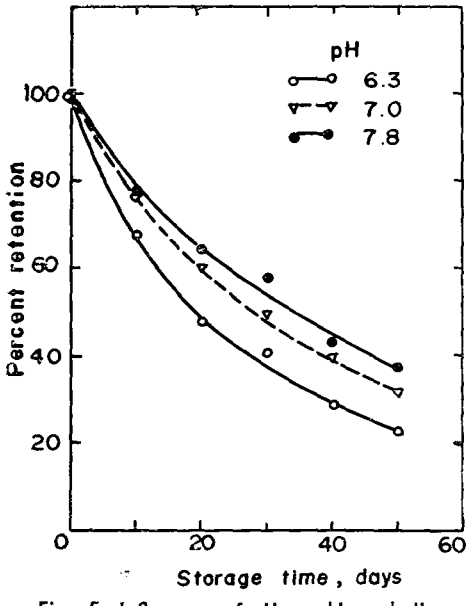


Fig. 5. Influences of pH on chlorophyll a retention during the storage of dried lavers

Effect of Additives on Uniformity and Surface Gloss of Dried Laver

As mentioned in earlier pages, preparation of thin films or sheets of lavers of uniform thickness requires trained skill. Customers often complain about the uniformity, rents, or lack of gloss with deep tint of colors. Reducing the size of chopped material is more or less effective in yielding a better uniformity of the films but it involves a problem of more pigment being lost during the preparation procedure due to the more finely chopped material. The gloss of the films in a fresh and carefully prepared product may be lost due to mechanical damages to algal tissues during chopping or the loss of certain water soluble components throughout the preparation.

From these points of view, the effects of several additives were tested. These were sodium

alginate, gum arabic, agar, and Teligen (product trade name). Chopped lavers were suspended in these solutions and dried sheets of lavers were prepared according to the usual method. Since the solutions of the additives are viscous, an adequate concentration of the solutions was determined first. The concentration of 10mg% of agar, gum arabic, and sodium alginate gave rapid drainage of excess water during the procedure, and a 1.5mg% solution of Teligen was also found to be convenient. As these solutions are very stable colloids, the suspension of laver pieces was interfered with when their concentrations were higher than that indicated above. The solutions greatly helped to both keep the suspension stable, and to keep uniform dispersion and spreading of chopped lavers. It also seemed that pigment loss, particularly water soluble biliproteins, was reduced compared with the result when tap water was used. In addition, gloss was also maintained or promoted as a thin film of polysaccharides remained on the surface of the lavers after drying. Agar solution seemed to give a better effect for gloss but its insolubility in cold water was a minor incovenience during the treatment.

Effect of Pigment Fixing Agents

It has been a well known fact that heavy metals like Cu, Mn, Ni, and Fe form complexes with chlorophylls thus stabilize the pigment. In our experiment, $CuSO_4$ was used for the pigment fixation. The result, however, was not clear. It was presumed that the time taken for preparation of laver sheets was not long enough for the required reaction of the fixing pigment because the preparation procedure must be done as fast as possible to avoid swelling of tissues and the loss of pigments.

II. Stability of Carotenoid Pigment in Lavers during Processing and Storage

Analysis of Carotenoids

Extraction of Carotenoids Carotenoid pigments were extracted by the same method used in the extraction of chlorophylls as described in the previous section using the methanol-petroleum ether mixture (2:1 v/v). As a further procedure for carotenoid pigment extraction, 50ml of methanol extracts were saponified for 20 minutes on a magnetic stirrer with the addition of 7.5g potassium hydroxide granules. Saponified pigment solution was transferred to a 500ml volume separation funnel and equal volumes of ether and 10% sodium chloride solution were added to partition chlorophylls into the methanolic hypophase. The extracts of pigments were washed

Table 10. Identity of Carotenoids in Lavers

Zone	Color	Solvent	Absorption maxima (mμ) Observed	Absorption maxima (mμ) Reported(*)	Pigment
Silica	**gel G-MgO(1:1)**				
1	yellow	n-hexane	483, 451, 424	483, 451, 423	zeaxanthin
2	〃	〃	476, 446, 419	477, 447, 420	lutein
Hyflo	**super cel-MgO(2:1)**				
1	yellow	petroleum ether	480, 450, 425	482, 451 (425)	β-carotene
2	〃	〃	473, 444, 420	473, 444, 422	α-carotene

(*) Goodwin (1965)

thoroughly five times with 50 ml of distilled water, dehydrated with an adequate amount of powdered sodium sulfate, and then evaporated to dryness in a rotary vacuum evaporator. The pigments were finally dissolved in petroleum ether making a volume of up to 10ml as the sample for the separation of carotenoids on the columns.

Determination of Pigment Contents Confectionary sugar columns were used for the separation of carotenes from xanthophylls. Sugar columns were prepared in the same way that the chlorophyll separation in the previous section was done except that straight sugar was used instead of sugar with 25% corn starch added. Columns were developed with 0.5% n-propanol petroleum ether or 5% acetone petroleum ether solution. As shown in Figure 6(a), carotenoids were resolved in four isolated zones but two of them remained unidentified. The rest of them were identified as a mixture of carotenes and xanthophylls. The zones of carotenes and xanthophylls were rechromatographed for further identification of individual carotenoids on both columns of silica gel G-MgO (1:1 w/w) and Hyflo-super cel-MgO (2:1 w/w). The former column was developed with 15 or 20% acetone petroleum ether to resolve lutein and zeaxanthin whose chromatogram is shown in Figure 6(b). And α-carotene and β-carotene were separated on the latter with 5 or 10% acetone petroleum ether solvent (see Figure 6-c). Identification of these pigments was done according to the absorption data described in Table 1 and Table 10, and Figure 7, and the chromatogram of authentic pigments isolated from spinach leaf. The solvents, absorption maxima, and extinction constants used for the determination of their relative contents in fresh lavers, are listed in Table 11. On the other hand, for the study of pigment loss or retention during processing and storage, two mixture zones of carotenes and xanthophylls resolved on sugar columns were individually dug out, eluted in a definite volume of refined acetone, and their optical densities then measured at 450mμ (Yamamoto et al., 1962).

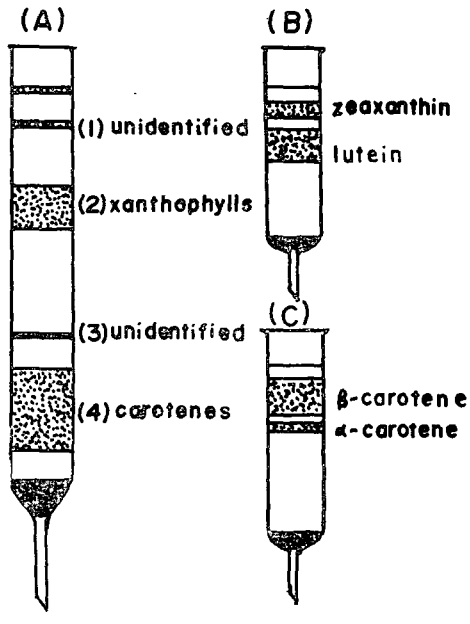


Fig. 6. Chromatograms of carotenoids in lavers.
Column:
(a) Sugar,
(b) Silica gel G-MgO(1:1),
(c) Hyflo super-cel-MgO(2:1)

Table 11. Absorption Maxima and $E^{1\%}_{1cm}$

Carotenoid	$E^{1\%}_{1cm}$	λmax	Solvent
α-carotene	2,800	445	petroleum erher
β-carotene	3,505	451	〃
lutein	2,380	456	chloroform
zeaxanthin	2,350	451	n-hexane

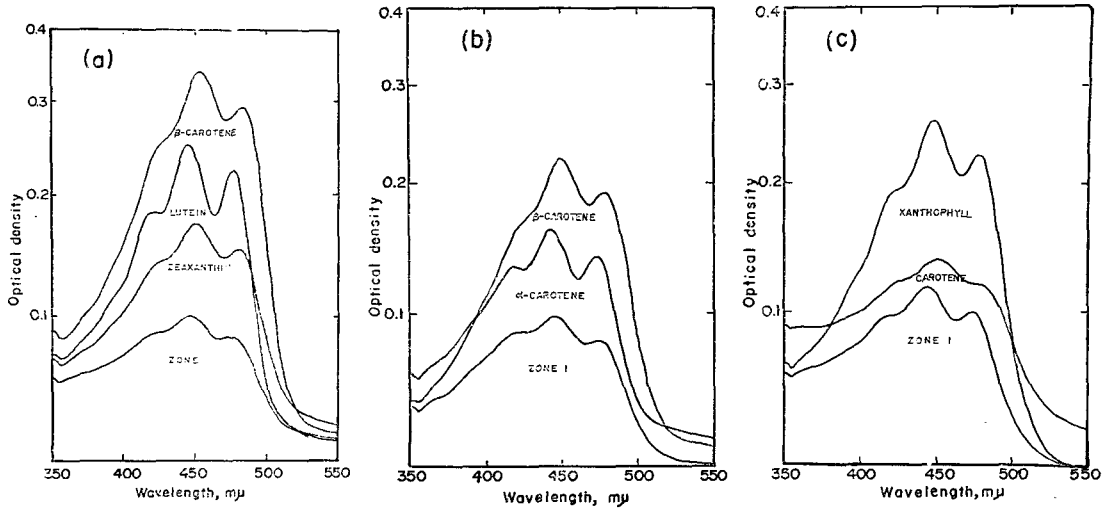


Fig. 7. Absorption spectra of pigments in lavers.
Solvents: (a) n-Hexane, (b) Petroleum ether, (c) Acetone

Results and Discussion

Carotenoid Composition of Laver

Heilbron and Lythgoe (1935) and Heilbron et al. (1936) early reported that β-carotene and lutein were two major carotenoids in red algae. In addition to these pigments, α-carotene and zeaxanthin were also found to be distributed in the algae by Strain (1958) from the results of chromatographic separation of carotenoids. Katayama (1964) recently measured the relative contents of these four carotenoids by thin layer chromatography and stated that β-carotene covered more than 60% of the total carotenoid content while the others were in little amount. In contrast, the results of this experiment showed that the contents of β-carotene, lutein, zeaxanthin, and α-carotene covered 33.7, 35.9, 12.2, and 5.8% respectively. Since several hours aged lavers from harvest were used for the experiment, it was presumed that certain conversion or degradation reactions might occur in shipping or storage of the materials. However, the conversion of epoxy carotenoid to hydroxy carotenoid occurred in seaweed post-mortem indicated by Goodwin (1965) could not be seen because of the lack of violaxanthin in *Porphyra tenera*. Moreover, Braarud and Sörensen (1956) confirmed in the same material that there was no change or interconversion of β-carotene and fucoxanthin to either violaxanthin or zeaxanthin. The results may suggest, therefore, that β-carotene is rapidly degraded in lavers even in a short time after harvest, and if not, the pigment composition of the material is to be different depending on the environmental conditions of cultivation fields.

Retention of Carotenoid Pigment during Storge at Low Temperatures

Changes in total carotene and xanthophyll in fresh lavers were measured during the storage at room temperature, 2 to 5°C, and −15°C and percent retention of the pigments is shown in Table 12. The loss of the pigments was greatly retarded at low temperature as the results show that at worst not less than 60% of carotenes were retained after 2 week storage in both cold storage and freezing. But a far lower rate of pigment retention was obtained after only 2 day storage at room temperature. It has been believed that freezing and thawing damage algal tissues through which the degradation reactions prevail, and that the treatment is not beneficial for the preservation of fresh lavers. It was also confirmed that cold storaged materials show more stability than in frozen materials. However, for weather preservation of fresh materials prior to processing, a few days storage at 2 to 5°C is advised. Relative stability of xanthophylls appeared more or less larger than that of carotenes in all cases.

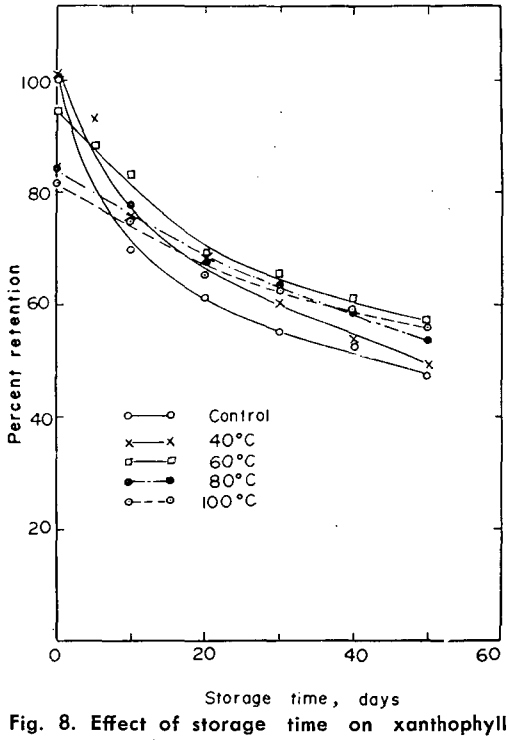


Fig. 8. Effect of storage time on xanthophyll retention.
Material: Heat treated sun dried lavers, Storage temp.: Rôôm temp.

Heat Lability of Carotenoids

Table 13 shows the results of carotenoid pigment retention and the change in moisture levels during heat treatment at various temperatures in both sun dried and wet lavers. As shown in the Table, carotenes appeared extremely labile to heat, particularly in sun dried materials. At worst more than 40% of the pigment was lost at 100°C while xanthophylls were comparaptively stable retaining above 80% at the same temperature. In wet materials, both xanthophyll and carotene were retained more than in sun dried materials. Moisture content in the

Table 12. Retention of Carotenoids in Fresh Lavers during Storage

Storage time (Days)	Percent pigment retention					
	Room temp.		2 to 5°C		−15°C	
	Xantho.	Carotenes	Xantho.	Carotenes	Xantho.	Carotenes
0	100.0	100.0	100.0	100.0	100.0	100.0
1	102.6	52.7	—	—	—	—
2	78.8	40.7	—	—	—	—
7	—	—	95.7	79.3	79.9	81.8
15	—	—	86.0	64.6	73.8	75.8

Table 13. Effects of Heat Treatment on Carotenoid Pigment Retention

Heat treatment (C°)	Sun dried materials			Wet materials		
	Moisture (%)	Xantho. (%)	Carotene (%)	Moisture (%)	Xantho. (%)	Carotene (%)
Control	11.7	100.0	100.0	15.2	100.0	100.0
40	7.7	101.2	76.1	10.8	92.7	88.5
60	6.5	94.4	66.0	9.6	92.2	82.8
80	5.0	84.1	57.8	6.8	86.7	78.8
100	3.4	81.3	59.5	4.0	83.0	70.4

wet was also 2 to 3% higher than in sun-dried materials. This suggests that the stability of the pigment during heat treatment was affected by the moisture levels in the materials.

Kimura (Kimura and Shioda, 1963a; Kimura et al. 1967) ran a model test for the acceleration of lipid oxidation in food with low moisture level reported by Salwin (1963), and confirmed the instability of carotenoid pigment in dehydrated food. He suggested that at an extremely low level of moisture, carotenoid existed in a crystalline state so that the pigment could easily come into contact with oxygen and thus oxidation was accelerated. If the moisture level is high in food the layers of water molecules can protect the pigment from the attack of oxygen. Pigment lability to heat can be influenced by moisture level and the state of food tissues. The result of carotenoid being lost much more in the sun dried materials than in the wet materials can be explained as in the case of oxidative degradation where certain colorless products were formed, because any charateristic colored zone did not appear on the sugar columns.

Our results were compared with the loss of carotenoid occuring in vegetables during hot air drying at 60 to 93°C and storage reported by other investigators (Hendel, 1953; DellaMonica and McDowell, 1965; Sibasaki, et al. 1966). Their results showed 10 to 40% pigment loss during heat treatment. Our results on sun dried materials turned out to be the worst case. This might result from the lower moisture level than in vegetables as well as mechanical damages of algal tissues through chopping in the preparation procedure.

Also noteworthy was the fact that carotenes were much more heat labile than xanthophylls, particularly in sun dried materials. A similar result was reported by Goodwin (1965) that β-carotene was more unstable than other epoxy carotenoids and xanthophylls.

Table 14. Effect of Storage Time on Xanthophyll Retention
Material: Heat treated sun dried lavers, Storage temp.: Room temp.

Storage time (Days)	Xanthophyll retention									
	Control		40°C		60°C		80°C		100°C	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0	321	100.0	325	101.2	303	94.4	270	84.1	261	81.3
5	—	—	302	94.1	283	88.2	—	—	—	—
10	223	69.5	242	75.4	267	83.1	250	77.9	238	74.1
20	197	61.4	218	67.9	220	68.5	214	66.7	209	65.2
30	183	57.0	192	60.0	212	66.0	206	64.1	204	63.5
40	173	53.9	169	53.0	195	60.9	186	58.0	187	59.0
50	151	47.0	156	48.6	182	56.7	170	53.0	176	54.8

Stability of Xanthophylls during Storage

The lutein-zeaxanthin zone composing total xanthophyll was isolated on the sugar columns and the changes in its content and percent retention were determined at steps during a fifty day storage period. The results from sun dried materials are shown in Table 14 and Figure 8 and wet materials in Table 15 and Figure 9. The results show that xanthophylls are more stable than chlorophyll a and carotenes. The cases of most rapid degradation of the pigment showed at 40°C as was true in the control sample, kept about 50% of pigment retention and more than 50 days of half life. Wet materials seemed to show slightly more retention than the sun dried ones but there was no outstanding difference between them. As in the case of chlorophyll, both samples treated at 60 and 100°C showed better retention than in other cases. This may suggest that the stability of the carotenoid pigment is closely related to the potentiality of other pigments as a whole system having influence each upon the other. The degradation of carotenoid pigment also involved two contrasting aspects; one was enzymatic degradation and the other was oxidative loss linked to the fat-oxidizing system as described in the former paragraph. A great amount of the pigment degradation at 100°C during heat treatment was due to accelerated oxidation in an extremely low moisture medium and the decreased rate of pigment loss during storage owes to the complete inactivation of enzymes. As mentioned earlier, oxidative reactions were influenced by the moisture

Table 15. Effect of Storage Time on Xanthophyll Retention
Material: Heat treated wet lavers, Storage temp.: Room Temp.

Storage time (Days)	Xanthophyll retention									
	Control		40°C		60°C		80°C		100°C	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0	218	100.0	202	92.7	201	92.2	189	86.7	181	83.0
10	170	78.0	166	76.3	182	83.4	172	79.1	170	78.0
20	147	67.4	151	69.4	163	74.9	153	70.4	152	69.7
30	132	60.5	137	63.1	147	67.4	144	66.2	145	66.3
40	125	57.3	122	55.9	141	64.7	137	63.0	142	65.1
50	123	56.4	122	55.9	134	61.4	130	59.2	139	64.0

Table 16. Effect of Storage Time on Carotene Retention
Material: Heat treated sun dried lavers, Storage temp.: Room temp.

Storage time (Days)	Carotene retention									
	Control		40°C		60°C		80°C		100°C	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0	235	100.0	177	76.1	155	66.0	136	57.8	140	59.5
10	149	63.4	146	62.1	134	57.0	124	52.8	126	53.6
20	116	49.3	111	47.5	113	48.0	114	48.5	114	48.5
30	99	42.1	101	43.0	107	45.5	110	46.8	107	45.5
40	92	39.1	93	39.5	104	44.3	107	45.5	102	43.4
50	85	36.1	89	37.8	100	42.5	90	38.3	102	43.4

level of the materials. This may explain why wet materials showed a slightly greater retention than sun dried materials during storage. The results also suggest that oxidative degradation is predominant over the other reactions like enzymatic activity.

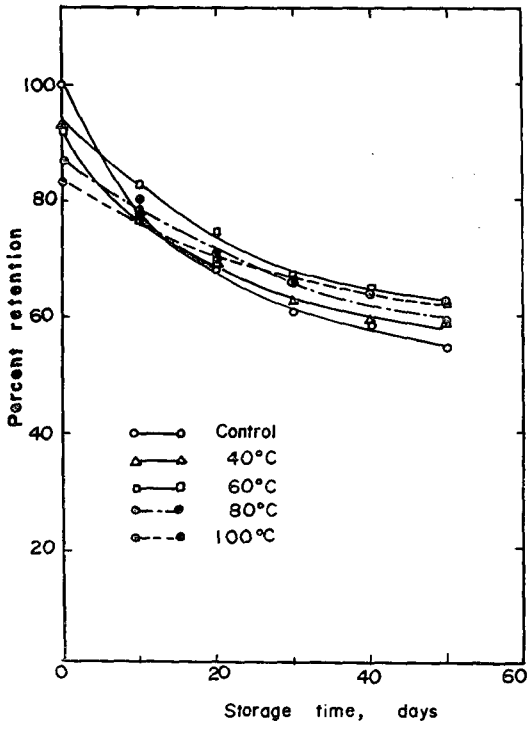


Fig. 9. Effect of storage time on xanthophyll retention. Material: Heat treated wet lavers, Storage temp.: Room temp.

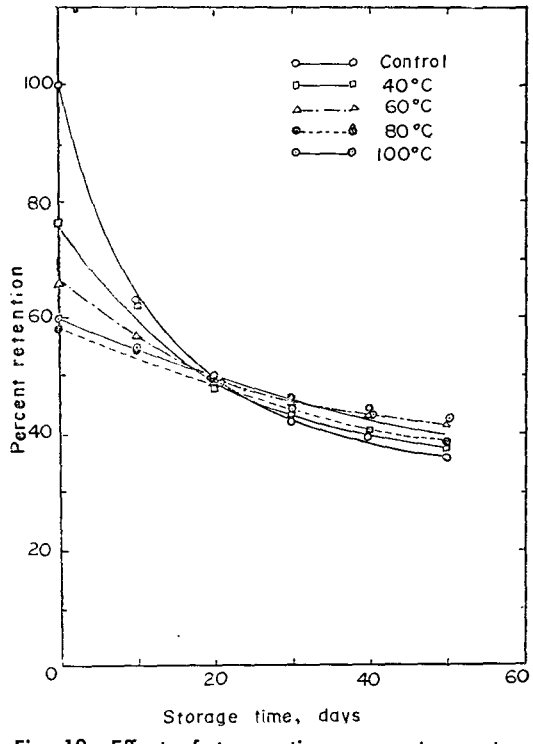


Fig. 10. Effect of storage time on carotene retention. Material: Heat treated sun dried lavers, Storage temp.: Room temp.

Table 17. Effect of Storage Time on Carotene Retention
Material: Heat treated wet lavers, Storage temp.: Room temp.

Storage time (Days)	Carotene retention									
	Control		40°C		60°C		80°C		100°C	
	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
0	140	100.0	124	88.5	116	82.8	110	78.8	98	70.4
10	112	80.0	108	77.3	105	75.0	105	74.8	95	68.2
20	93	66.2	96	68.6	100	71.3	98	70.4	89	64.0
30	87	62.5	90	64.5	98	70.4	93	66.4	88	62.7
40	83	59.0	85	60.7	91	65.0	86	61.4	84	60.0
50	76	54.3	79	57.1	88	53.0	77	55.0	80	57.4

Stability of Carotenes during Storage

A mixture zone of α-carotene and β-carotene was analysed as total carotenes in the same way as in xanthophylls. The results are shown in Table 16 and Figure 10 from sun dried materials and in Table 17 and Figure 11 from the wet ones. It is noteworthy that wet materials showed much greater carotene retention during storage than sun dried ones although the general tendency was not far from the results in both the cases of chlorophyll a and xanthophylls. This result suggests that the moisture content retained after heat treatment is critical to the stability of carotenes and that the pigment is degraded mainly by oxidative reactions since moisture in algal tissues

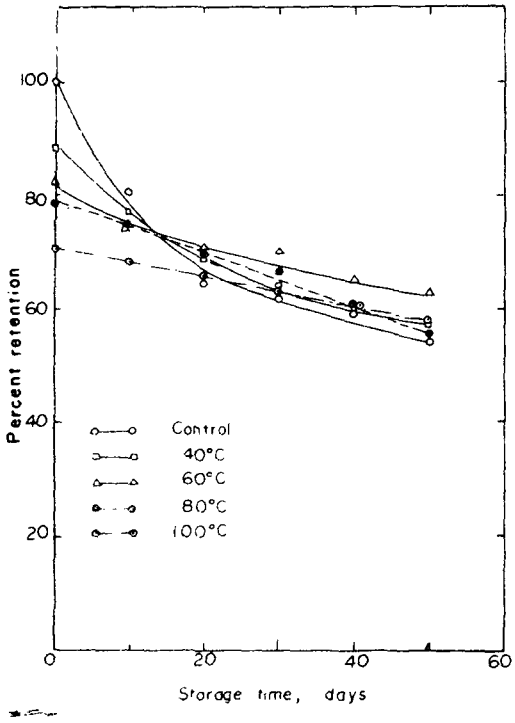


Fig. 11. Effect of storage time on carotene retention. Material: Heat treated wet lavers, Storage temp.: Room temp.

has a protection effect upon pigment degradation as indicated in the results of heat lability. Carotene is one of the major carotenoids in red algae as confirmed in the analysis of pigment in fresh lavers. It was reported by Goodwin (1965) that carotene degraded more rapidly than epoxy carotenoids and xanthophylls during the storage of plant tissues. The present result confirmed this as the sun dried materials showed only a 20 day half life of the pigment degradation reaction.

From the results of carotenoid pigment retention during storge, it is concluded that oxidative degradation reactions predominate over other reactions so that an adequate moisture retention should be maintained for their protection effect on the pigment stability. Carotenes were more unstable than xanthophylls and chlorophyll a in both heat treatment and storage. It was also noticed that both carotenes and xanthophylls were retained more in the materials treated at 60°C than in the others even though a considerable amount of carotene loss was unavoidable at that temperature.

Effect of Antioxidants

Many researchers (Deobald et al., 1964; Purcell, 1962; Johnson et al., 1964) have reported that the application of antioxidants retards the oxidative degradation of carotenoid pigment in plant tissues. Such antioxidants as BHA, BHT, PG, or α-tocopherol were commonly used with the addition of synergistic compounds like ascorbic acid and citric acid. Deobald et al. (1964) reported that the loss of carotenoids was minimized in dried sweet potato when 200 ppm of Tenox IV, the mixture of BHA and BHT (50:50 w/w), with 100 ppm of citric acid was used, and that the effect was enforced by the control of the container atmosphere. Purcell (1962) also similarly reported that the effect of antioxidants is obviously promoted when the atomosphere of the containers is replaced with nitrogen gas. Johnson et al. (1964) obtained a year extention of shelf life of dried apple sauce by the use of antioxidants. On the other hand, Kimura and Shioda (1963b) proved the prevention of oxidative degradation of carotenoids using 150mg% BHA with 150 to 300mg% of isoascorbic acid in freeze dried tomato juice and they developed an indirect addition method which resulted in better retention of carotenoids than in the direct method.

In this experiment, BHA and BHT were examined for their effect in retarding the loss of carotenoids during storage.

Sheets of lavers were prepared with the materials soaked in 300ppm solutions of both anti-

oxidants, dried and stored at room temperature. The results, however, are uncertain. It is presumed that the antioxidants can not be uniformely distributed on the surface of the materials in such a short period of time during the preparation procedure. And the viscous suspension of chopped materials can also be a factor preventing the uniform distribution of the agents.

III. Color Stability of Biliproteins in Lavers during Processing and Storage

Analysis of Phycobilin Pigments

The characteristic red and blue color of lavers are presented by three kinds of biliproteins namely phycoerythrin, phycocyanin, and allophycocyanin. The spectral properties of the pigments from *Porphyra tenera* were studied by Hattori and Fujita (1959a). They crystallized the biliproteins from different kinds of algae (Hattori and Fujita, 1959b) and measured the absorption maxima and their extinction constants at the maxima (Fujita and Hattori, 1960). The phycobilins obtained from lavers showed the absorption maxima at 560, 620, and 650mμ for phycoerythrin, phycocyanin, and allophycocyanin respectively.

In this experiment, the color loss of the phycobilins was measured by the decreases in optical densities of the pigment extracts at the absorption maxima during processing and storage of lavers. For the extraction of the biliproteins, 5g of fresh or 1.5g of dried lavers were blended for 5 minutes in a Waring blender with 100ml acetone cooled previously at $-15°C$. The blend was transferred into a 250ml Erlenmyer flask and stored for 2 days in a freezer at $-15°C$, in order to extract fat soluble chlorophylls and carotenoids. The acetone extracts of these pigments were filtered through a fine glass filter and the residue was thoroughly washed out with an adequate amount of acetone until the filtrate became clear. The residue washed out with acetone was then put into a 25ml flask and the biliproteins were extracted overnight in a freezer at $-15°C$ by the addition of 200ml phosphate buffer solution keeping pH controlled at 6.5. The pH of the buffer was adjusted with the mixture of M/15 Na_2HPO_4 and M/15 KH_2PO_4 (3:7 v/v). A portion of the extracts of biliproteins was decanted and the optical densities of the pigment solution were measured at 560, 620, and 650mμ. These wavelengths correspond to the absorption maxima of phycoerythrin, phycocyanin, and allophycocyanin respectively. With the measurement of optical densities at the maxima at the steps of processing or storage, the percent retention of the pigment was computed and the stability of the phycobilins is discussed herein.

Results and Discussion

Phycobilin Retention in Fresh Lavers during Storage at Low Temperatures

Fresh lavers were stored for 2 weeks at 2 to 5°C and $-15°C$ respectively. The percent retention of biliproteins was computed and shown in Table 18. The results show that the biliproteins are also more stable in cold storaged materials than in frozen ones as was shown in the case of other pigments like chlorophyll a and carotenoids. It is generally believed that the biliproteins are vulnerable to the effects of temperature changes since the pigments are combined with proteins. The loss of the pigments during storage at low temperature resulted possively due

to the denaturation of proteins by the effects of temperature. The stability of biliproteins at low temperature appeared to be lower than that of chlorophyll a and carotenoids. Phycocyanin was degraded much more than phycoerythrin in both cold storaged and frozen materials. In a 2 week period of storage, more than 70% of phycocyanin was lost even at 2 to 5°C. It is, therefore, suggested that the weather preservation of fresh lavers should not be longer than 5 days as far as the stability of bilibroteins is concerned.

Heat Lability of Biliproteins

The sun dried materials were heat treated at various temperatures as was done in the previous experiments. From the results of the experiment shown in Table 19, it is indicated that, in contrast to the result at low temperature, phycoerythrin is more heat labile than the others. Phycocyanins were retained slightly more than phycoerythrin but they were also quite heat unstable. Comparing their stability to that of carotenes, phycobilins in sun dried materials appeared to have better retention during heat treatment. An important aspect in heat lability of phycobilins, however, is that the pigments tend to degrade rapidly even at low temperatures whereas carotenes show considerable stability at low temperatues.

Table 18. Phycobilin Retention in Fresh Lavers during Storage.

Storage time (Days)	Percent retenton of optical denstity					
	At 2 to 5°C			At −15°C		
	PE	PC	APC	PE	PC	APC
0	100.0	100.0	100.0	100.0	100.0	100.0
4	94.0	89.7	89.7	88.6	84.3	85.1
7	89.5	61.4	66.9	81.2	60.9	61.7
10	78.8	49.7	47.3	64.3	41.3	47.8
15	64.5	32.2	38.8	52.7	29.6	34.8

Remark; PE: Phycoerythrin
PC: Phycocyanin
APC: Allophycocyanin

Table 19. Effects of Heat Treatment on Phycobilin Retention

Heat treatment (°C)	Percent retention of optical density		
	Phycoery-thrin	Phycocy-anin	Allophyco-cyanin
Control	100.0	100.0	100.0
40	88.6	86.1	88.8
60	84.1	77.5	87.7
80	75.4	73.8	77.4
100	6.0	69.5	69.8

Retention of Phycobilins during Storage

Figure 12 (a–c) shows the percent retention of phycobilins during a 40 day period storage of sun dried materials. It is noticed from the results that biliproteins are very rapidly degraded during storage but retained slightly more than in the case of chlorophyll a. It is also a remarkable point that heat labile phycoerythrin shows higher stability than phycocyanins in storage of materials. The effects of heat treatment on the stability of the pigments was not obviously detected as was shown in the case of chlorophyll a. It is believed that discoloration of biliproteins is caused by denaturation of proteins linked to the

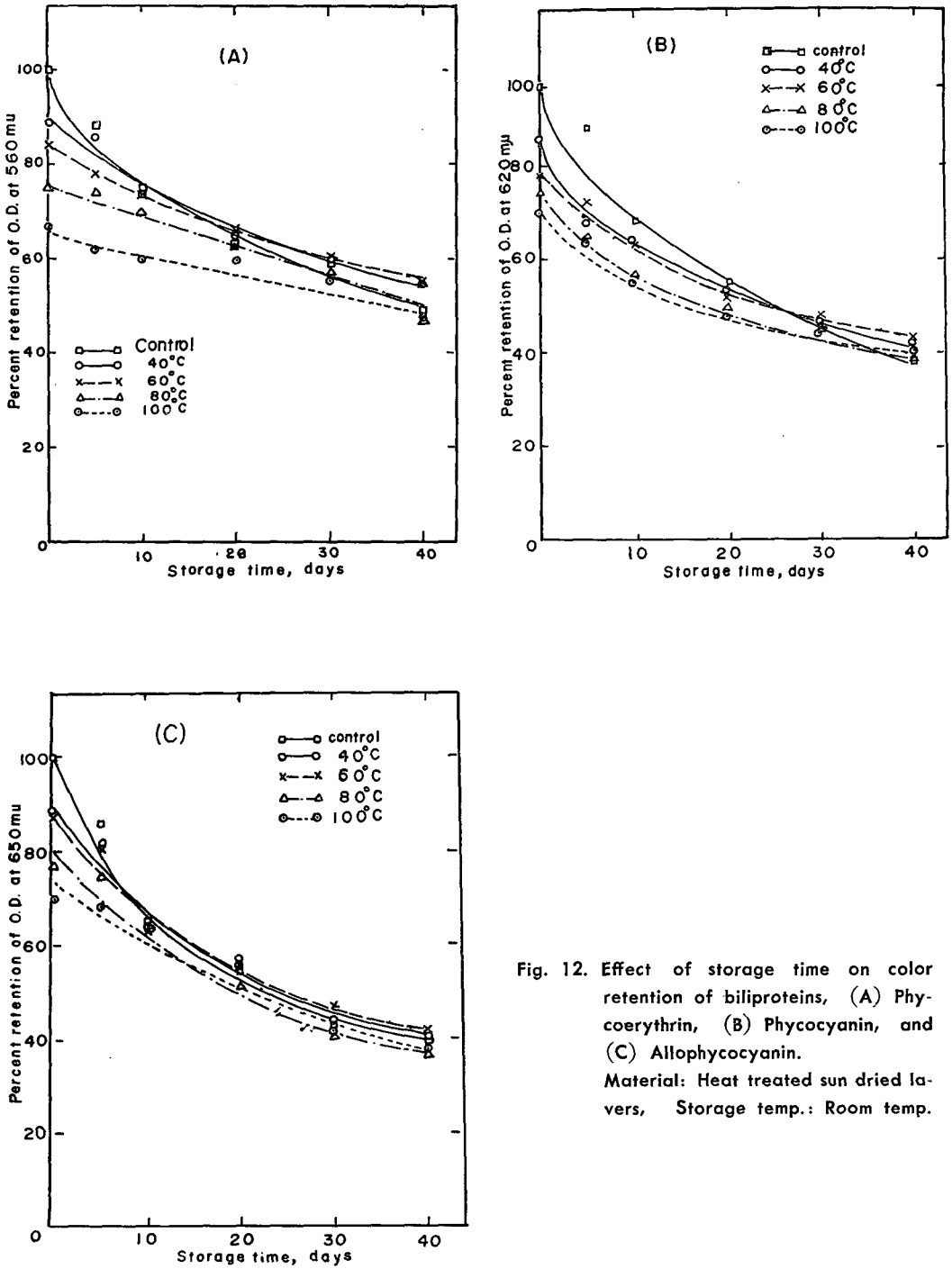


Fig. 12. Effect of storage time on color retention of biliproteins, (A) Phycoerythrin, (B) Phycocyanin, and (C) Allophycocyanin.
Material: Heat treated sun dried lavers, Storage temp.: Room temp.

pigments. The denaturation of proteins are generally promoted by heat and dehydration so that heat treated lavers at high temperatures are to be in low retention of phycobilins. The facf that a fast rate of the pigment loss appeared also at low temperature, may postulate that the factors other than denaturation of protein involved in the discoloration of the phycobilins. The enzymes which are active for hydrolysis of proteins can play a role in the discoloration of the biliproteins. In addition, acid hydrolysis of porteins can also be responsible to the discoloration.

Summary

The stability of three major pigments in lavers, namely chlorophylls, carotenoids, and phycobilins, during processing and storage was studied. The results of the pigment retention in fresh lavers during storage at low temperatures, of heat lability during heat treatment, and of the pigment loss during storage are summarized as follows:

1. The contents of chlorophyll a, xanthophyll (lutein+zeaxanthin), and carotene (α-+β-carotene) averaged 1,525, 627, and 409 μg/g of dry samples respectively. The comparative composition of four carotenoids, lutein, β-carotene, zeaxanthin, and α-carotene, was 35.9, 33.7, 12.2, and 5.8% respectively.

2. In storage of fresh lavers at room temperature (20—25°C), 2 to 5°C, and −15°C, the pigments generally were retained more at 2 to 5°C than others while carotene only showed more retention at −15°C than at 2 to 5°C. Phycocyanin tended to decrease more rapidly than phycoerythrin at low temperatures.

3. In regard to heat treatment of both dried and wet materials at 40, 60, 80,100°C respectively, the pigments were more stable at lower temperatures than at higher temperatures in both cases of materials. Carotenoids were retained more in wet materials than in the sun dried. Xanthophylls showed higher heat stability than carotenes. Phycoerythrin was considered more heat labile than phycocyanin, particularly at higher temperatures.

4. In storage of heat treated lavers for 50 days at room temperature, the retention of pigments appeared differently in accordance with the temperatures of treatment. The materials treated at 60°C showed better retention of pigments than those treated at the other temperatures. Degradation rate of xanthophylls, carotenes, and phycobilins was decreased in the materials treated at higher temperatures, showing a tendency of great pigment retention even for a long period storage. Chlorophyll a was retained more in sun dried materials whereas xanthophyll and carotene were retained more in the wet.

5. In order to maintain a higher pigment stability during storage, the materials must be treated at 60°C in a dryer with rapid ventilation until the moisture level reaches about 6.0%. In case of treatment at a higher temperature than 60°C, they must be treated for a short period of time to minimize pigment degradation by heat.

6. The lavers treated with the solutions of pH around 7.8 showed a higher retention of chlorophyll a. When treated with the solutions of algal extracts such as agar and alginic acid, uniform layer of laver sheets and better surface gloss of dried lavers were obtained.

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