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SHORT-TERM STABILITY STUDY OF RETINOL α-TOCOPHEROL AND VITAMIN C IN CANDIDATE REFERENCE MATERIALS RM 380 AND RM 383

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ABSTRACT

SHORT-TERM STABILITY STUDY OF RETINOL α-TOCOPHEROL AND VITAMIN C IN CANDIDATE REFERENCE MATERIALS RM 380 and RM 383

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P.C.H. Hollman and J.H. Slangen

As part of a Community Bureau of Reference (BCR) certification project on food reference materials for nutrients, stability of retinol and α-tocopherol in milk powder (RM 380) and of vitamin C in haricots verts beans (RM 383) was studied. The objectives of the study were to assess stability of these vitamins when exposed to short-term high ambient temperatures as may arise during adverse transport conditions and to assess long-term stability of retinol in RM 380 which had been stored for 3 years at different temperatures (-40°C, -18°C, and 4°C).

Short-term stability was monitored by exposing samples to a series of increasing temperatures (25°C, 30°C, and 42°C) for periods of each two weeks. Stability of retinol, α-tocopherol and vitamin C proved to be excellent, whereas at 42°C degradation was found. It was concluded that samples can be safely transported provided that the temperature of the samples does not exceed 30°C.

Long-term stability of retinol in RM 380 proved to be adequate after storage for 34 months at -18°C and 4°C.

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4 tables, 3 figures, 1 annex

Key words: reference materials, vitamins, retinol, α-tocopherol, vitamin C, foods, food analysis, stability, BCR

CONTENTS

1 INTRODUCTION

As part of a certification project on major dietary components and major elements in 5 food reference materials (CRM 380-384), the feasibility of future certification of vitamins in food was studied. Results of an interlaboratory using three of these materials, pork muscle, haricots verts beans and milk powder, showed good agreement for α-tocopherol, retinol, ß-carotene, vitamin B1, vitamin C, and niacin [1]. It was concluded that these data on the content of vitamins in these reference materials would be very useful for food laboratories.

However beforehand, stability of vitamins in these foods had to be proven. Long-term stability (24 - 30 months) of α-tocopherol, retinol, vitamin B1 and vitamin C in samples stored at -18°C and 4°C was studied and proved to be adequate [2]. Retinol in milk powder rapidly decreased during the first six months of storage but stabilised afterwards. However, it was decided to extend the stability study of retinol covering a period of 3 years. Except for vitamin B1, no data were available on the stability of these vitamins when exposed to short-term high ambient temperatures as may arise during adverse transport conditions.

BCR contracted RIKILT-DLO to carry out the long-term stability of retinol in milk powder and the short-term stability study of certain vitamins in milk powder and haricots verts. In this report the results of these studies are described.

2 MATERIALS AND METHODS

2.1 Materials

Whole (full-cream) milk powder (RM 380) and freeze-dried haricots verts beans (RM 383) were provided by Leatherhead Food RA in heat sealed laminated foil sachets of 100 g.

2.2 Short-term stability study

Sachets of milk powder (RM 380) and haricots verts beans (RM 383) which had been stored at Leatherhead Food RA were sent to RIKILT in September 1991. These samples were packed in a box containing dry ice. Upon receipt of the samples at RIKILT, samples were still frozen. Sachets were immediately stored at -18°C and 25°C. After 2 weeks part of the samples which had been stored at 25°C were stored at 30°C. After 2 weeks part of the samples which had been stored at 30°C were stored at 42°C. After six weeks, all samples thus stored at different time-temperature conditions as summarised in Table 1, were analysed. All analyses of each vitamin were caried out in one series to minimise within laboratory variation. Samples stored at -18°C served as analytical control samples. As was proved previously [2] retinol and vitamin C show excellent stability in these materials.

Table 1. Scheme of analyses

Temperature of storage	0	1	2	3	4	5	6
42°C					x	x	x
30°C			x	x	x	x	x
25°C	x	x	x	x	x	x	x

Time of storage (weeks)

2.3 Long-term stability study

Directly after receipt of the RMs in the beginning of September 1988, sachets were stored at -40 ± 2°C, -18 ± 2°C, and +4 ± 2°C for 34 months. The freezers (-40°C and -18°C) and the refrigerator (+4°C) were provided with an alarm set at 2°C above the target temperature. No anomalies were detected during the study.

2.4 Methods of analysis

At each time/temperature point, duplicate measurements of vitamins in one sachet for each RM were performed using the methods described in this section. Repeatability criteria as determined previously [2] were applied. Whenever results did not meet these repeatability criteria, analyses were repeated in duplicate. All results were expressed on dry matter.

- Dry weight

Milk Powder: 2 g sample is dried at 103 °C during 4 hours.

Haricots Verts Beans: 5 g sample is dried at 103 °C during 4 hours.

- Retinol

Milk Powder: 10 g sample is saponified in alkaline ethanol and extracted with dichloroethane according to Grimm [3]. Retinol is determined with reversed phase HPLC (C18) and UV-detection at 325 nm.

- α-Tocopherol

Milk Powder: 10 g sample is saponified in alkaline ethanol and extracted with dichloroethane according to Grimm [3]. α-Tocopherol is determined with reversed phase HPLC (C18) and fluorescence detection at 290/326 nm.

- Vitamin C

Haricots Verts Beans: 2 g sample is extracted with metaphosphoric acid 1%, followed by enzymatic oxidation of ascorbic acid with ascorbate oxidase at 37 °C for 5 min. A fluorescent quinoxaline is formed by reaction of dehydroascorbic acid with o-phenylenediamine for 30 min at 37 °C. The quinoxaline is separated with reversed HPLC (C18) and determined with fluorescence detection at 355/425 nm.

3 RESULTS AND DISCUSSION

Short-term stability study

Results of the short-term stability study are presented in Figures 1-3 and Tables 2 and 3 (Annex I). In the Figures the values "average" and "s_m" given represent the mean and standard deviation of the vitamin content of the samples stored at -18°C (analytical control). This standard deviation represents both the analytical variation and the variation between different sachets of one RM. So only results of sachets stored at elevated temperatures differing by more than 2 x s_m from the average, indicate statistically significant ($p = 0.05$) deterioration. Individual results of these controls are given in Table 2 ("control at -18°C") and in Figures 1 - 3 (data at storage time 0).

Retinol and α-tocopherol in RM 380 show excellent short-term stability at both 25°C and 30°C for 6 weeks (Fig. 1 and 2). From these data it is also evident that storage at 30°C does not show a tendency towards lower vitamin contents. However, storage at 42°C for two weeks shows degradation of both vitamins.

Vitamin C in RM 383 shows excellent short-term stability at both 25°C and 30°C for at least 5 weeks (Fig. 3). Storage at 42°C even for 1 week shows detoriation of vitamin C.

Long-term stability study

Results are summarised in Table 4. Differences between duplicate samples and between different storage periods are smaller than 2 x s_m as determined in control samples at -18°C (Table 2, s_m = 0.017 mg/100 g dry weight). Thus, results confirm stability of retinol in RM 380 after storage at -18°C and 4°C for 34 months. Results also indicate that the retinol content of milk powder stabilizes at a different level depending on the temperature of storage. Comparing results of control samples at -18°C (Table 2) with results given in Table 4, it is likely that samples at Leatherhead Food RA have been stored at a temperature > 4°C.

4 CONCLUSIONS

Retinol and α-tocopherol in milk powder (RM 380) and vitamin C in haricots verts beans (RM 383) are stable at 25°C and 30°C for 6 weeks. However, storage at 42°C induces degradation of retinol and α-tocopherol after 1 week, whereas deterioration of vitamin C probably starts immediately. It is concluded that samples can be safely transported, provided that the temperature of the samples does not exceed 30°C.

Retinol in milk powder (RM 380) proves to be stable for at least 34 months after storage at -18°C and 4°C. Long-term storage at 4°C is possible, however storage at -18°C has to be preferred.

5 REFERENCES

1. Hollman P.C.H., Slangen J.H. Intercomparison of methods for the determination of vitamins in foods. RIKILT-DLO Report 91.11.
2. Hollman P.C.H., Slangen J.H. Homogeneity and stability of major nutrients and vitamins in five food candidate refernce materials. RIKILT-DLO Report 91.31.
3. Grimm, Tiews: Z. Landwirtsch. Forsch. (1972) 27: 42

Figure 1. Retinol (mg/100 g dry weight) monitored at regular intervals in RM 380 stored at different temperatures

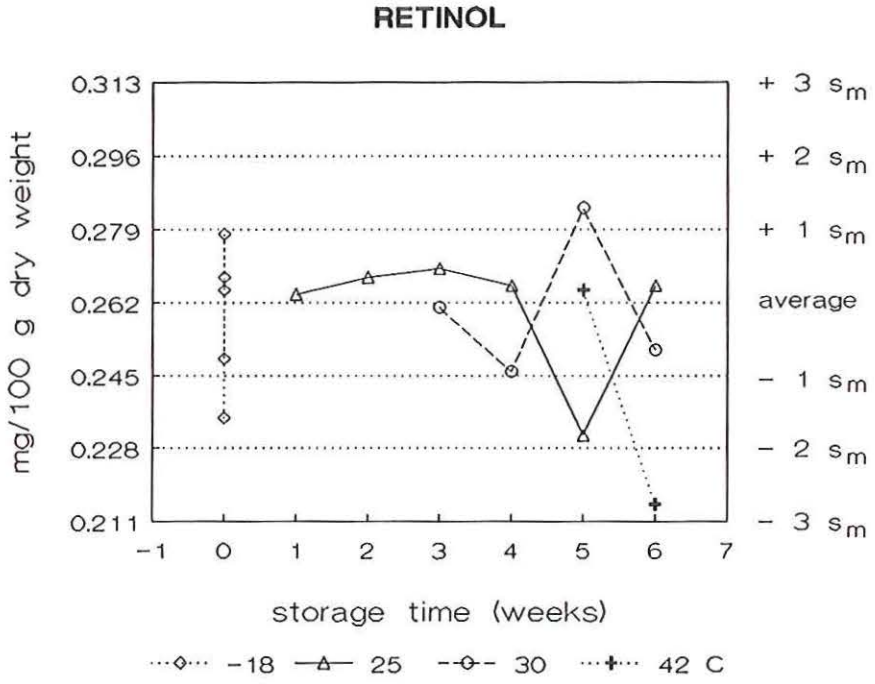

Figure 2. α-Tocopherol (mg/100 g dry weight) monitored at regular intervals in RM 380 stored at different temperatures

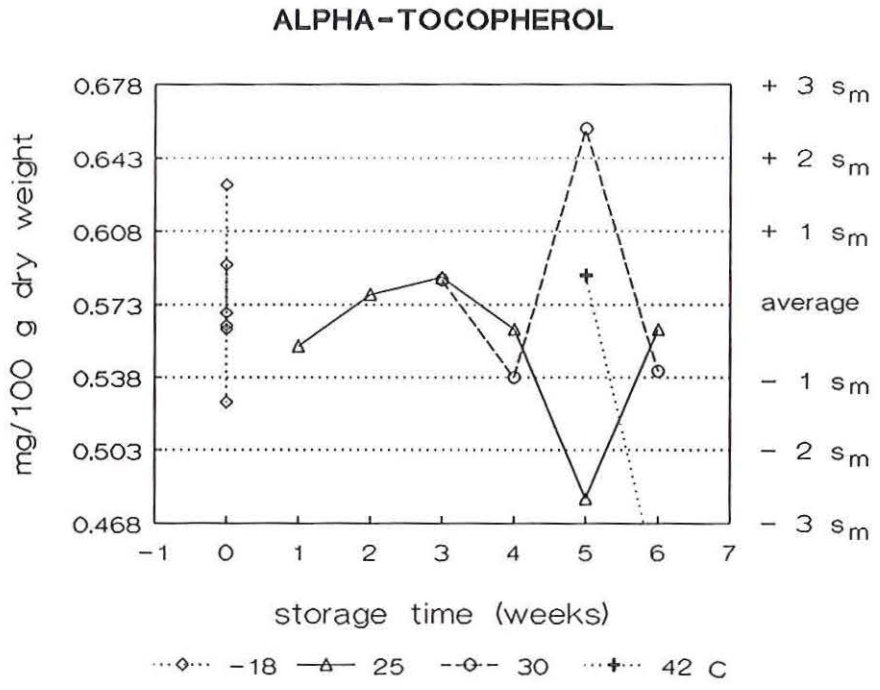

Figure 3. Vitamin C (mg/100 g dry weight) monitored at regular intervals in RM 383 stored at different temperatures

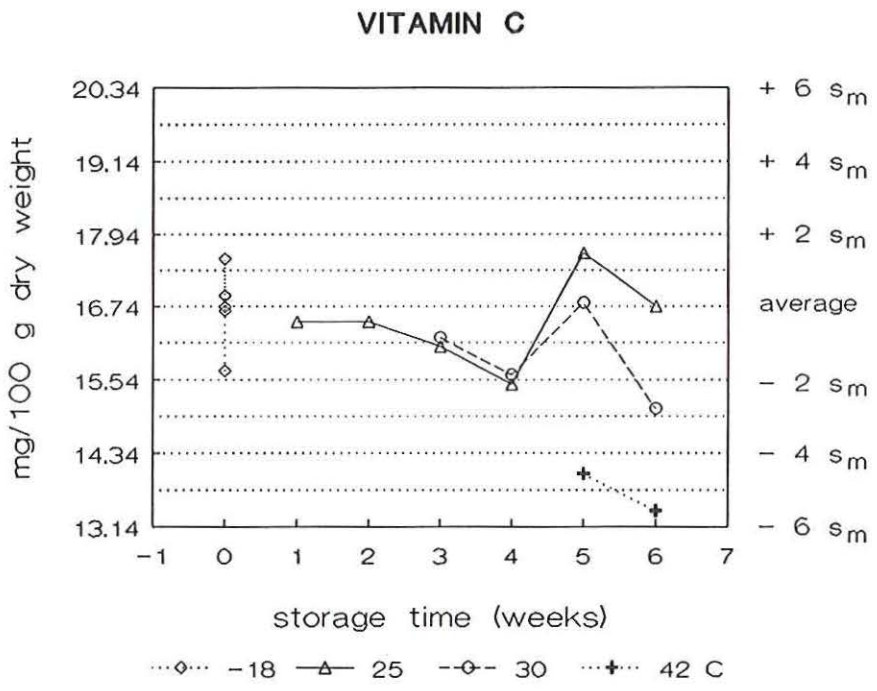

Table 2. Mean of duplicate determinations of dry matter (g/100 g), retinol and α-tocopherol content (mg/100 g dry weight) in RM 380 stored at different elevated temperatures and monitored at regular intervals.

Storage conditions (weeks-temperature)	Sachet code	Dry matter (g/100 g)	Retinol (mg/100 g dry weight)	α-tocopherol (mg/100 g dry weight)
1-25	506	98.10	0.264	0.553
2-25	17	98.21	0.268	0.578
3-25	340	98.00	0.270	0.586
4-25	510	98.06	0.266	0.561
5-25	48	98.18	0.231	0.480
6-25	549	98.00	0.266	0.561
*2-25+1-30	141	98.00	0.261	0.585
2-25+2-30	842	97.69	0.246	0.538
2-25+3-30	773	97.66	0.284	0.657
2-25+4-30	72	98.06	0.251	0.541
2-25+2-30+1-42	779	97.88	0.265	0.587
2-25+2-30+2-42	68	98.14	0.215	0.441
control at -18°C	260	98.04	0.235	0.526
control at -18°C	719	97.82	0.278	0.630
control at -18°C	834	97.66	0.249	0.561
control at -18°C	805	97.55	0.268	0.563
control at -18°C	209	97.96	0.278	0.592
control at -18°C	243	97.96	0.265	0.569
Average			0.262	0.573
s_m			0.017	0.035

* 2-25+1-30: sample stored at 25°C for two weeks, followed by storage at 30°C for one week

Table 3. Mean of duplicate determinations of dry matter (g/100 g) and vitamin C content (mg/100 g dry weight) in RM 383 stored at different elevated temperatures and monitored at regular intervals.

Storage conditions (weeks-temperature)	Sachet code	Dry matter (g/100 g)	Retinol (mg/100 g dry weight)
1-25	528	96.41	16.49
2-25	534	96.40	16.49
3-25	403	96.42	16.08
4-25	422	96.40	15.46
5-25	721	96.44	17.63
6-25	468	96.46	16.74
*2-25+1-30	797	96.13	16.23
2-25+2-30	117	96.36	15.62
2-25+3-30	732	96.40	16.80
2-25+4-30	948	95.86	15.07
2-25+2-30+1-42	813	95.99	14.01
2-25+2-30+2-42	155	96.27	13.40
control at -18°C	149	96.33	15.68
control at -18°C	832	96.05	16.92
control at -18°C	835	96.10	17.53
control at -18°C	847	95.96	16.73
control at -18°C	872	96.08	16.91
control at -18°C	917	96.00	16.67
Average			16.74
s_m			0.60

* 2-25+1-30: sample stored at 25°C for two weeks, followed by storage at 30°C for one week

Table 4. Mean of duplicate determinations of dry matter (g/100 g) and retinol content (mg/100 g dry weight) in RM 380 stored at different elevated temperatures and monitored after 24 * and 34 months.

Storage conditions Temperature	24 months *	34 months		
	Retinol (mg/100 g dry weight)	Retinol (mg/100 g dry weight)	Sachet code	Dry matter (g/100 g)
-40°C		0.384	802	97.47
		0.379	857	97.40
Average	-	0.382		
-18°C		0.373	438	97.93
		0.346	440	97.89
Average	0.342	0.360		
+4°C		0.348	64	98.00
		0.328	907	97.78
Average	0.333	0.338		

* details are given in RIKILT report 91.31 [2]