行政院國家科學委員會專題研究計畫成果報告

以螢光分析法檢測粉末食品之氧化劣變

Fluorescence Analyses of Oxidation Deterioration of Powdered Foods

計畫編號：NSC87-2314-B-041-003

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一、摘要

黃豆粉氧化產生之螢光性成分可分離至氯仿-甲醇(CM)萃取液之有機層中，其最大激發波長(ex.)為350nm、放射波長(em.)為440nm。黃豆油單獨存在時，氧化產物螢光之變化不明顯；與脫脂黃豆粉混合時，氧化產物之螢光強度隨油脂含量增加而上升。氧化黃豆油與大豆蛋白作用後，會造成蛋白質之游離氨基含量、溶解度、及可溶性蛋白質之疏水性下降。此類螢光性成分與氧化油脂和蛋白質之作用有關。黃豆粉於上述波長之螢光強度隨時間上升，並分別對過氧化價(r=0.951)和TBA值(r=0.966)存在線性關係。

全脂奶粉以reflectance螢光法分析時，氧化試樣之ex.=365nm、em.=435nm。此氧化產物可分離至CM之有機層中，以transmission螢光法分析得ex.=350nm、em.=440nm。以reflectance或transmission法測定特徵波長之螢光強度，可用於分辨不同氧化程度之奶粉；其中transmission法分析值之變異係數較小(4~10%)。市售奶粉(八種)開封後於37℃儲存，各試樣過氧化價和TBA值隨時間之變化並無明顯的關係；但其中全脂奶粉(五種)之螢光強度對油脂含量之比值隨時間上升，經迴歸分析後可得到統一的線性關係式(r=0.885)，可供全脂奶粉氧化劣變之檢測。

關鍵詞：螢光分析；氧化；黃豆粉；奶粉

Abstract

The fluorescent compounds formed in oxidized soybean flour were soluble in the organic layer of chloroform-methanol (CM) extracts. The spectra of the organic layers showed excitation maximum (ex.) at 350nm and emission maximum (em.) at 440nm. The fluorescence intensity of bulk soybean oil did not change significantly with increasing oxidation. However, the intensity increased during storage in proportion to the oil content in the soybean flour/soybean oil system. The oxidized soybean oil interacted with soy protein isolate was found to contribute to decreased protein solubility, free amino group content, and the soluble protein hydrophobicity. This occurance would suggest the interaction of oxidized soybean oil and soy proteins resulted in the formation of intrinsic fluorescence. As the extent of oxidation proceeded, the fluorescence intensity of soybean flour increased with time and exhibited linear correlation with peroxide value (r=0.951) and TBA value (r=0.966).

The front-surface fluorescence of oxidized whole milk powder showed ex. 365nm and em. 435nm. The oxidized fluorescent compounds could be extracted to the organic layer of CM solvent, and the corresponding solution showed ex. 350nm and em. 440nm in a transmission spectrofluorometer. The extent of oxidative deterioration of milk powders could be determined by measuring either front surface fluorescence or transmission fluorescence. The transmission method, however, yield better results with less coefficient of variances (around 4~10%). Commercial milk powders (8 different kinds) were oxidized at 37 ℃ under air in the dark. The peroxide value and TBA value did not exist significantly correlation with storage time. Of

all the whole milk powders (5 kinds) investigated, the ratio of fluorescence intensity to lipid content increased with storage time. The correlation coefficient (r=0.885) and regression line of the relationship were presented and provided a means for the determination of oxidative deterioration in whole milk powders.

Keywords:spectrofluorometry; oxidation; soybean flour; milk powder

二、計畫緣由與目的

市售含油脂之蛋白質食品種類相當多，儲存期間品質之劣變及對健康之危害值得重視。本計畫針對黃豆粉、奶粉等大宗民生用品，以螢光分析法檢測氧化劣變程度。分析方式採用粉末試樣直接分析之 reflectance 螢光測定法，及針對萃取液分析之 transmission 螢光測定法。目的在探討試樣氧化產生之特徵螢光成分，及分析其螢光強度對儲存時間或氧化指標之關係，結果將有助於本類食品品質之檢驗，以杜絕劣質品之違規使用。

三、研究方法

市售黃豆粉、奶粉等以 reflectance spectrofluorometry 分析不同氧化程度粉末之 front-surface 螢光，並以溶劑萃取氧化產物，以 transmission spectrofluorometry 分析溶液螢光隨時間之變化(1)。另外測定過氧化價、TBA 值(2)，以及分析蛋白質性質之變化(3)，以探討氧化劣變相關之螢光性成分，及其對儲存時間和氧化程度之關係。

四、結果與討論

1. 黃豆粉

與新鮮者比較，氧化黃豆粉 front-surface 螢光在激發波長 350nm 、放射波長 440nm 之強度明顯上升。此螢光特徵之氧化產物可分離至氯彷-甲醇萃取液之有機層中，以 transmission 螢光法分析時(圖一)，有機層於 ex. 350nm 、 em. 440nm 出現尖峰；新鮮黃豆粉之最大激發波長在 270nm 附近，且 em. 440nm 之螢光強度較低。

黃豆油本身在氧化過程中之螢光變化不明顯；若定量脫脂黃豆粉中混合不同比率黃豆油時，則氧化產物 CM 有機層於 ex. 350nm 、 em. 440nm 之螢光強度隨黃豆油含量增加而上升(圖二)。精製大豆分離蛋白混合黃豆油(9:1,w/w ；試樣 PO)於 60 ℃儲存時，有機層於 ex. 365nm 、 em. 450nm 之螢光強度隨時間上升。儲存期間蛋白質之溶解度、游離氨基含量、及可溶性蛋白質之疏水性隨時間下降。於 PO 中添加抗氧化劑 BHT 時(試樣 POBHT)，可抑制螢光強度與蛋白質性質之變化(圖三)。此螢光性成分與氧化黃豆油和大豆蛋白之作用有關。

市售黃豆粉氧化過程中 ex. 350nm 、 em. 440nm 之螢光強度隨時間呈線性上升(圖四)，此螢光強度並隨過氧化價和 TBA 值之上升而變大(圖五)，其線性相關係數(r 值)分別為 0.951 和 0.966。此螢光性成分可作為黃豆粉氧化劣變之指標。

2. 奶粉

全脂奶粉開封後於 37 ℃儲放，粉末以 reflectance 螢光法測定時， ex. 365nm 、 em. 435nm 之螢光強度隨氧化程度增加(圖六)。利用本法檢測三種不同氧化程度之奶粉試樣時，試樣間之螢光強度測定值達到極顯著差異，但各試樣測定值之變異係數介於 20~30%(表一)，是屬於較不穩定之測定方式，其主要原因為分析時試樣之鬆密度不同所致。若將試樣先均勻分散於不具螢光之介質中再測定，則可改善此現象。當試樣分散於甘油/水中後以 reflectance 螢光法測定時，氧化奶粉之特徵波長出現在 ex. 350nm 、 em. 440nm 附近(圖七)。不同氧化程度試樣之螢光強度測定值列於表二，各試樣測定值之變異係數介於 13~23%。

上述螢光特徵之氧化產物可萃取至 CM 之有機層中(圖八)，以 transmission 法測定不同試樣於 ex. 350nm 、 em. 440nm 之螢光強度可分辨氧化程度(表三)，其中各試樣測定值之變異係數低於 10%，是三種測定方式中最理想者。

市售奶粉(八種)開封後於 37 ℃儲存，定時取樣分析過氧化價、 TBA 值，以及 CM 萃取液有機層之螢光強度。儲存期間過氧

化價對時間並無顯著之關係(圖九)。TBA值於530nm之吸收度趨近於0，此原因為奶粉油脂中三個(含)以上雙鍵之脂肪酸含量較低；當TBA反應液改測450nm之吸收度時，其測定值對時間之相關性仍不佳。

圖十為儲存期間各奶粉試樣CM萃取液有機層於ex. 350nm、em. 440nm之螢光強度。其中脫脂奶粉之螢光強度較低，且幾乎不隨時間而變；全脂奶粉之螢光強度較大，且隨時間有上升趨勢。若以螢光強度對油脂含量之比值對時間迴歸，則所探討的不同品牌全脂奶粉(五種)可得到統一的線性關係式(圖十一)，其線性相關係數(r值)為0.885，此結果可供全脂奶粉開封後氧化時間之檢測。

五、計畫成果自評

本研究之內容與原計畫相符，並達到預期之目標如下：探討黃豆粉、奶粉等氧化劣變指標之螢光性成分；分析其螢光強度對儲存時間和氧化指標之關係。

六、參考文獻

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2. 梁哲豪：以螢光分析法評估花生粉中油脂之氧化程度。中國農業化學會誌, 34(6):715-722 (1996).
3. J.H. Liang:Fluorescence due to interaction of oxidizing soybean oil and soy proteins. Food Chem. (in press)

七、圖表

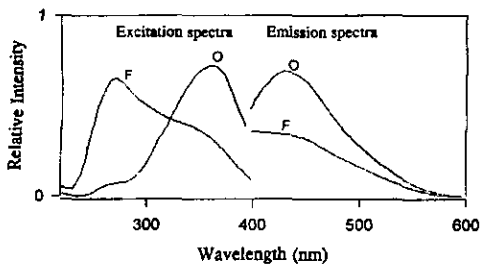

Fig. 1. Transmission fluorescence spectra of the organic layers from chloroform-methanol extracts of soybean flour. F, fresh; O, oxidized at 60 ℃ for 30 days.

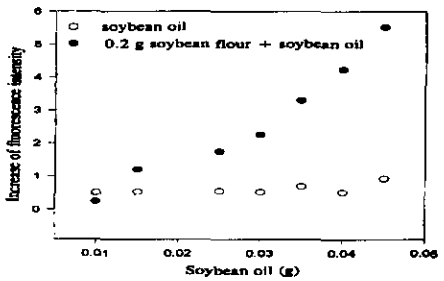

Fig. 2. The increase of fluorescence intensity (arbitrary units) during storage of bulk soybean oil and mixture of soybean flour/soybean oil at 60 ℃ for 9 days.

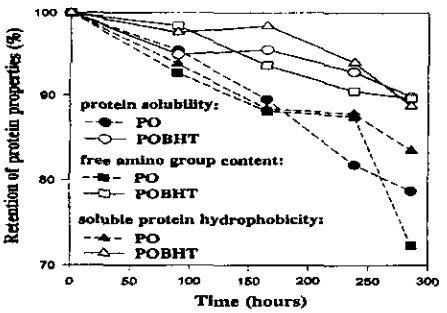

Fig. 3. Retention of protein properties in the samples during storage at 60 ℃ in the dark.

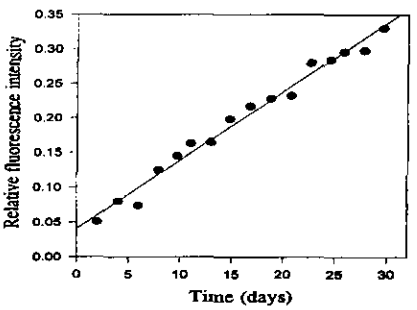

Fig. 4. Time course of fluorescence intensity (ex. 350nm, em. 440nm) of the organic layers from chloroform-methanol extracts of soybean flour during storage at 60 ℃ in the dark.

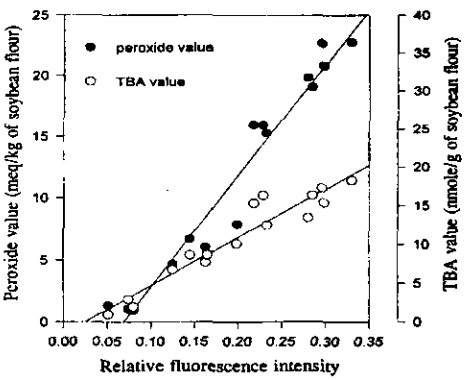

Fig. 5. Relationship of peroxide value and TBA value to the fluorescence intensity during storage of soybean flour at 60 ℃ in the dark.

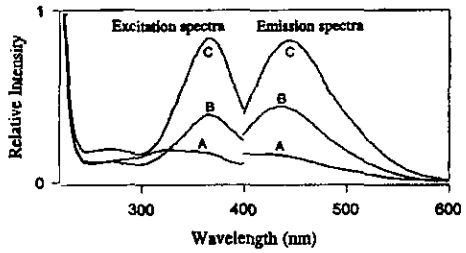

Fig. 6. Front-surface fluorescence spectra of the milk powders. A, fresh whole milk powder; B, whole milk powder stored at 37 ℃ under vacuum for 8 months; C, whole milk powder stored at 37 ℃ under air for 8 months.

Table 1. Repeatability of front-surface fluorescence intensity (ex. 365nm, em. 435nm) measured with reflectance spectrofluorometry for milk powders.

Sample *	FI	n	CV(%)
A	230.2 ± 62.4	12	27.08
B	513.5 ± 120.4	12	23.55
C	1094.7 ± 273.0	12	24.94

* Samples A, B, C are the same as indicated in Fig. 6.
FI,fluorescence intensity with standard derivation;
n, number of trials; CV, coefficient of variation.

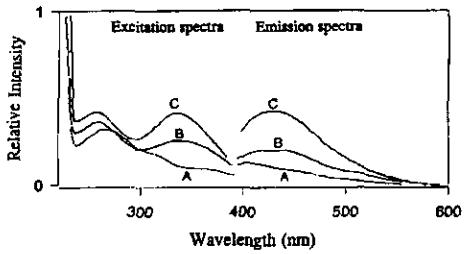

Fig. 7. The fluorescence spectra of milk powder dispersion in glycerol/water. A,B,C are the same as indicated in Fig. 6.

Table 2. Repeatability of fluorescence intensity (ex. 350nm,em. 440nm) measured with reflectance spectrofluorometry for milk powders suspension in glycerine/water.

Sample *	FI	n	CV(%)
A	248.2 ± 33.0	12	13.31
B	406.5 ± 103.3	12	22.44
C	955.8 ± 151.4	12	15.84

* Samples A, B, C are the same as indicated in Fig. 6.
FI, fluorescence intensity with standard derivation;
n, number of trials; CV, coefficient of variation.

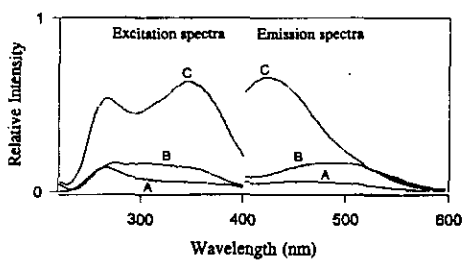

Fig. 8. Transmission fluorescence spectra of the organic layers from chloroform-methanol extracts of milk powders. A,B,C are the same as indicated in Fig. 6.

Table 3. Repeatability of fluorescence intensity (ex. 350nm,em. 440nm) measured with transmission spectrofluorometry for the organic layers from CM extracts of milk powders.

Sample *	FI	n	CV(%)
A	146.7 ± 14.4	12	9.80
B	308.0 ± 12.9	12	4.09
C	1289.0 ± 110.1	12	9.01

* Samples A, B, C are the same as indicated in Fig. 6.
FI, fluorescence intensity with standard derivation;
n, number of trials; CV, coefficient of variation.

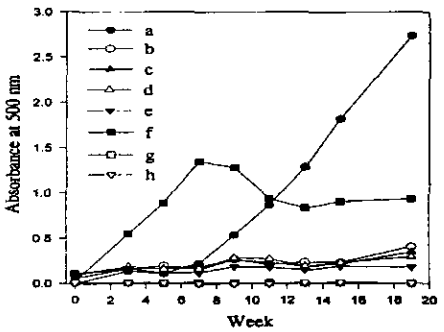

Fig. 9. Time courses of peroxide value of milk powders during storage at 37 ℃ under air in the dark. a,b,c,d,e, whole milk powders; f,g,h, skim milk powders.

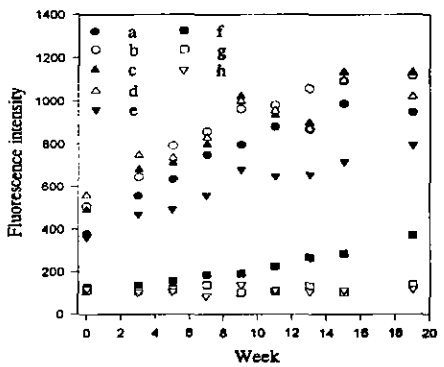

Fig. 10. Increase of fluorescence intensity (ex. 350nm, em. 440nm) *in the organic layer of chloroform-methanol extracts of milk* powders during storage at 37 ℃ in the dark. a,b,c,d,e, whole milk powders; f,g,h, skim milk powders.

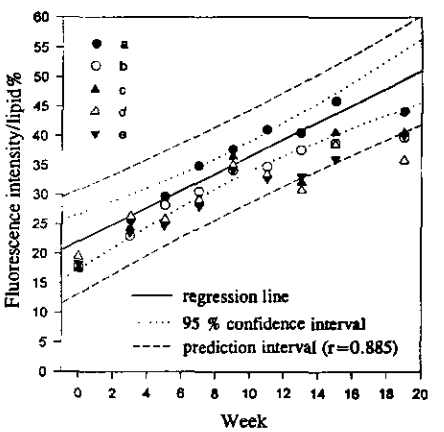

Fig. 11. Changes in the ratios of fluorescence intensity to lipid content during storage of whole milk powders, sample a, b, c, d, e