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The Correlation of CO_2 Content with Non-Enzymatic Browning Color in Non-Fat Dried Milk

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脫脂粉乳에서 CO_2 含量과 非酵素的 褐變色素와의 相關關係

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抄 錄

脫脂粉乳에서 마이야르 反應(Maillard reaction)의 速度를 간단히, 그리고 効果的으로 測定하기 爲한 指標로서 包裝上位空間內의 CO_2含量을 利用하였고, CO_2量과 褐變色素形成 사이의 相關關係를 調査하였다. 褐變色素와 CO_2含量사이에는 높은 相關關係를 나타냈으며, 試料의 上位空間內의 CO_2量을 測定함으로써 마이야르 反應을 豫測할 수 있는 빠르고 간단한 方法이 될 수 있었다. 揮發成分은 貯藏期間이 增加할수록 增加하였고, 酸素含量과는 反比例하였다. 리신(lysine)은 反應의 觸媒로 글루코스보다 더 効果的이었으며, 試料를 55°C와 65°C에 넣어 貯藏할 때 反應을 促進시켜 試驗期間을 短縮시켰으며 75°C에서는 빨리 變質되었다.

Introduction

The Maillard reaction has a significant effect on all types of food products. The points from Nursten[1] illustrates its far reaching effects such as production of color, production of flavors or off-flavors, reduction in nutritional value, toxicity through possible formation of the Amadori compounds-imidazoles and N-nitroso derivatives and antioxidant properties-reductions or heavy metal chelation. While some of these effects are desirable in certain foods, the Maillard reaction decreases the quality of milk products by producing undesirable flavor and color. Both amino groups and reducing sugars are present in milk and the reaction will proceed rapidly. Dried milk is a special problem because if the extended shelf-life of this product and harsh conditions of temperature and humidity it may be subjected to, which accelerate this reaction.

The absence of water and fat leave little to protect non-fat dried milk from the Maillard reaction. Fat and water were shown to protect the amino-carbohydrate complex from initial stages of non-enzymatic browning (Schroeder[2]).

Lysine and glucose are of particular interest in the study of the Maillard reaction. These substances were used by Maillard to describe the reaction initially. Lysine is a particularly susceptible amino acid because of the presence of an E-amino group in its structure. Lysine is also important because it is an essential amino acid and is often the limiting amino acid in the proteins of foodstuffs. Many studies like Namiki et al[3~10] have been done using glucose and/or lysine as the reactants.

Monitoring the Maillard reaction has been the subject of much research. Color is the obvious primary indicator and many methods have been used to measure the brown color formed (Choi[9]). Unfortunately, brown color appears the reaction is in its final stages. Many investigators have searched for substances or properties which appear earlier in the reaction to use as monitoring or predicting devices. This has included such things as furosine (Moller[12], Finot[13]).

Available or free lysine (Waletzko[14]) and derivitogram of amino acid weights among others. These techiques have been successful except for the expensive and sometimes complex methodology involved in retrieving and identifying these substances.

Gas chromatography is a valuable evaluation tool for analyzing volatile formation in the headspace of packaged samples. By identifying a substance, which we can measure with the gas chromatogram, that is produced or consumed in the initial portion of the Maillard reaction we can monitor the reaction. If this substance can be correlated with a more visible, objective indication of the reaction, such as color or flavor, we can show the validity of this new monitoring procedure. An amino group was used as an early indication of protein degradation by Eichner and Ciner-Douk[15]. Isovalderaldehyde, a product of the Strecker degradation of leucine was measured using gas chromotagraphy, in the head space of stored vegetable products. The formation of this substance was shown to proceed the production of off-flavors and colors. As browning and flavor development continued in these tomato and carrot products, isovalderaldehyde concentration increased at a similar rate. While isovalderaldehyde concentration increased at a similar rate. While isovalderaldehyde may be related to isolated products, carbon dioxide appears to be liberated by the Maillard reaction in all foods.

Materials and Methods

The volatile evaluation were performed on a Hewlett-Packard 5880A gas chromatogram with a 10% SE-30 coated on Tenax GC column. The headspace samples were obtained from 25 gram of product stored in 120ml glass bottles, which were kept at 55°, 65° and 75°C in controlled environment incubators. The 25 gram samples were made up of commercial non-fat dried milk, and for added lysine and glucose samples, the combined weight of lysine or glucose and NFDM was 25 grams. The lysine was L-lysine, 182.7 m.w.u., extra pure, from Sigma Company and the glucose was D-glucose, 180.16 m.w.u., extra pure, from Fisher Scientific Company. The data obtained from the chromatograms and the brown color was measured by reflectance spectrophotometry at 520 nm using an SP-20 spectrometer. The statistical evaluations were completed by the statistical analysis system (SAS) program.

Results and Discussion

The results of this experiment show that adding lysine or glucose to a product does catalyze the Maillard reaction. In figures 1 and 2, the gas chromatograms illustrate the general trends that occurred during storage. Oxygen content varied inversely with carbon dioxide content and decreased with storage time. Volatile compounds increased with sto-

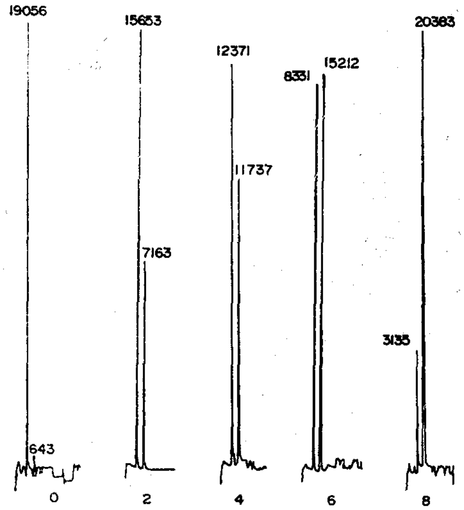

Fig. 1. Gas chromatograms of oxygen and carbon dioxide contents in the headspace of dry milk bottles during storage.

Table 1. Reproducibilities of oxygen, carbon dioxide, volatile compounds and brown color analyses of dry milk

Trials	Oxygen	Carbon dioxide	Volatiles	Brown color
1	19458	820	1512	98.8
2	19428	821	1418	98.6
3	19264	817	1422	99.0
4	19160	815	1489	99.2
Average	19327	818	1460	98.9
S	140	2.7	47	0.34
C.V.	0.72	0.30	3.22	0.35

rage time and acetone and pentane were identified in the headspace. Evans et al[16,17] reported that pentane is related to the flavor quality of oil containing foods. Acetone has been previously identified in the volatiles of dry whole milk and fresh fluid milk. Table 1 shows the multiple trial results for determining the reproducibility of this method. The coefficient of variance and standard deviation show that the methods used have little sample variation. Only one sample was used to obtain

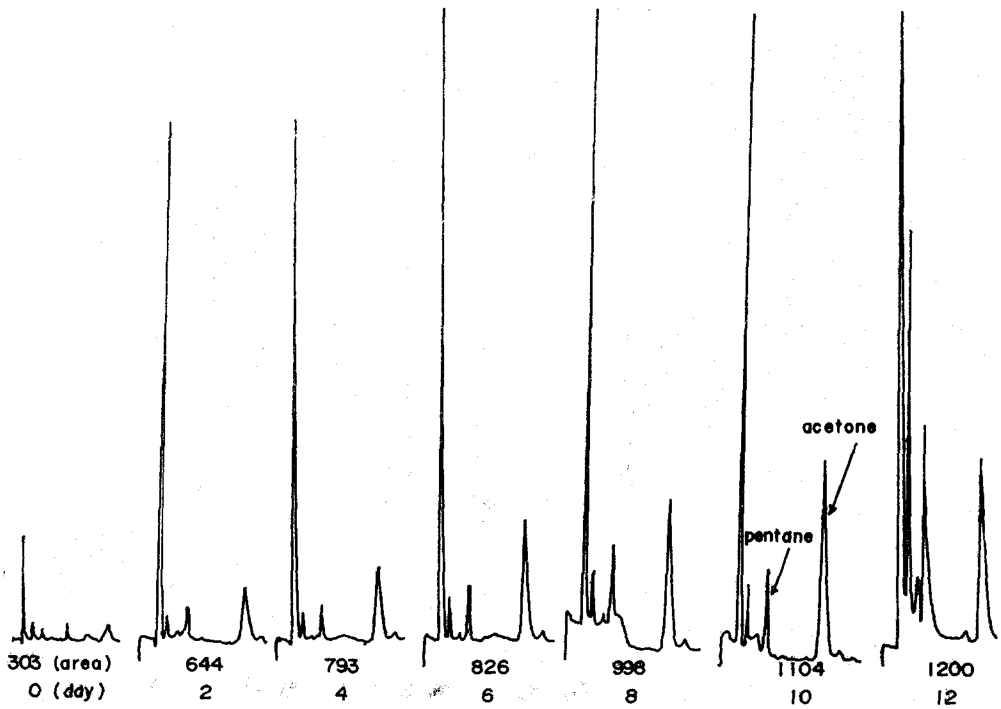

Fig. 2. Gas chromatograms of volatile compounds in the headspace of dry milk bottles during storage.

the following data because of time and space restrictions and this inadequate for accuracy based on Table 1.

Lysine addition

The data for remained oxygen, flavor (volatile) content, carbon dioxide and brown color for samples with added lysine is presented figures 3~6 in graphs. These figures show that adding lysine enhances the changes shown and as oxygen decreases, volatiles, carbon dioxide and brown color all increase. Brown color percent is equal to 1-reflective index at 520 nm times one hundred so that correlation shows brown color increasing (instead of reflectance decreasing) with storage time(Table 2). Table 3 shows the high correlation between brown color and carbon dioxide content. The very high correlation indicates that this method can be used to evaluate the Maillard reaction rate. As another check on these results a linear regression was used to analyze flavor content vs. carbon dioxide content(Table 4).

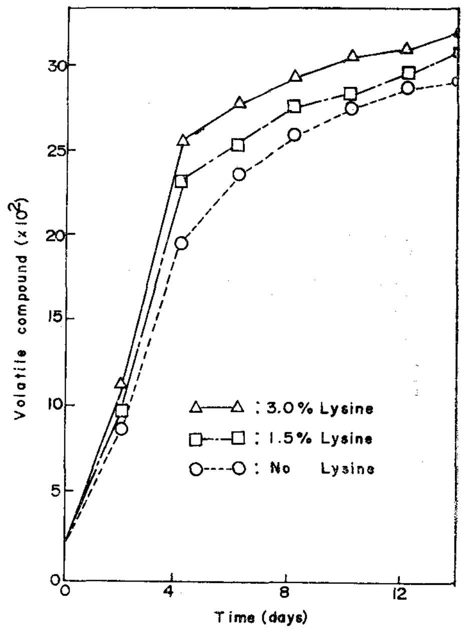

Fig. 4. Effects of added lysine levels on the volatile compound in the headspace of dry milk bottle during storage.

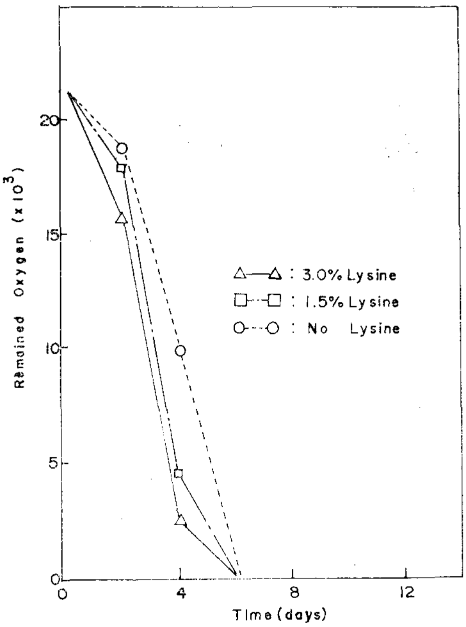

Fig. 3. Effects of added lysine levels on the oxygen content in the headspace of dry milk bottle during storage.

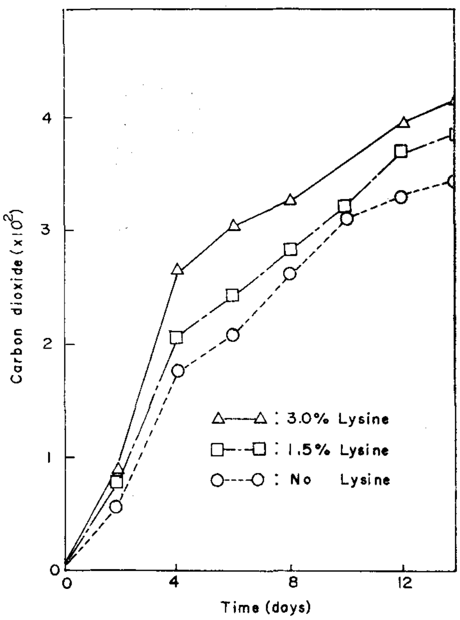

Fig. 5. Effects of added lysine levels on the carbon dioxide content in the headspace of dry milk bottle during storage.

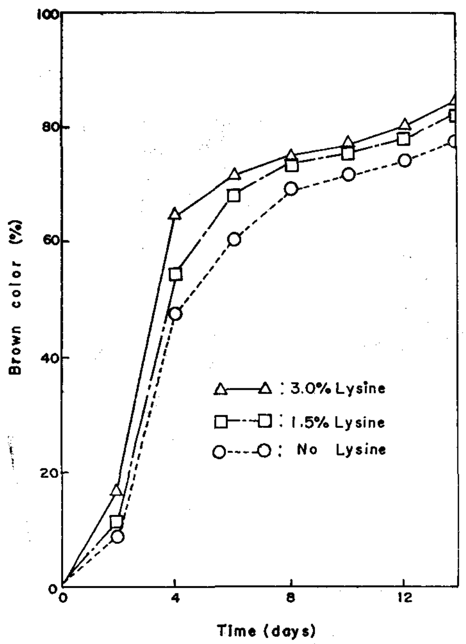

Fig. 6. Effects of added lysine levels in the brown color of milk packed in air during storage.

Table 2. Linear regression equation for brown color as a function of storage time at 55°C, 65°C and 75°C

Storage temp.	Added lysine (%)	Correl-ation coefficient	Equation
55°C	0	.98	Y= 0.1083+0.7613 X
	1.5	.99	Y= 0.4583+0.7666 X
	3.0	.95	Y= 1.2083+0.9398 X
65°C	0	.91	Y=11.8166+5.6029 X
	1.5	.89	Y=15.0833+5.7827 X
	3.0	.84	Y=27.8382+4.6851 X
75°C	0	.75	Y=39.0666+4.5369 X
	1.5	.73	Y=41.4666+4.4619 X
	3.0	.69	Y=44.2250+4.3160 X

Once again, very high correlation coefficients were obtained. The Duncan's multiple range test results show no significant difference is caused by addition of lysine but the data supports the same trends shown in the previous tables and graphs.

Table 3. Linear regression equation for brown color as a function of carbon dioxide content at 55°C, 65°C and 75°C

Storage temp.	Added lysine (%)	Correl-ation coefficient	Equation
55°C	0	.94	Y= 0.0026+0.0026 X
	1.5	.96	Y=−0.9135+0.0026 X
	3.0	.93	Y=−0.4156+0.0031 X
65°C	0	.98	Y= 2.2123+0.0022 X
	1.5	.97	Y= 2.7259+0.0022 X
	3.0	.98	Y= 4.2418+0.0020 X
75°C	0	.97	Y= 7.8132+0.0022 X
	1.5	.97	Y= 6.4371+0.0022 X
	3.0	.97	Y= 6.1423+0.0022 X

Table 4. Linear regression analysis(correlation coefficient and regression equations) for flavor content vs. carbon dioxide content at 55°C, 65°C and 75°C

Storage temp.	Sample	Correl-ation coefficient	Equation
55°C	L-0	.95	Y= 8.61+0.1496 X
	L-15,000	.96	Y= 24.45+0.1463 X
	L-30,000	.98	Y= 78.08+0.1664 X
65°C	L-0	.98	Y=299.61+0.0810 X
	L-15,000	.97	Y=274.61+0.0809 X
	L-30,000	.98	Y=259.05+0.0774 X
75°C	L-0	.98	Y=166.83+0.0867 X
	L-15,000	.98	Y=153.75+0.0831 X
	L-30,000	.99	Y=159.24+0.0814 X

Y=flavor content, X=carbon dioxide
L-0 : No lysine
L-15,000 : 15,000 ppm lysine
L-30,000 : 30,000 ppm lysine

Glucose additions

The data for remained oxygen, flavor(volatile) compounds, carbon dioxide and brown color is presented in figures 7~9 in graphic form. This data also shows oxygen decreasing

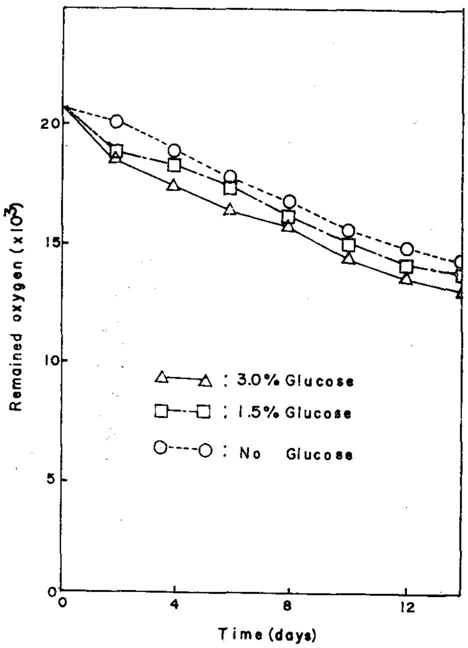

Fig. 7. Effects of added glucose levels on the oxygen content in the headspace of dry milk bottle during storage.

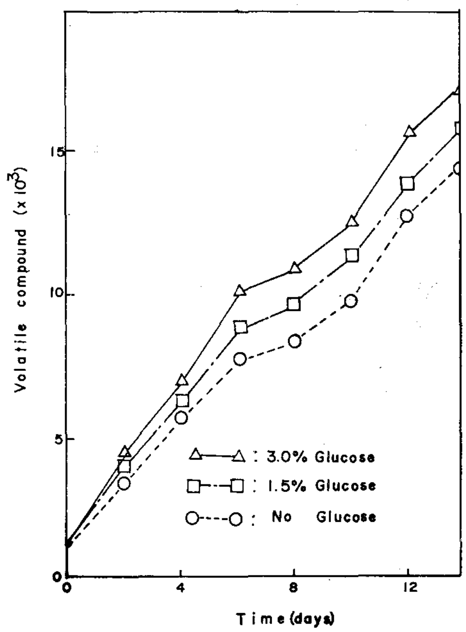

Fig 8. Effects of added glucose levels on the volatile compound in the headspace of dry milk bottle during storage.

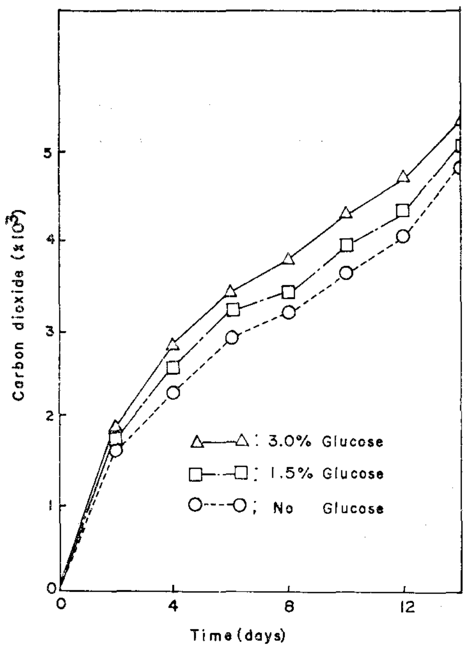

Fig. 9. Effects of added glucose levels on the carbon dioxide content in the headspace of dry milk bottle during storage.

as volatiles, carbon dioxide and brown color increase, but at less than half the change that lysine produced. These changes occur in all of the samples (0, 1.5 and 3 ppm additions) and indicates that the lot of non-fat dried milk used for lysine additions may have had a higher Aw that the lot used for glucose additions. This difference would have caused the rate difference found in the results. Duncan's Multiple Range test results again show no significant changes due to glucose additions, but support the previously noted trends. The correlation of brown color with storage time shows a direct relationship and also shows the instability of the samples stored at 75°C (Table 5). Visual observations showed that samples stored at 75°C had experienced rapid deterioration (possibly caramelization) and appeared badly browned (light coffee color) by the sixth or eighth day of storage. Table 6 again shows the high correlation between brown color and carbon dioxide and the lower

Table 5. Linear regression equation for brown color as a function of storage time at 55°C, 65°C and 75°C

Storage temp.	Added glucose (%)	Correl-ation coefficient	Equation
	0	.98	Y= 0.7166+0.5494 X
55°C	1.5	.99	Y= 0.9750+0.5910 X
	3.0	.97	Y= 1.4583+0.6283 X
	0	.95	Y= 1.5833+0.6505 X
65°C	1.5	.94	Y= 2.0833+0.6577 X
	3.0	.94	Y= 2.5250+0.7625 X
	0	.76	Y=39.2000+4.8107 X
75°C	1.5	.72	Y=43.4250+4.5089 X
	3.0	.69	Y=45.5250+4.3785 X

Table 6. Linear regression equation for brown color as a function of carbon dioxide content at 55°C, 65°C and 75°C

Storage Temp.	Added glucose (%)	Correl-ation coefficient	Equation
	0	.96	Y= 1.4806+0.0036 X
55°C	1.5	.94	Y= 1.9138+0.0036 X
	3.0	.93	Y= 2.4219+0.0037 X
	0	.94	Y= 0.2726+0.0021 X
65°C	1.5	.96	Y= 0.4393+0.0020 X
	3.0	.98	Y= 0.4933+0.0022 X
	0	.84	Y=28.5740+0.0013 X
75°C	1.5	.82	Y=30.4939+0.0013 X
	3.0	.80	Y=33.0126+0.0012 X

correlation numbers at 75°C show the effects of this deterioration.

Abstract

In order to develop a simple and effective method for determining the rate of the Maillard reaction in non-fat dry milk, the carbon dioxide content of the headspace as an indicator were used and the amount of correlation between CO_2 content and brown color development were determined by the gas chromatograph. There is a high correlation between brown color and CO_2 content. The use of gas chromatography to analyze the CO_2 in the headspace of samples is a quick, simple and effective method of monitoring the Maillard reaction. Volatile concentration increases with storage time and varies inversely with oxygen content. Lysine is more effective than glucose in catalyzing the Maillard reaction. Product samples can be stored at 55° and 65°C to accelerate the rate of the Maillard reaction and shorten testing period, but product stored at 75°C is degraded too rapidly to be of any real use.

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