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Effects of Water Activity on the Non-Enzymatic Browning Reaction of Dry Milk

Kyu-Seob Chang, Yeong-Il Chang, Ki-Chul Chun and Seong-Gu Lee

Department of Food Science and Technology
Chungnam National University, Daejeon 300-31, Korea

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水分活性度가 粉乳의 非酵素的 褐變에 미치는 影響

張奎燮 · 張榮一 · 千基哲 · 李成求

忠南大學校 食品加工學科

抄　錄

水分活性度가 粉乳의 非酵素的 褐變反應에 미치는 影響을 밝히기 爲하여 全脂粉乳와 脫脂粉乳를 各各 55°C에서 貯藏하면서 이때 發生 또는 消滅되는 O_2, CO_2 및 H_2O를 Gas Chromatograph에 의해 測定하였고, Maillard 反應에 의해서 生成된 褐色化 程度는 Reflective Spectrophotometer로 測定하였으며, 貯藏期間에 따른 O_2, CO_2, 褐變化의 關係를 回歸方程式으로 얻었다. 즉, 貯藏期間동안 水分活性度가 0.4 以上 褐變物質과 異臭生成도 增加하였으며, 全脂 및 脫脂粉乳 모두 水分活性度가 0.33과 0.44사이에서는 큰 變化를 나타내지 않았다. Carbonyl-amine 反應에 의해 일어나는 褐色化는 脫脂粉乳보다 全脂粉乳가 높았으며, 酸素는 褐色과 異臭가 增加함에 따라 감소하였다.

Introduction

The food industry could benefit from a stable dry whole milk(DWM) product that be stored under warehouse conditions without developing a musty and unacceptable odor. It has been reported that moisture content is the most important factor in the rate and extent of browning reaction and storage temperature is the second most important factor[1]. To these two parameters add the variable of a high fat content of 29%, and the product will become a host for a wide variety of oxidation and browning induced flavors.

Major reactants in the nonenzymatic browning reaction in milk powders are casein, lactose and oxygen. At a low water activity (Aw), the water is bound and unvailable for chemical reactions[2].

The breakdown products of the browning of milk system include short-chain acids, aldehydes, alcohols, brown pigments, CO_2, H_2O, a variety of unpleasant browning, bitter odors and flavors[3,4]. Improvement in the quality control methods for detecting "off-flavors" in milk powders and better warehouse storage conditions are needed to make dry whole milk a marketable products.

The purpose of this research was to study the effects of water activity on the nonenzymatic browning reactions of dry milk powders. The brown color which developed from the Maillard reaction was also measured by using a reflective spectrophotometer.

Materials and Methods

Materials

The Dry Whole Milk(DWM) and Nonfat Dry Milk(NFDM) were purchased in the market. These were spray dried using a tower drier.

Methods

1. Adjusting the water activity

The dry milk samples were placed in dessicators with salt solutions in the bottom. The water activities of the six salt solution were as follows at near 25°C (Table 1).

Table 1. Water activity on different salt solution

Chemical salt	Water activity
LiCl	0.11
CH_3COOK	0.23
$MgCl_2$	0.33
$CaCl_2$	0.35
$Mg(NO_3)_2$	0.53
NaCl	0.75

2. Equilibration of milk powders

There were 10 grams of milk samples weighed into about 7.5cm diameter aluminium weighing dishes. The dry milk was kept in the dessicator for 40 hours at near 25°C. This was assumed to be the equilibrium time for all except the highest water activity. Holding the samples for longer than 40 hours produce a browning reaction at the highest water activities. This would make it difficult to study the initial stages of browning. Preliminary work showed that 40 hours was acceptable for water uptake without browning.

The dessicators were opened and 5 grams of the milk was weighed into 50m*l* reaction vials. This weighing took approximately 3 minutes for each sample. It was assumed that there were negligible changes in moisture content in the sample. The vials were covered with a teflon/rubber septa and an aluminium cap which was crimped to form an airtight seal.

3. Storage conditions

The vials were stored 55°C in forced air ovens under dark.

4. Gas chromatographic determination of oxygen, carbon dioxide in the headspace

The volatile compounds, oxygen and carbon dioxide in the headspace of vials were determined using a Hewlett-Packard gas chromatograph HP-5880. One milliliter of headspace gas was drawn by a gas tight syringe and then analysed on a 0.32cm×30cm stainless steel column packed with 80/100 mesh Tenax GC coated with 10% SE-30 at 120°C using a flame ionization detector. For carbon dioxide and oxygen analyses, one milliliter of head space gas was analysed on the same column at 35°C using a thermal conductivity detector.

5. Color determination

Color of samples were determined as a refletive index of product using spectroscopy at 520nm to estimate nonenzymatic browning reaction.

6. Statistical analysis

Duncan's multiple range test was chosen to determine if the variation among the means were statistically different at $P \leq 0.05$. The temperatures analyzed were 35°C and 55°C. The two temperatures and the six water activities were the independent variables. The O_2, CO_2 and brown color were the dependent variables.

Linear regression equations[5] and the correlation coefficients were determined for O_2 vs. storage, CO_2 vs. storage and brown color vs. storage period.

Results and Discussion

A. Moisture absorption isotherm of dry milk

The average fat content of the duplicate DWM and NFDM samples were 29.01% and 1.97%, respectively. The NFDM results were above the 1.50%, which was recommended in the requirements for NFDM.

Moisture content for the DWM powder was 3.61%.

The moisture isotherm of NFDM and DWM are shown in Figure 1. Heldman et al.[6] reported the milk powders below 0.44 Aw absorb moisture on polar sites of the lactose. The 0.44 Aw appears to be the transition area where the lactose is fully saturated and the protein constituents take over in absorbing the moisture.

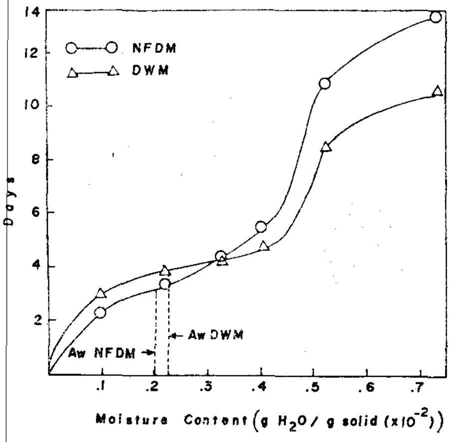

Fig. 1. Moisture isotherms for NFDM and DWM.

Saltmarch and Labuza[7] also noticed a break at the 0.33~0.44 Aw. They concluded that in this range there is a formation of a tightly packed crystalline lactose structure which gives up water. This is an explanation for the plateau at the moisture content of the expected 0.44 Aw.

The DWM did not seem to cake to the extent that the NFDM did. This could affect how efficiently the water was transported by capillary action to the inner layer of the milk powders at the 0.52 and 0.75 water activities.

B. Gas chromatographic determination of O_2, CO_2 and H_2O.

Samples stored at 25°C, 35°C and 55°C were analyzed for O_2, CO_2 and H_2O. The 25°C showed very little browning reaction in the 14 day period.

This is expected at such a low temperature. At 35°C the browning reaction was visible but it was decided to use 55°C samples to best illustrate the browning trends. The water activities were labeled as 0.12, 0.22, 0.33, 0.44, 0.52 and 0.75 in the graphs. These are only expected water activities and it is difficult to say that these water activities were maintained throughout the entire experiment. It is more likely that the water activity decrease initially as the system was heated to 55°C and changed continually throughout the browning reaction.

1. Oxygen

Figure 2 shows the oxygen level in NFDM. The oxygen level in DWM is shown in Figure 3. Compairing these two figures demonstrates slightly less oxygen depletion in the DWM at the 0.12~0.44 Aw range. The NFDM appears to have more of a depletion of oxygen at 0.52 Aw and 0.75 Aw. At low Aw the water seems to prevent lipid oxidation in the DWM system.

This could be explained by the fact that there is 36% protein in NFDM compaired to

26% in DWM. There is also a 51% lactose in NFDM as opposed to only 38% in DWM[8]. The lactose and casein are major reactants of the browning reaction which may be a major contributor of oxygen depletion.

Oxygen depletes as the milk powders undergo the browning reaction. The rate of browning will increase as there is more free water available. Therefore, more O_2 should be depleted at the higher water activities. This trend appears nonlinear because the 0.44 Aw gives an unexpected low moisture reading.

The 0.44 Aw equilibrated sample did not deplete oxygen as rapidly as the 0.33 Aw. The 0.35 to 0.45 water activity range is a transitional period for lactose. During these water activities the OH groups of the lactose are binding water. When the lactose is fully hydrated, it undergoes crystallization[7]. During the the hydrated stage the water is bound tightly to the lactose. Bound water can not be used as a solvent for chemical reactions[9]. There would be less browning reaction and DWM may have more lipid oxidation occuring less oxygen depletion in the 0.35 to 0.45 Aw range.

Halton and Fisher[10] in 1973, proposed that water forms a protective barrier which prevents oxygen from reacting with unsaturated fatty acids. Therefore, at low water activity, than the high Aw samples.

Carbonyls that are formed from lipid oxidation can react with amines and form browning reaction products. Harper and Hall[8] noted that the breakdown products of milk fat oxidation are a carrier for off-flavors.

Oxygen depletion is a combination of several simultaneous in milk powder systems. Two of the major reaction are nonenzymatic browning and lipid oxidation.

Statistical analyses of O_2 depletion for NFDM and DWM is shown in Table 2. Duncan's Multiple Range Test was performed at $P \leq 0.05$. The water activity made a significant difference in oxygen depletion in both NFDM and DWM.

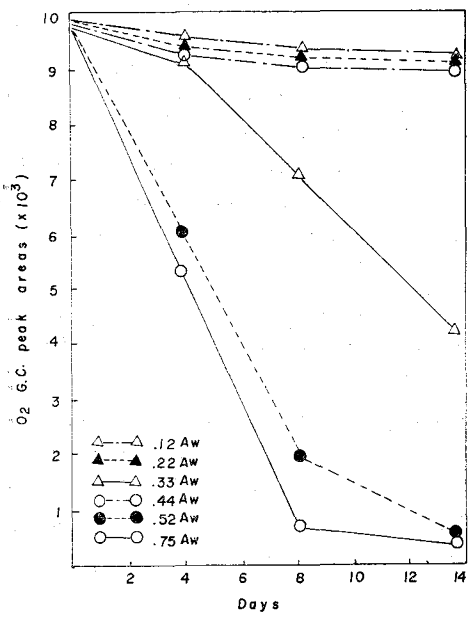

Fig. 2. Effects of water activity on oxygen depletion in NFDM vs. storage time in days at 55°C.

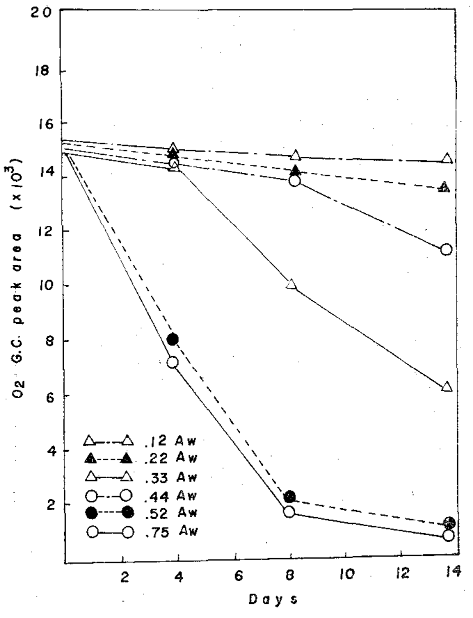

Fig. 3. Effects of water activity on oxygen depletion in DWM vs. storage time in days at 55°C.

Table 2. Duncan's multiple range test for the effects of water activity on O_2 depletion in NFDM and DWM storage at 55°C using NFDM and DWM samples

Water Activity	Mean O_2 Peak Area	
	NFDM	DWM
0.12	9,506A	14,627A
0.22	9,486A	14,606A
0.33	7,915B	11,167B
0.44	9,504A	13,994A
0.52	4,417C	5,317C
0.75	3,382C	5,395C

Note : Mean O_2 with different postscripts are significantly different ($P \leq 0.05$).

2. Carbon dioxide

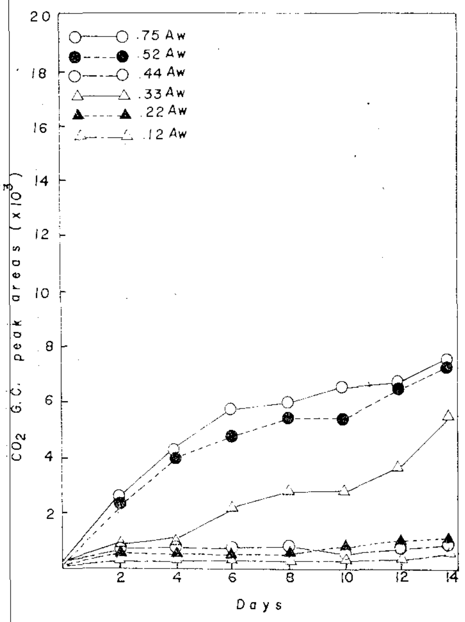

Fig. 4. Effects of water activity on CO_2 formation in NFDM vs. storage time in days at 55°C.

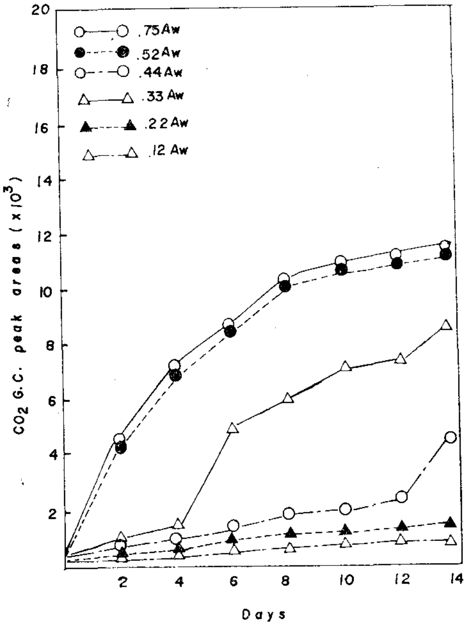

Fig. 5. Effects of water activity on CO_2 formation in DWM vs. storage time in days at 55°C.

Figure 4 and Figure 5 show the effects of Aw on CO_2 in NFDM and DWM, respectively. Carbon dioxide is one of the end products of nonenzymatic browning via Strecker degradation. Its rate of formation is a good indication of the amount of browning in the milk powder system[11]. The CO_2 was eluted and the peak printed at approximately 0.97 minutes. Initially, there were only small peaks and they were integrated manually. Water activities 0.12, 0.22 and 0.44 showed no significant increase in CO_2 concentration. in NFDM. The 0.44 Aw did increase slightly in DWM, which

Table 3. Duncan's multiple range test for the effects of water activity on CO_2 formation during storage at 55°C using NFDM and DWM samples

Water Activity	Mean O_2 Peak Area	
	NFDM	DWM
0.12	287C	513D
0.22	446C	860D
0.33	2,376B	4,517B
0.44	333C	1,844C
0.52	4,638A	7,980A
0.75	4,857A	7,930A

Note : Mean CO_2 formation with different postscripts are significantly different ($P \leq 0.05$).

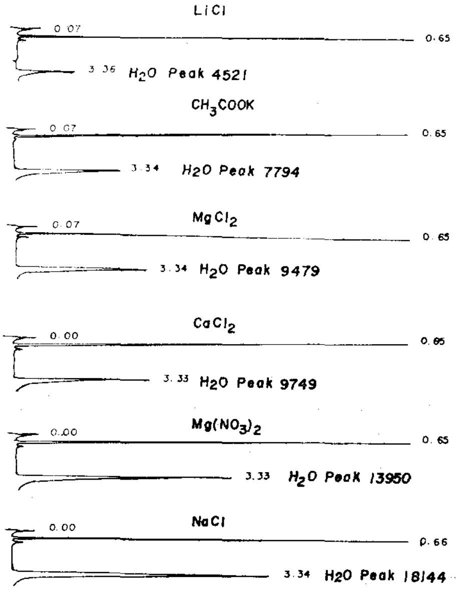

Fig. 6. Gas chromatograms showing oxygen and water content of the headspace of the salt solutions.

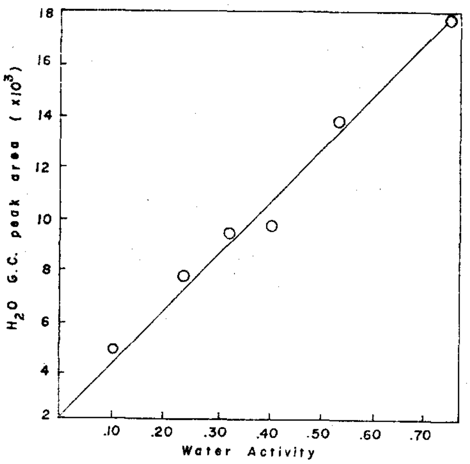

Fig. 7. Standard curve of the gas chromatographic peaks vs. the water activity of the salt solutions.

is shown in Figure 5. Again, in both samples, there was the inversion of the equilibrated 0.33 and 0.44 water activities. This again may be due to the formation of lactose crystals and the decreased free water available for chemical reactions in the milk system near

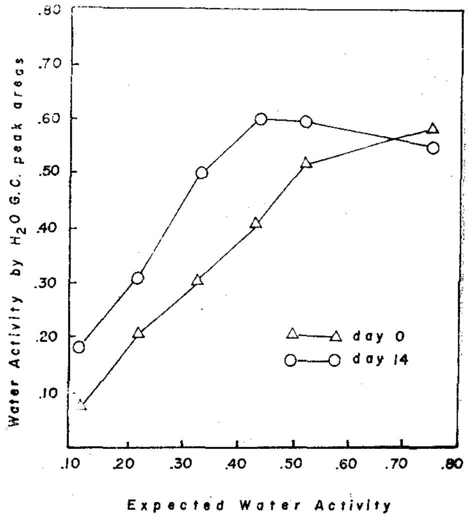

Fig. 8. Water activity from the standard cnrve of H_2O G.C. peak areas vs. expected water activity of DWM stored at 55°C for 0 and 14 days.

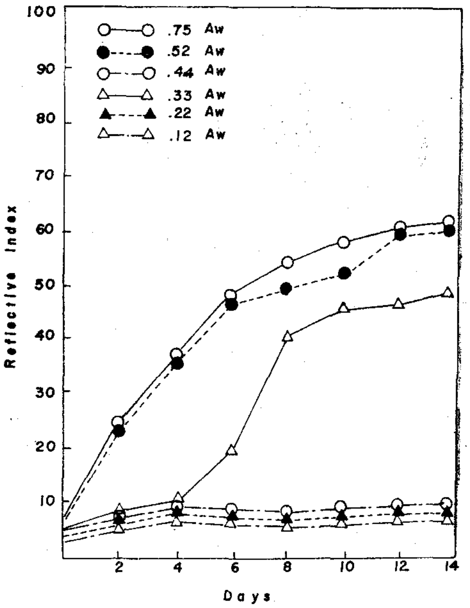

Fig. 9. Effects of water activity on brown color formation in NFDM vs. storage time in days at 55°C.

the 0.44 Aw. The water activity of the 0.44 equilibrated samples had probably changed due to the 55°C storage and the water was being used as a solvent in browning and other chemical reactions.

The comparisons of the CO_2 produced at the 0.33, 0.44 and 0.75 Aw shows that DWM had a faster rate of formation than NFDM. This indicates that DWM browns quicker than NFDM.

Table 3 shows that water activity appears to have a significant difference at $P \leq 0.05$ in CO_2 formation in NFDM samples and DWM samples as analyzed by Duncan's Multiple Range Test.

Samples were equilibrated over K_2CO_3 as a check of the 0.46 Aw range and compared to samples equilibrated over $MgCl_2$ (0.33 Aw). There was slightly less CO_2 produced and less O_2 depleted in milk samples equilibrated over K_2CO_3 (0.46 Aw) than the samples equilibrated over $MgCl_2$ (0.33 Aw). These results are similar to the $CaCl_2$ equilibrated samples (0.44 Aw).

3. Water activity of salt solution by G.C.

Determination of moisture content by G.C. method was studied by sampling the headspace of the 6 separate salt solutions used to equilibrated the milk powders. Results of this study are in Figure 6. Salts with the low water activities produced small peak heights. The water peak is eluted 3.3 minutes after injection of headspace sample.

A standard curve for H_2O G.C. peak areas versus expected water activity of the salt solutions is shown in Figure 7 and the correlation coefficient is 0.98. The G.C. water peak areas correlate to water activities of the salt solution.

4. Water peaks

The results show that the water content detected by the G.C. seems to vary. This is explained by the importance of the release and uptake of water during the different stages of the browning reaction. This make it difficult to use as a predictive parameter for the browning reaction. Figure 8 shows the water activity readings which were obtained by G.C. H_2O peak areas versus the expected Aw for DWM. The "s" shape isotherm is very evident at day 0. There is a wide variation of the Aw through day 14. One possible explanation is as lactose crystallizes it is releasing water.

C. Reflective index by spectrophotometry

Brown color formation is an indication of the extent of browning. Figure 9 is a graph of reflective index reading versus days storage at 55°C for NFDM. Figure 10 is a similar graph for DWM. Brown color is formed in the later stages of the Maillard reation and CO_2 is given off[12]. Spectophotometry gives an indication of the amount of brown color formed. The reflectance reading of 100 would indicate no brown color present.

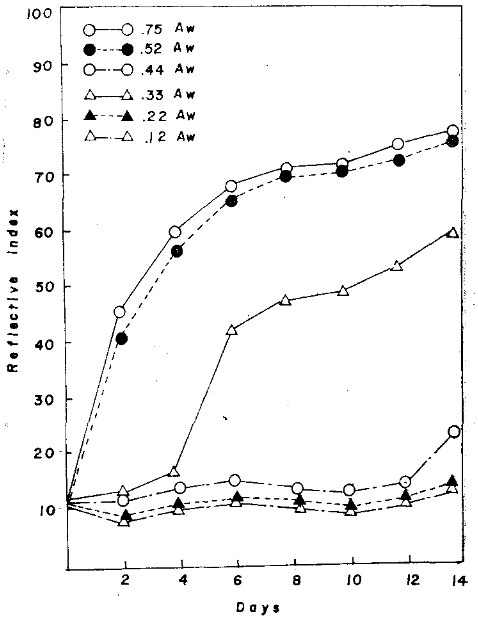

Fig. 10. Effects of water activity on brown color formation in DWM vs. storage time in days at 55°C.

A comparison of CO_2 formation to reflective index reading show good correlation between the two techniques as shown in Table 4 and 5. Confirming the idea that G.C. techniques

Table 4. Correlation coefficents and linear regression equations to predict brown color formation as a function of carbon dioxide development in NFDM stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	0.87	$Y=0.021X+-0.061$
0.22	0.60	$Y=0.005X+3.94$
0.33	0.90	$Y=0.009X+5.67$
0.44	0.84	$Y=0.009X+7.19$
0.52	0.99	$Y=0.009X+2.34$
0.75	0.99	$Y=0.008X+2.87$

$X=CO_2$ formation by G.C.
Y=Browning by spectrophotometry

Table 5. Correlation coefficients and linear regression equations to predict brown color formation as a function of carbon dioxide development in DWM Stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	0.77	$Y=0.0056X+6.56$
0.22	0.74	$Y=0.003X+7.00$
0.33	0.99	$Y=0.006X+6.09$
0.44	0.97	$Y=0.006X+3.74$
0.52	0.99	$Y=0.006X+11.08$
0.75	0.98	$Y=0.006X+15.00$

$X=CO_2$ formation by G.C.
Y=Browning by spectrophotometry

can be used to indicate the extent of non-enzymatic browning. The only caution being that some CO_2 was already produced before samples were analyzed and before samples were analyzed and before any actual brown color could be detected.

Dry whole milk gave higher reflective index readings and produced more browning than NFDM.

Statistical analyses indicated water activity had significant effects on the brown color formation at $P<0.05$. This is shown in Table 6 for NFDM and DWM samples.

Table 6. Duncan's multiple range test for the effects of water activity on mean reflective index during storage at 55°C using NFDM and DWM samples

Water activity	Mean reflective index	
	NFDM	DWM
0.12	5.35C	9.51C
0.22	6.24C	9.71C
0.33	26.98B	34.48B
0.44	10.46C	13.00C
0.52	42.35A	57.11A
0.75	42.15A	59.41A

Note : Mean reflective indexes with different postscripts are significantly different ($P\leq 0.05$)

Table 7. Correlation coefficients and linear regression equation to predict oxygen depletion as a Function of storage time in DWM Stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	−0.84	$Y=-84.00X+15,255.00$
0.22	−0.67	$Y=-67.00X+15,125.00$
0.33	−0.97	$Y=-701.00X+16,077.00$
0.44	−0.91	$Y=-253.00X+12,676.00$
0.52	−0.92	$Y=-1,013.00X+12,543.00$
0.75	−0.92	$Y=-1,000.00X+12,480.30$

X=Days stored at 55°C
$Y=CO_2$ by G.C.

D. Statistical Analysis

Correlation coefficients and linear regression equations to predict O_2 depletion as a function of storage time are shown in Table 7. There appears to be a linear relationship between O_2 depletion and storage time at 55°C. The correlation coefficients ranged from −0.67 to −0.97, in DWM. They range from −0.29 to 0.97 for NFDM and are shown in Table 8.

Table 9 show the linear regression equations to predict CO_2 formation as a function of storage time. Correlation coefficients range

Table 8. Correlation coefficients and linear regression equation to predict oxygen depletion as a function of storage time in NFDM stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	−0.29	Y=−18.20X+9,603.00
0.22	−0.66	Y=−41.49X+9,777.30
0.33	−0.93	Y=−363.00X+1,045.70
0.44	−0.46	Y=−30.600X+9,717.00
0.52	−0.97	Y=−676.00X+9,150.00
0.75	−0.92	Y=−7,000.67X+8,281.00

X=Days stored at 55°C
Y=O_2 by G.C.

Table 9. Correlation coefficients and linear regression equation to predict CO_2 formation as a function of storage time in DWM stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	0.93	Y=34.66X+270.80
0.22	0.98	Y=84.00X+278.50
0.33	0.97	Y=657.00X−90.91
0.44	0.91	Y=253.00X−62.91
0.52	0.89	Y=692.00X+2,990.00
0.75	0.92	Y=729.00X+2,808.00

X=Days stored at 55°C
Y=CO_2 by G.C.

Table 10. Correlation coefficients and linear regression equation to predict CO_2 formation as a function as a function of storage times in NFDM storage at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	0.52	Y=6.76X+244.67
0.22	0.56	Y=22.66X+239.75
0.33	0.96	Y=381.00X+286.00
0.44	0.25	Y=5.26X+293.41
0.52	0.96	Y=469.00X+1,342.42
0.75	0.93	Y=462.00X+1,626.27

X=Days stored at 55°C
Y=CO_2 by G.C.

Table 11. Correlation coefficients and linear regression equation to predict brown color formation as a function of storage time in DWM stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	0.73	Y=0.23X+7.92
0.22	0.66	Y=0.66X+8.08
0.33	0.95	Y=4.08X+5.91
0.44	0.80	Y=0.86X+6.83
0.52	0.87	Y=4.02X+28.92
0.75	0.87	Y=3.91X+32.00

X=Days stored at 55°C
Y=Brown color by spectrophotometer

Table 12. Correlation coefficients and linear regression equation to predict brown color formation as a function of storage time in NFDM stored at 55°C

Expected water activity	Correlation coefficient	Equation
0.12	0.69	Y=0.20X+4.00
0.22	0.67	Y=0.24X+4.58
0.33	0.95	Y=3.74X+0.92
0.44	0.68	Y=1.45X+0.33
0.52	0.94	Y=4.00X+13.80
0.75	0.93	Y=3.81X+15.42

X=Days stored at 55°C
Y=Brown color by spectrophotometer

from 0.89 to 0.97, in DWM. In NFDM, they ranged from 0.25 to 0.96 and are shown in Table 10.

Linear regression equations to predict brown pigment formation as a function of storage time is displayed in Table 11. Correlation coefficients of NFDM were 0.67 to 0.95 respectively.

Abstract

This study was carried out the effects of water activity on the nonenzymatic browning reactions of dry milk powders. Samples (Dry Whole Milk DWM, Nonfat Dry Milk NFDM)

stored at 55°C were analyzed for O_2, CO_2 and H_2O by Gas chromatographic method. The brown color which developed from the Maillard reaction was also measured by a reflective spectrophotometer. And linear regression equations and the correlation coefficients were determined for O_2, CO_2 and brown color vs. storage, and the results are as follows. The amount of brown and off-flavor development increase as the water activities increase about 0.44 Aw during storage. Both DWM and NFDM milk products show a plateau in water absorption between the 0.33 and 0.44 water activities. DWM produced more browning than NFDM which may be partially due to the carbonyl amine reaction. The $CaCl_2$ solution may exude a water activity lower than 0.44 and varies greatly with temperatures. Oxygen is depleted as brown color development.

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