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Reasons for the High Proportion of Pheophytin in Edible Plant Oils

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To reduce the pheophytin (Phy) level in oils, it is necessary to understand the reasons of the high proportion of Phy in refined edible oils. In this investigation, the compositional changes in chlorophyll (Chl) and Phy during extraction and some refining processes were evaluated by photofluorometric analysis.

The composition of Chl pigments in crude oils was examined when Canola seed oil was extracted with 6 kinds of organic solvents. The proportion of Phy was found to be the highest (97%) in oil extracted with *n*-hexane, but 38% in hexane-ethanol (3 : 1) extracts, and 13% in chloroform-methanol (2 : 1) extracts. Especially, when the residue following extraction with *n*-hexane was further extracted with chloroform-methanol (2 : 1), the proportion was only 3%. From these results, it is concluded that the use of *n*-hexane as a solvent reduces the extraction of Chl from oil seeds.

Next, Canola seed oil was extracted with *n*-hexane for different extraction times and at different temperatures. Moreover, the changes in Chl during acid treatment of the crude oil were investigated in a model system. A portion of the Chl was converted into Phy during heat or acid treatments. However, in our previous study, a relatively constant compositional ratio between Chl pigments was observed in rapeseed oils throughout the process of mass production. The conversion of Chl to Phy during heat or acid treatment in model experiments may thus be assumed insignificant in practical refining for mass production.

1 Introduction

It is well known that the oxidative deterioration of fats and oils is greatly enhanced by light, especially in the presence of chlorophyll (Chl)s. Our previous reports^{1),2)} have shown that the four pigments of Chl and pheophytin (Phy) are present, in general, in concentrations of 0.06 to 0.3 ppm in refined edible plant oils and a high proportion of the pigments, over 80%, was observed to be Phy. In addition, Phy showed a higher prooxidant activity and higher stability through the photooxidation of triglycerides than did Chl³⁾⁻⁵⁾. Therefore, it was concluded that the Phy content must be noted, when considering the oxidative stability of edible oils.

In order to reduce the Phy level in oils and to improve their quality, it is necessary to understand the reasons for the high proportion of Phy in refined edible oils. It is interesting to see if a high proportion of Phy are present

in oil seeds, or if it is caused during production processing. In this study, the compositional changes occurring in Chl and Phy during extraction and some of the oil refining processes were evaluated by photofluorometric analysis.

2 Experimentals

2-1 Extraction of oils

Oil was extracted from Canola seed with 6 kinds of organic solvent. That is, 3g of Canola seeds (moisture content 5.8%) were homogenized in 20 mL of organic solvent for 10 s using an ULTRA TURRAX (Janke & Kunkel KG Ika-Werk), and filtered with suction and washed. Oil yields, phosphorus content⁶⁾, and concentration and composition of Chl pigments of this crude extracted oil were then evaluated.

Moreover, Canola seed oil was extracted with *n*-hexane for different extraction times and different temperatures, followed by a similar analysis of the pigment composition by the photofluorometric method²⁾.

2.2 Acid treatment of oils

Three kinds of 10% (wt/wt) acid aqueous solution (oxalic, citric and phosphoric acids) were added to 25 g of methyl linoleate with 2.2×10^{-7} mol of Chl a, to the level of 0.2%, and heated at 80°C with mixing. In addition, phosphoric acid aqueous solution was added to two kinds of crude rapeseed oil to the same concentration and treated in a similar manner.

3 Results and Discussion

3.1 Composition of chlorophyll pigments of oils extracted with several solvents

First, how the pigment composition of an extracted oil would vary kind with of organic solvent used was investigated. As shown in **Table-1**, the proportion of Phy reached 97% in oil extracted with *n*-hexane, while it was 38% in the hexane-ethanol (3 : 1) extract, and 13% in the chloroform-methanol (2 : 1) extract. When the residue after extraction with *n*-hexane was further treated with chloroform-methanol, the Phy was only 3%. This result indicated that in the oil seeds Chl is present in a much higher proportion, over 90% of pigments, and the use of a polar solvent

increased the proportion of Chl in the crude oil.

Next, by the addition of surfactant to the solvents to the 1% level, variation in Chl composition was investigated. Addition of Triton X-100 caused an increase in the proportion of Chl (**Table-2**). In this Table, the total pigment concentration after the addition of surfactant is indicated as being lower than that without surfactant. This seemed to be caused by surfactant remaining in the oils.

It is generally believed that Chl is bound to protein in the chloroplast lamellae, which is the normal location for Chl in plant tissue⁷⁾. From these experimental results, it is presumed that Phy is not situated in the chloroplast lamellae and are released into stroma in the process of catabolism, so that the use of *n*-hexane as a solvent is seen to reduce the extraction of Chl from oil seeds.

A number of oil seeds extraction processes employing isopropyl alcohol and ethyl alcohol as solvents have been tried on a laboratory scale, mainly from the standpoint of oil yield and quality of defatted protein. However, there has been no report on the pigment composition in oil extracted by a mixed solvent

Table-1 Composition of chlorophyll pigments in oils extracted with several organic solvents.

Solvent*	Yields (%)	P Content (%)	chl a	Chl b	Phy a	Phy b	Total #	Ratio
H	22	0.0083	0.14	0	3.22	0.80	4.16	3 : 0 : 77 : 20
H-E (3 : 1)	27	0.066	23.31	7.78	13.35	5.39	50.03	47 : 15 : 27 : 11
HP-E (3 : 1)	30	0.088	40.40	14.79	15.43	7.61	78.23	51 : 19 : 20 : 10
C-M (2 : 1)	37	0.057	45.92	14.51	7.22	2.13	69.78	66 : 21 : 10 : 3
HP-E (3 : 1) after H	12	0.15	88.03	32.64	27.68	17.89	166.24	53 : 19 : 17 : 11
C-M (2 : 1) after H	17	0.15	92.01	24.77	1.78	2.35	120.91	76 : 20 : 1 : 2
C-M-W (1 : 2 : 0.8)	23	0.14	12.22	5.43	8.20	1.22	27.07	45 : 20 : 30 : 5
Acetone-W (4 : 1)	18	0.20	1.27	12.26	59.16	10.30	82.98	2 : 15 : 71 : 12

* Ab. : H, Hexane ; HP, Heptane ; E, Ethanol ; C, Chloroform ; M, Methanol ; W, Water ; # mg/kg

Table-2 Effect of surfactant on solvent extraction for 24 h at room temperature.

Solvent	Chl a	Chl b	Phy a	Phy b	Total #	Ratio
H	0.43	0.65	4.45	0.98	6.51	7 : 10 : 68 : 15
+ Triton X-100*	0.63	0.30	2.04	0.33	3.30	19 : 9 : 62 : 10
H-E (3 : 1)	31.64	11.97	17.02	9.21	69.84	45 : 17 : 28 : 21
+ Triton X-100*	27.46	9.45	13.71	7.01	58.13	47 : 17 : 24 : 12

* Addition level, 1% # mg/kg

process. In this experiment, we estimated quantitatively for the first time the Chl composition in oils extracted with different solvents.

3.2 Variation of chlorophyll composition with temperature and time for heating

The heat treatment of seeds during the extraction process is thought to be a second reason for the high proportion of Phy, because Chl decomposes or partly changes to Phy on heating^{8),9)}. Johansson and Appelqvist¹⁰⁾ reported that the drying of the moist seeds caused a transformation of some Chl to Phy.

After extraction of oil from Canola seed with 3 kinds of solvent for different extraction times and at different temperatures, the Chl composition of the crude oils was analyzed (Fig.-1). A high proportion of Chl was observed in oil extracted in hexane for 2 h, compared with that extracted for 0.5 h, or at 60°C, compared with that at room temperature. Moreover, with the other solvents, we found a similar tendency, although a high proportion of Chl was observed, even in extraction at room temperature.

The chlorophyll composition of crude oils obtained from rapeseed at different temperatures and for different pre-heating times was investigated. Before extraction with *n*-hexane, rapeseeds were heated at 40 to 105°C for 30 to 90 min (Fig.-2). The results suggest that the proportion of Chl increased during heating below 60°C, while on heating at higher temperatures, as indicated in the 105°C case, a portion of the Chl in the rapeseed decomposes or is converted to Phy.

Fig.-1 Variation of chlorophyll composition in crude rapeseed oils with extraction temperatures and times.

Fig.-2 Variations of chlorophyll composition in crude oils with temperature and time of pre-heating of rapeseeds.

In general, a heating process at 105°C for 30 min, the so-called "cooking", has been widely practiced before solvent extraction in the production of commercial edible oil. Thus, high proportion of the Phy in edible plant oils may be partly caused by heat during the extraction process.

3.3 Variation of Chlorophyll Composition with Acid Treatment

It is a well known fact that Chl is converted into Phy, by losing Mg under acidic conditions, and citric or phosphoric acids may often be used in the degumming of crude extracted oils. Therefore, in order to learn the effect of acid treatment on the Chl composition in oils, we added acid aqueous solutions such as oxalic, citric and phosphoric acids to methyl linoleate with Chl and heated at 80°C.

From the results of compositional analysis of Chl pigments in the methyl linoleate mixture treated with 3 kinds of acid aqueous solution, larger amounts of Chl are converted into Phy in proportional to heating time, regardless of the kind of acid (Fig.-3).

In the next experiment, the effect of phosphoric acid on crude rapeseed oil extracted with two different solvents was investigated. As shown in Fig.-4, a portion of the Chl was converted into Phy with the passage of time during acid treatment of the hexane-ethanol extracts, which contained a relatively high proportion of Chl. On the other hand, there were no changes in the hexane extracts, which contained a high proportion of Phy.

These results suggest that during the de-

After 10% (wt/wt) acid aqueous solution was added to methyl linoleate containing Chl a and Phy a, at the level of 0.2%, the mixture was shaken and heated at 80°C.

Fig. 3 Change in chlorophyll and pheophytin composition during acid treatment of methyl linoleate.

Fig. 4 Conversion of chlorophyll to pheophytin during phosphoric acid-treatment of crude rapeseed oils.

gumming process part of the Chl may be converted into Phy, in extracted oils with a high proportion of Chl.

In our previous paper¹⁾, a relatively constant pigment composition in rapeseed oil was observed through a series of the processes in mass production, although one of our co-workers observed last year that the adsorption efficiency of the four pigments on a bleaching clay decreased in the following order; Chl a > Phy a > Chl b > Phy b¹¹⁾. It can therefore be presumed that the conversion of Chl to Phy during the heating and acid treatment of seeds or crude oil in the model experiments may be insignificant in practical refining for mass production.

Johansson and Appelqvist¹⁰⁾ reported the content of green pigments in rapeseeds oil (assayed at A_{670} or "chlorophyll") as effected by seed storage, mode of drying and nature of the extraction solvents. They also inferred

a high proportion of Phy in heptane-ethanol (3:1) extracts from the decrease in quotient between the peak heights (at 671/536 nm), although the Phy content was not determined quantitatively. Our observations in this experiments confirm their assumption.

To summarize the results of this study, in the oil seeds, Chl is present in a much higher proportion, over 90% of the pigments, and is bound to protein. In contrast, Phy is present in the free state, so that it is predominantly extracted at a level of over 85% of total pigments with *n*-hexane. During the extraction and degumming processes, only part of the Chl can be converted in to Phy. However, in the final refined oil, Phy is present in lower concentrations but in the same proportion to Chl as in crude oil, when *n*-hexane is used as the extraction solvent.

Through these experiments the reasons for the high proportion of Phy in edible plant oils were manifested. We can achieve low levels of Phy in refined oils when extraction is carried out at lower temperatures and when the exposure to heat is minimized during the following purification. Moreover, the Chl-free oil can be prepared using column chromatography¹²⁾ and supercritical gas extraction^{13),14)} on a laboratory scale. However, in industrial production, the Chl and Phy levels in the refined oils should be considered from the viewpoint of production cost.

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食用植物油にフェオフィチンが 高比率で存在する理由

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油脂の光酸化促進性及び油脂の酸化時の安定性がクロロフィル (Chl) より高いフェオフィチン (Phy) が精製食用植物油になぜ Chl より高比率で存在するかについて検討した。なたね種子 (カノーラ種) に含まれる油脂を6種の溶媒で抽出し、色素量とその組成を蛍光定量によって分析した。その結果、ヘキサンを用いた際に Phy が 97% も占めたが、溶媒の極性をあげると Chl 抽出量が増えることがわかった。このことは、ヘキサン抽出では Chl とタンパク質の結合が切れないために Phy が優先的に抽出されたものと推定された。

なたね粉砕物を加熱あるいはなたね抽出油を酸処理した際の色素組成の変化を検討したところ、Chl の一部が Phy に変化していることが認められたが、実際の製油工程では始めから Phy の比率が圧倒的に高いので、この変化はあまり問題にはならないと考えられる。

◎本年1月号より、米国油化学協会 (AOCS) で昨年4月以来毎月刊行されている国際油脂情報 (International Newsletter on Fats and Oils, 略称 I.N.F.O.) から選んで要約し、本誌に掲載することにいたしましたのでご利用下さい。

国際油脂情報

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