STUDIES OF ANTIOXYGENIC ACTION OF VITAMIN C ON THE CONSTITUENTS OF DRIED WHOLE MILK I. EFFECT OF HYDROQUINONE AND cis-ISOSAFROEUGENOL

STUDIES OF ANTIOXYGENIC ACTION OF VITAMIN C ON THE CONSTITUENTS OF DRIED WHOLE MILK
I. EFFECT OF HYDROQUINONE AND *cis*-ISOSAFROEUGENOL

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(*Received June 20, 1952*)

During the storage of the dried whole milk the oxidized flavour often develops and vitamin A, B_1, C and etc. reduce, especially in air-packing. Many investigations have been made to prevent or retard the development of this oxidized flavour and the reduction of vitamin A, B_1, C and etc. by addition of various antioxidants or by gas-packing. It is of late recognized that addition of a certain amount of *l*-ascorbic acid inhibits the oxidation of dried whole milk.[1) 2) 3) 4)] Hydroquinone is well known as an effective antioxidant, but *cis*-isosafroeugenol is quite unknown.

We carried out this experiment to research the antioxygenic action of vitamin C on lipids, vitamins etc. and the effect of the addition of hydroquinone and *cis*-isosafroeugenol with vitamin C, in manufcaturing process and storage.

Experimental Procedure

The raw milk containing chemical composition as shown in Table 1 was standardized to fat content of 2.8% and preheated at 76°C for 1 minute and condensed in an usual vacuum pan at 24–25 in. of vacuum and 49~55°C of temperature.

Concentrated milk was divided into 2 portions and the one was cooled as the control (sample 1) and to the other was added *l*-ascorbic acid at rate of 20 mg. per liter, calculated on the reconstituted basis and cooled. The *l*-ascorbic acid

added concentrated milk was subdivided into 3 portions and given the following 3 treatments: no treatment (sample 2); addition of hydroquinone at rate of 0.025 g. per 100 g. of fat (sample 3); addition of *cis*-isosafroeugenol at same rate (sample 4). And the chemical composition of the concentrated milk is shown in Table 2.

Table 1. Chemical composition of the raw milk.

Watar %	Fat %	Protein %	Lactose %	Ash %	Sp. gravity	Acidity %
88.68	3.26	3.0	4.34	0.72	1.030	0.15

Tadle 2. Chemical composition of the concentrated milk.

Water %	Fat %	Protein %	Lactose %	Ash %
61.91	10.59	10.15	14.72	2.28

The milk was condensed and dried on the same day, and drying was done with the Merrell-Soule spray dryer. Within an hour after drying, the powders were sieved on a 60-mesh screen and packed in tins containing about 450 g. per can.

The moisture of powders was determined by the vacuum-oven method. The fat was estimated by the Roese-Gottlieb's method, and protein, lactose and ash were analyzed by normal methods. Peroxide value was measured by Wheeler's method[5]. Vitamin A potency was determined by Oshima's method using the Carr-Price reaction[6], vitamin B_1 was determined by Sakurai's method[7] and vitamin C was estimated by Fujita's method[8]. pH was determined by quinhydrone electrode. Solubility of powders was determined by Modified

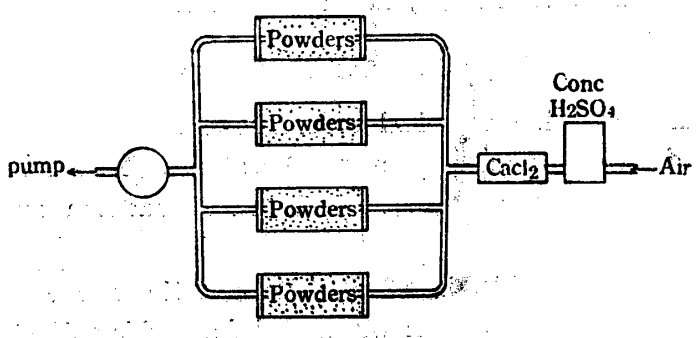

Fig. 1. Aeration apparatus.

Howat's method[9]. And estimation of diameter of particles of powders was done using microscope.

Four samples of the dried whole milk were storaged under the following four conditions: (a) in cans at room temperature and at 37°C; (b) in desiccator ($CaCl_2$) at room temperature and at 37°C; (c) in cans sealed with papers at room temperature; (d) aerated in such an apparatus as Fig. 1.

Results

The chemical and physical properties of four samples of dried whole milk are shown in Table 3. And reduction of vitamin A and C in the manufacturing process of dried whole milk is shown in Table 4.

Table 3. Chemical and physical properties of samples of dried whole milk.

	Sample 1 (Control)	Sample 2	Sample 3	Sample 4
Moisture %	2.06	1.79	1.92	1.76
Fat %	27.84	27.88	27.90	28.11
Protein %	25.77	25.95	25.11	25.51
Lactose %	37.50	37.41	37.63	37.53
Ash %	6.16	6.36	6.47	6.40
Acidity cc/100g.	131	127	128	118
pH	6.38	6.33	6.32	6.33
Peroxide value	0	0	0	0
Vitamin A I.U./100g.	1065	1466	1200	1335
B_1 γ/100g.	188	222	222	219
C mg./100g.	7.65	20.33	14.09	16.53
Solubility %	99.42	99.50	99.29	99.48
Mean diameter of particles μ	85.0	85.2	86.2	85.1

Table 4. Reduction of Vitamin A and C in manufacturing process of the dried whole milk.

	Sample 1	Sample 2	Sample 3	Sample 4
Vitamin A potency per g. of fat of concentrated milk I.U.	52	52	52	52
Vitamin A potency per g. of fat of dried whole milk I.U.	38	52	42	47
Reduction of Vitamin A in drying process %	26	0	18	5
Vitamin C content per 1. of milk mg.	7	27	27	27
Vitamin C content per 100 g. of dried whole milk mg.	7.65	20.33	14.09	16.53
Reduction of Vitamin C indrying process %	26	24	47	38

Percentage of destruction of vitamin A was lowest in sample 2 among the four samples. If the percentage of destruction of vitamin A in sample 2 is

supposed as nul, that in sample 1 was 26%, showing the highest value. Those in sample 3 and sample 4 were 18% and 5%, respectively. Vitamin B_1 content was 222γ per 100 g. in sample 2 and sample 3, 219γ in sample 4 and 180γ in sample 1. Vitamin C content was 20.33 mg. per 100 g. in sample 2, 16.53 mg. in sample 4, 14.09 mg. in sample 3 and 7.65 mg. in control (sample 1). Thus, we believe that vitamin C has protective action against oxidative destruction of vitamin A and B_1 in drying process of milk, and hydroquinone and *cis*-isosafroeugenol have little antioxygenic action on vitamin C and A.

Results obtained in storage in four conditions are as follows:

1. *Storage in Cans at Room Temperature and* 37°C.

Four samples of dried whole milk packed in cans were storaged at room temperature and 37°C. Results obtained are shown in Table 5 and Table 6. And percentage of remaining amount of vitamin A and C during storage are shown in Fig. 2 and Fig. 3. In this case, as a rule, destruction of vitamin A and C was strongly stimulated during storage at 37°C as compared with room temperature. Remaining amount of vitamin A was reduced to 55~80% in 4 months and to about 20% in 12 months at room temperature, but reduced to 20~30% for 4 months at 37°C. And *l*-ascorbic acid added samples, such as sample 2, sample 3 and sample 4, retained more vitamin A and C than in control (sample 1), especially so, at 37°C. But effect of hydroquinone and *cis*-iso-

Table 5. Changes of characters in samples during storage in cans at room temperature.

	Sample No.	Storage (months)				
		0	2	4	9	12
Acidity cc/100 g.	1	131		140		
	2	127		144		
	3	128		142		
	4	118		152		
Peroxide value	1	0	0	+	0.45	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Vitamin A I.U./100 g.	1	1065	950	600	480	180
	2	1466	1230	1000	610	350
	3	1200	1200	970	520	250
	4	1335	1200	970	700	350
B_1 γ/100 g.	1	188	188	149		
	2	222	226	172		
	3	222	212	156		
	4	219	207	173		
C mg./100 g.	1	7.65	6.66	5.02	3.62	0.41
	2	20.33	18.37	15.54	9.25	0.60
	3	14.09	13.33	10.05	8.05	0.75
	4	16.53	14.78	11.00	6.50	0.60

Tadle 6. Cnanges of characters in samples during storage in cans at 37°C.

	Sample No.	Storage (months)				
		0	1	2	3	4
Acidity cc/100 g.	1	131	132	142	146	140
	2	127	136	134	143	144
	3	128	132	134	143	146
	4	118	138	130	134	146
Peroxide value	1	0	0	0	+	+
	2	0	0	0	0	0
	3	0	0	0.9	+	0
	4	0	0	0	0	0
Vitamin A I.U./100 g.	1	1065	730	600	200	220
	2	1466	1265	1000	480	480
	3	1200	1130	670	200	270
	4	1335	1200	870	400	400
B_1 γ/100 g.	1	188	182	120	128	138
	2	222	205	127	126	133
	3	222	202	133	133	123
	4	219	207	120	132	123
C mg./100 g.	1	7.65	4.96	4.24	0.41	3.21
	2	20.33	15.68	12.57	4.45	9.20
	3	14.09	10.22	9.57	2.00	7.65
	4	16.53	13,27	12.57	2.97	9.20
Oxidized flavour	1	−	−	−	+	+
	2	−	−	−	−	−
	3	−	−	−	+	+
	4	−	−	−	−	−
Solubility %	1	99.42	99.40		99.21	99.37
	2	99.50	99.46		99.30	99.44
	3	99.29	99.29		99.18	99.46
	4	99.48	99.45		99.38	99.34

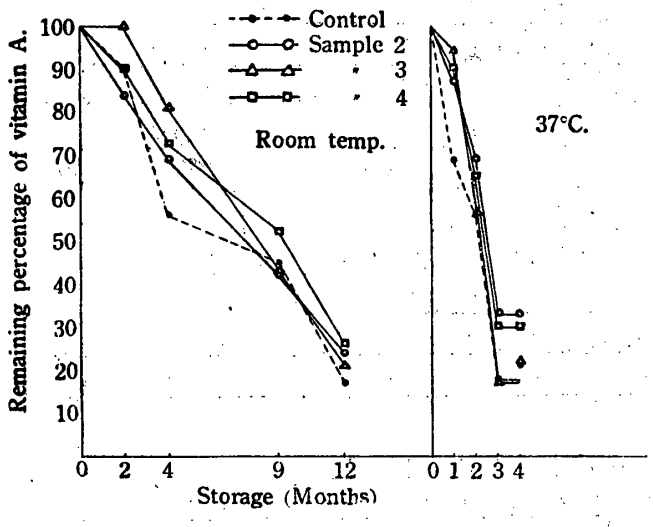

Fig. 2. Changes of vitamin A in samples (dried whole milk) during storage in cans at room temperature and at 37°C.

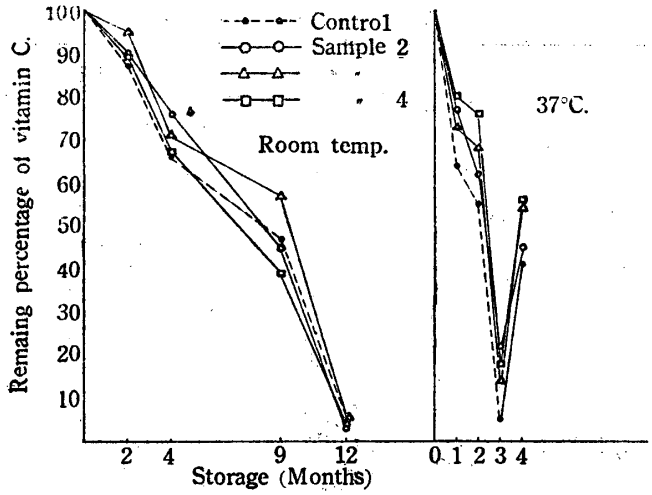

Fig. 3. Changes of vitamin C in samples (dried whole milk) during storage in cans at room temperature and at 37°C.

safroeugenol was not so appreciable as expected.

Peroxide value was positive after 4 months and 0.45 after 9 months in control sample at room temperature, but nul in other three samples. And at 37°C, peroxide value was positive in control after 3 and 4 months, 0.9 in smaple 3 after 3 months and nul in sample 2 and sample 4 for 4 months.

Oxidized flavour developed only in control sample for 4 months at room temperature, and at 37°C did so in the control and sample 3 after 3 months and did not so in others. Thus, hydroquinone rather stimulated oxidation after 2 months at 37°C.

Solubility was slightly lowered in all cases.

2. *Storage in Desiccator ($CaCl_2$) at Room Temperature and* 37°C.

Cans of powders were opened and storaged in desiccator ($CaCl_2$) at room temperature and 37°C and tested. Results obtained are shown in Table 7 and Table 8. And percentage of remaining amount of vitamin A and C during storage are shown in Fig. 4 and Fig. 5.

Generally, reduction of vitamin A and C during storage was less in desiccator than in cans air-packed both at room temperature and at 37°C and especially, destruction of *l*-ascorbic acid added samples was less than that of the control both at room temperature and at 37°C. Reduction of vitamin B_1 was also a little less.

Peroxide value did not develop in all samples at room temperature, but developed at 37°C in the control and a trace also in sample 3 after 3 months and did not in others.

Moisture content reduced a little, but solubility was little affected.

Table 7. Changes of characters in samples during storage in desiccator at room temperature.

	Sample No.	Storage (months)			
		0	1	2	3
Moisture %	1	2.06	1.88	1.90	1.40
	2	1.79	1.90	1.91	1.69
	3	1.92	1.78	1.83	1.23
	4	1.76	1.64	1.71	1.21
Acidty cc/100 g.	1	131	131	135	132
	2	127	127	131	132
	3	128	128	130	129
	4	118	118	122	132
Peroxide value	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
Vitamin A I.U./100 g.	1	950	670	650	550
	2	1230	1200	1170	1070
	3	1200	1070	990	900
	4	1200	1140	1100	970
B_1 γ/100g.	1	188	157	148	136
	2	226	177	170	196
	3	212	174	170	150
	4	207	183	178	168
C mg./100 g.	1	6.66	6.67	7.65	5.40
	2	18.37	17.71	18.57	15.42
	3	13.33	12.86	13.00	10.76
	4	14.76	15.43	16.77	14.89
Solubility %	1	99.42	99.40	99.36	99.32
	2	99.50	99.47	99.45	99.40
	3	99.29	99.37	99.35	99.31
	4	99.48	99.53	99.53	99.47

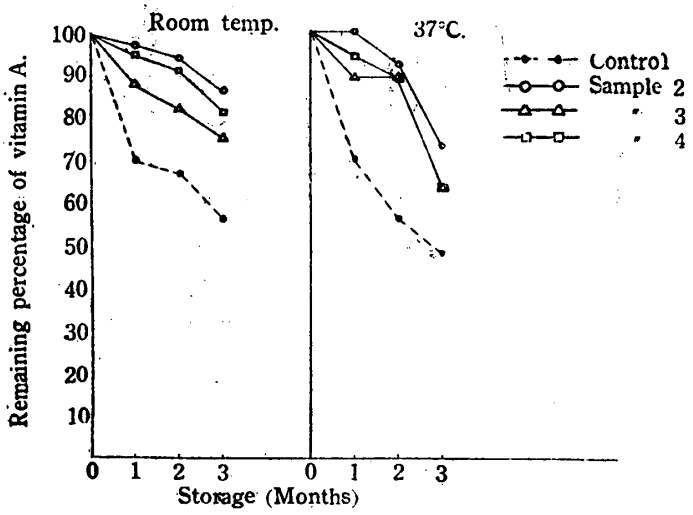

Fig. 4. Changes of vitamin A in samples (dried whole milk) during storage in desiccator at room temperature and at 37°C.

Table 8. Changes of characters in samples during storage in desiccator at 37°C.

	Sample No.	Storage (month)			
		0	1	2	3
Moisture %	1	2.06	1.82	1.77	
	2	1.79	1.87	1.80	
	3	1.92	1.72	1.68	
	4	1.76	1.60	1.75	
Acidity cc/100 g.	1	131	131	133	132
	2	127	127	131	130
	3	128	128	130	131
	4	118	118	120	132
Peroxide value	1	0	0	0	+
	2	0	0	0	0
	3	0	0	0	±
	4	0	0	0	0
Vitamin A I.U./100 g.	1	950	670	530	460
	2	1230	1230	1130	900
	3	1200	1070	1070	750
	4	1200	1135	1070	790
B_1 γ/100 g.	1	188	153		
	2	226	175		
	3	212	175		
	4	207	177		
C mg./100g.	1	6.66	4.74	2.70	3.65
	2	18.37	12.79	12.50	10.24
	3	13.33	10.18	6.25	6.40
	4	14.78	11.45	10.00	8.00
Solubity %	1	99.42	99.40	99.36	99.32
	2	99.50	99.47	99.45	99.40
	3	99.29	99.37	99.35	99.31
	4	99.48	99.53	99.53	99.47

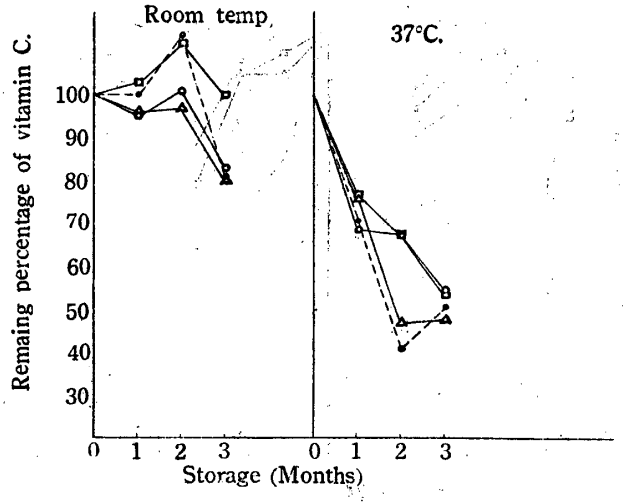

Fig. 5. Changes of vitamin C in samples (dried whole milk) during storage in desiccator at room temperature and at 37°C.

3. *Storage in Cans Sealed with Papers at Room Temperature.*

Cans were opened and sealed with papers and storage-tested at room temperature. Results obtained are shown in Table 9. The Percentage of remaining vitamin A and C are shown in Fig. 6. In this case, reduction of vitamin A was 30~40% for 14 days and rather less in control than in the others. Reduction of vitamin C was slightly in all cases.

Peroxide value was positive in the control after 7 days and 0.8 in sample 4, but nul in sample 2 and sample 3 for 14 days. The oxidized flavour developed only in the control after 11 days.

Moisture was absorbed to about 4% for 14 days and solubility was reduced by about 1% in all cases.

Table 9. Effect of absorbing moisture.

	Sample No.	Storage period (day)					
		0	4	7	9	11	14
Moisture %	1	2.06	2.60	3.11	3.44	3.71	4.70
	2	1.79	2.96	3.19	3.46	3,56	4.26
	3	1.92	2.87	3.89	2.91	3.30	4.06
	4	1.76	3.15	3.39	3.58	3.59	3.88
Acidity cc/100 g.	1	131	132	132	126	126	132
	2	127	128	128	134	132	132
	3	128	130	128	128	130	130
	4	118	130	132	130	128	130
Peroxide value	1	0		+			+
	2	0		0			0
	3	0		0			0
	4	0		0.8			0.8
Vitamin A I.U./100 g.	1	950		870		760	630
	2	1230		900		860	800
	3	1200		800		730	670
	4	1200		870		830	800
B_1 γ100 g.	1	188		162			135
	2	226		178			164
	3	212		180			143
	4	207		175			160
C mg./100 g.	1	6.66	6.90	7.14	6.90	6.59	6.59
	2	18.37	18.18	17.91	18.12	18.12	17.57
	3	13.33	13.04	12.63	12.61	12.47	12.47
	4	14.78	15.79	15.58	16.34	14.32	15.47
Oxidized flavour	1	−	−	−	−	+	+
	2	−	−	−	−	−	−
	3	−	−	−	−	−	−
	4	−	−	−	−	−	−
Solubility %	1	99.42	99.37	99.20	99.09	98.89	98.56
	2	99.50	99.41	99.37	99.28	99.18	99.10
	3	99 29	99.20	99.16	99.14	99.04	99.03
	4	99.48	99.32	99.25	99.10	99.01	98.96

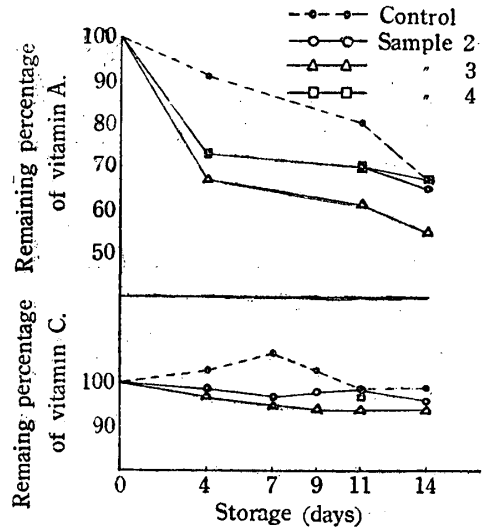

Fig. 6. Changes of vitamin A and C in samples (dried whole milk) absorbed moisture.

4. *Aeration-test.*

Four samples were put in such an apparatus as Fig. 1 and aerated for 5 hours daily (from 10 o'clock a.m. to 3 o'clock p.m.) and tested for 16 days. Cylinders of powders were shaken timely for areation. Results obtained are shown in Table 10. The Percentage of remaining amount of vitamin A and C is shown in Fig. 7. And reduction of vitamin A was about 30~40% for 16 days (aeration of 80 hours) and more in the control than in the others.

Tadle 10. Effect of aeration.

	Sample No.	Aeration period (days)					
		0	4	6	9	12	16
Room temperature C°			14 19	16 18	15 17.5	11 17	13 18
Moisture %	1 2 3 4	2.06 1.79 1.92 1.76	2.65 2.46 2.18 2.56	2.35 1.90 2.20 2.31	2.92 2.49 2.59 3.13	3.57 2.95 3.03 4.10	5.05 4.29 3.77 5.12
Acidity cc/100 g.	1 2 3 4	131 127 128 118	131 127 128 118		130 134 138 128		131 130 142 128
Peroxide value	1 2 3 4	0 0 0 0			+ 0 0 0		+ 0 0 0

pH	1	6.38	6.38		6.39		6.36
	2	6.33	6.33		6.33		6.33
	3	6.33	6.33		6.38		6.36
	4	6.33	6.33		6.33		6.36
Vitamin A I.U./100 g.	1	1065	760	690	610	610	540
	2	1446	1300	1220	1070	1070	760
	3	1200	1070	1070	920	840	760
	4	1335	1230	1230	920	920	770
B_1 γ/100 g.	1	188				159	160
	2	222				172	175
	3	222				161	159
	4	219				175	175
C mg./100 g.	1	7.65	6.05	5.76	5.60	5.00	4.56
	2	20.32	19.16	17.86	16.80	16.38	14.76
	3	14.09	12.43	11.11	10.24	10.69	9.39
	4	16.53	15.86	14.70	13.33	13.75	12.92
Oxidized flavour	1	–	–	–	–	–	–
	2	–	–	–	–	–	–
	3	–	–	–	–	–	–
	4	–	–	–	–	–	–
Solubility %	1	99.42	98.92	99.38	99.27	99.16	99.05
	2	99.50	98.96	99.36	99.28	99.23	99.11
	3	99.29	98.26	99.45	99.35	99.27	99.22
	4	99.48	98.72	99.45	99.35	99.18	99.05

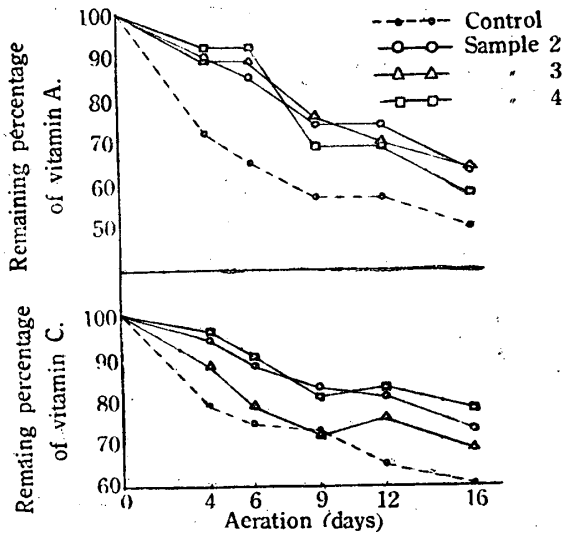

Fig. 7. Changes of vitamin A and C in samples (dried whole milk) aerated.

Peroxide value developed only in the control after 9 days. Oxidized flavour was not appreciable in all cases.

Moisture increased to almost 5% and solubility was slightly affected.

Discussion

Henry, J. D. et al.[10] reported that there is no noticiable loss of vitamin A in the manufacture of dried whole milk and also none in storage for one year. Miyawaki[11] described that vitamin A is destroyed by prolonged exposure to air in the manufacture of dried milk. In the present experiment, destruction of vitamin A is 26%, if that in the powders added with *l*-ascorbic acid alone (sample 2) is supposed to be nul. Vitamin A content of samples added with both *l*-ascorbic acid and hydroquinone or *cis*-isosafroeugenol was less than that of the sample added with *l*-ascorbic acid alone. This is believed to be effect of twice homogenization through Gaulin Homogenizer in the case of their addition before drying milk. Thus, we found difference of content of vitamin A in samples of powders and think that some amount of vitamin A may be destroyed in drying milk at least in latest Japanese factory, but it may be avoided by addition of 20 mg. of *l*-ascorbic acid before drying.

Vitamin B_1 content of powders added with *l*-ascorbic acid was slightly more than that of the control and it is believed to be the effect of *l*-ascorbic acid added.

Krukovsky et al.[12] demonstrated experimentally that partial oxidation of vitamin C in milk stimulates the development of tallowy flavour, while complete quick oxidation of this vitamin inhibits the production of tallowy flavour. Hollender et al., Findlay et al. and Jack et al.[1) 2) 3)] reported that ascorbic acid in amount of 0.1%~0.2% inhibits the development of tallowy flavour and Wright et al. reported that ascorbic acid in amount of 0.004%~0.016% in gas-packed powders do so. In this experiment, ascorbic acid in amount of 0.02% inhibited the development of peroxide value and tallowy flavour in dried whole milk at room temperature for one year and at 37°C for 4 months. *cis*-isosafroeugenol was a little effective as antioxidant, and hydroquinone also was a little effective at room temperature, but it stimulated oxidation at 37°C and in existence of relatively large amount of moisture. Aeration for 16 days (80 hours) developed peroxide in the control but did not in the others and in this case deterioration in flavour was not appreciable.

Addition of vitamin C lowered slightly pH, but did not affect solubility.

Summary

Four samples of dried whole milk were manufactured and the effect of vitamin C (*l*-ascorbic acid) with hydroquinone or *cis*-isosafroeugenol on the constituents of the milk was examined.

Vitamin A potency in the powder added vitamin C was larger by 26% than that of control powder and a little larger than those of the samples added with hydroquinone or *cis*-isosafroiugenol. Therefore, the addition of vitamin C to

concentrated milk before drying prevents the destruction of vitamin A during drying process and the addition of hydroquinone or *cis*-isosafroeugenol with vitamin C has more effect.

In storage, 0.02% vitamin C in the dried whole milk inhibited the development of oxidized flavour for 12 months at room temperature, and for 4 months at 37°C and protected vitamin A and B_1 to a certain extent. The addition of hydroquinone or *cis*-isosafroeugenol slightly increased the effect of vitamin C. But hydroquinone stimulated the development of peroxide or tallowy flavour at 37°C and in existence of relatively large amount of moisture.

Thus, we believe that the addition of vitamin C before drying milk inhibits oxidation of the constituents of dried whole milk, especially lipids and vitamin A. A simillar relation occurs with such synergists as tocopherols, citrate and etc. The addition of hydroquinone or *cis*-isosafroeugenol with vitamin C, however, slightly increases the antioxygenic action of vitamin C.

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