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PREPARATION, HOMOGENEITY AND STABILITY OF MILKPOWDERS CONTAINING POLYCHLORODIBENZO-P-DIOXINS AND POLYCHLORODIBENZOFURANES

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ABSTRACT

Preparation, homogeneity and stability of milkpowders containing polychlorodibenzo-p-dioxins and polychlorodibenzofuranes

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9 tables, 4 references, 15 pages

A non-enriched, a low dioxin and a medium dioxin level milkpowder was prepared for intercomparison purposes. The dioxin level, determined with international (I) accepted TEF values, is respectively about 3, 9 and 14 pg I-TEQ/g milkfat. The homogeneity and the stability of the milkpowders were checked by analysis of the fat, Kjeldahl-N and PCB content. The homogeneity of the milkpowders was shown to be excellent. The stability tested over a period of about one year at three temperatures (-20 °C, +20 °C and +37 °C) shows that the materials, based on the analysis of the fat and Kjeldahl-N content are stable.

Keywords: dioxins, milkpowder, homogeneity, stability

SAMENVATTING

Voor het Community Bureau of Reference (BCR) werden een "natuurlijk" gecontamineerd, een laag en een medium niveau gecontamineerd melkpoeder bereid. Van eenzelfde batch boterolie werden drie aparte batches bereid. Aan twee batches werd een verschillende hoeveelheid dioxine toegevoegd. Uitgaande van ondermelk werden met behulp van de drie batches boterolie drie batches melk bereid. Het dioxinengehalte in de drie batches, bepaald met behulp van internationaal geaccepteerde TEF waarden, bedraagt resp. ca. 3, 9 en 14 pg I-TEQ/g melkvet. Een deel van elke batch melk werd gevriesdroogd en de rest werd verstoven. De aldus bereide gevriesdroogde melkpoeder werd gecontroleerd op homogeniteit en stabiliteit.

Voor de homogeniteit werden het vetgehalte, Kjeldahl-N en PCB gehalte bepaald. Uit de resultaten van de verschillende batches bleek dat de homogeniteit voor zowel de gevriesdroogde als de verstoven melkpoeder homogeen was.

De stabiliteit werd gecontroleerd in de gevriesdroogde melkpoeders die gedurende ca. één jaar bij -20°C, +20°C en 37°C waren opgeslagen. Uit de resultaten van de vet- en Kjeldahl-N-bepaling bleek dat de gevriesdroogde melkpoeders stabiel zijn.

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1 INTRODUCTION

The determination of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in food products (e.g. milk) poses specific difficulties due to the low concentration of the compounds to be measured, the matrix and the presence of other more concentrated contaminants such as polychlorinated biphenyls. However, in view of the recent developments and the public concern about food (e.g. milk) contamination, it was decided in a meeting of the BCR in Brussels, 19 June 1990 to improve the measurement of PCDDs and PCDFs in food by preparing milkpowder samples containing different concentrations of PCDDs and PCDFs (dioxins) for intercomparison studies.

2 MATERIALS AND METHOD

2.1 Preparation of samples

A dioxin solution, containing 12 isomers, prepared at the Studie Centrum voor Kernenergie (SCK), Mol, Belgium shall be used for the enrichment of milk with dioxins (Table 1). In the meeting of BCR in Brussels it was decided to prepare a non spiked milk (batch A) a low level contaminated milk (batch B) and a medium level contaminated milk (batch C).

Isomer	Concentration (ng)/6.941 g isooctane
2378 TCDD	45.0
12378 PCDD	106.9
123478 HCDD	57.3
123678 HCDD	160.1
123789 HCDD	56.3
2378 TCDF	52.9
12378 PCDF	5.9
23478 PCDF	232.5
123478 HCDF	135.3
123678 HCDF	121.6
123789 HCDF	3.2
234678 HCDF	155.1

For the preparation of the milk samples a batch of skimmed-milk and a batch of butteroil shall be selected. The butteroil shall contain a low incurred "dioxin" content e.g. 2 to 3 pg I-TEQ/g fat.
In total three batches milk shall be prepared respectively non-spiked (batch A), spiked at the 6 pg I-TEQ/g level (batch B) and spiked at the 12 pg I-TEQ/g level (batch C) on a fat basis. All batches shall contain an equivalent fat content.
The skimmed milk shall be separated into three batches for the preparation of three batches milk, each 400 l.
From each batch (A, B and C) 80 l of the preparared milk shall be transported to the Central Bureau for Nuclear Measurements (CBNM), Geel, Belgium where they shall be freeze dried. The freeze dried milk shall be ground, mixed and sieved to pass a sieve with apertures of 100 μm. The sieved fraction shall be homogenised in a mixing drum for 24 hours. In total 160 bottles containing each about 65 g milkpowder shall be filled.
The last 320 l milk shall be spray dried at the Netherlands Institute for Dairy Research (NIZO) and transported to CBNM where it shall be treated as the freeze-dried milk and bottled.

2.2 Homogeneity study

The between bottle homogeneity of all three freeze dried and three spray dried milkpowders shall be verified by determining in each of five bottles selected along the bottling procedure of each of the six materials the content of the natural occuring chlorobiphenyls (PCB). Also the Kjeldahl-N and the fat content shall be determined.

2.3 Stability study

The stability study will only be conducted on the freeze dried samples. The Kjeldahl-N content in three bottles of each of the three batches milkpowders shall be determined immediately after preparation. Also three bottles of the unspiked and spiked samples shall be stored at -20 °C, +20 °C and +37 °C until the end of the intercomparison exercise on the freeze dried batches. Also the fat content shall be determined at both occasions.

2.4 Method

The chlorobiphenyl content of the milkpowders shall be determined in the milkfat using saponification for extraction and clean-up. After a final clean-up on basic alumina the chlorobiphenyls shall be determined by capillary gas chromatography using electron capture detection (Tuinstra).
The fat content shall be determined according to IDF standard 9C: 1987 (Röse Gottlieb procedure) and the Kjeldahl-N-content shall be determined according to IDF standard 20A: 1986 (Kjeldahl method) and shall be expressed on a dry mass basis.

3 RESULTS AND DISCUSSION

3.1 Preparation of samples

The butteroil used for the preparation of the milk is a part of a batch butteroil with a low incurred dioxine content used at RIKILT-DLO as a control sample in the dioxin analyses.

The mean is 2.60 pg I-TEQ/g milkfat, the standard deviation 0.31 pg I-TEQ/g milkfat and the CV 11.8% (n=32).

For batch A, B and C 500 g butteroil each was used. To batch B 2.3856 g of the dioxin solution (see 2.1) and to batch C 4.3918 g of the dioxin solution (see 2.1) was dosed. The dose and the theoretical final concentration in the milkpowders of the dioxins, expressed in pg I-TEQ/g milkfat is summarized in table 2.

Both spiked butteroils and a batch original butteroil were transported to NIZO, Ede, the Netherlands for the preparation of the three batches milkpowder.

Table 2: Theoretical dose (ng) and concentration of dioxins in the milkpowders (pg I-TEQ/g milkfat)

Isomer	Batch B		Batch C	
	ng	I-TEQ (pg/g)	ng	I-TEQ (pg/g)
2378 TCDD	15.4665	0.9667	28.4728	1.7796
12378 PCDD	36.7415	1.1482	67.6388	2.1137
123478 HCDD	19.6940	0.1231	36.2553	0.2266
123678 HCDD	55.0264	0.3439	101.2999	0.6331
123789 HCDD	19.3503	0.1209	35.6227	0.2226
2378 TCDF	18.1817	0.1136	33.4714	0.2092
12378 PCDF	2.0278	0.0063	3.7331	0.0117
23478 PCDF	79.9103	2.4972	147.1095	4.5972
123478 HCDF	46.5026	0.2906	85.6083	0.5351
123678 HCDF	41.7939	0.2612	76.9398	0.4809
123789 HCDF	1.0998	0.0069	2.0247	0.0126
234678 HCDF	53.3079	0.3332	98.1363	0.6134
	total I-TEQ 6.21		total I-TEQ 11.44	

Batch A was prepared by adding 16 kg of the butteroil as such to 384 kg skimmed milk. The homogenisation was carried out at a temperature of 60°C in a first step at a pressure of 200 bar and in a second step at a pressure of 10 bar. The same conditions were also used for the spikes batches B and C.

Batch B was prepared by adding 15.5 kg of the butteroil to 384 kg skimmed milk and 0.5 kg of spiked butteroil containing 2.3858 g of the dioxin solution (see 2.1). By weighing back it was determined that only 494.4 g instead of 500 g spiked butteroil was added.

Batch C was prepared by adding 15.5 kg of the butteroil to 384 kg skimmed milk and 0.5 kg of spiked containing 4.3918 g of the dioxin solution. By weighing back it was determined that only 498.6 g instead of 500 spiked butteroil was added.

The final concentration of the dioxins in the milkpowder is summarized in table 3.

Table 3: Final (ng) dose and concentration of dioxins in the milkpowders (pg I-TEQ/g milkfat)

Isomer	Batch B		Batch C	
	ng	I-TEQ (pg/g)	ng	I-TEQ (pg/g)
2378 TCDD	15.2933	0.9558	28.3930	1.7745
12378 PCDD	36.3300	1.1353	67.4494	2.1078
123478 HCDD	19.4734	0.1217	36.1538	0.2259
123678 HCDD	54.4101	0.3401	101.0163	0.6313
123789 HCDD	19.1336	0.1196	35.5230	0.2220
2378 TCDF	17.9781	0.1124	33.3776	0.2086
12378 PCDF	2.0051	0.0063	3.7227	0.0116
23478 PCDF	79.0153	2.4692	146.6976	4.5843
123478 HCDF	45.9818	0.2874	85.3686	0.5335
123678 HCDF	41.3258	0.2583	76.7244	0.4795
123789 HCDF	1.0998	0.0068	2.0190	0.0126
234678 HCDF	52.7108	0.3294	97.8614	0.6116
	total I-TEQ 6.14		total I-TEQ 11.40	

The three batches were pasteurized. From each batch (A, B and C) of the prepared milk, 320 l was spray-dried immediately after pasteurisation. After concentration to 45-47% dry matter in a few temperature steps from 75°C to 58°C, the concentrate was sprayed at a temperature of 75°C and dryed to a final water content between 2.15 - 3.10%.

From each batch of the prepared milk, 80 l was separated for freeze drying. The pasteurised milk and homogenised milkpowder were transported to CBNM Geel in well closed polyethelene drums. The three milk batches were stored for a maximum of three days at 4°C. The milk batches A and B were frozen in the freeze-drier Epsilon 2 85D at -25°C and stored at -20°C. The third milk batch was frozen at -25°C in 39 hours and freeze dried using the following programme: 48 h. -20°C, 24 h. -10°C, 24 h. 0°C and 48 h. secondary drying at 20°C. The batches stored in the freezer were freeze dried two weeks later using the same procedure and programme.

The freeze dried batches A, B and C have been ground at the Company Alpin in Augsburg, Germany. Preliminary experiments carried out with freeze dried milk had shown the need to grind using a Pulverising mill Type 100 UPZ cooled with liquid nitrogen to temperatures below -50°C. At higher temperature the machine was blocked by burned particles. The results of sieve analyses are summarized in table 4.

Table 4: Particle size measured after grinding with Mill 100 UPZ von Alpine

Batch	Particle size, % smaller than			
	0.050 mm	0.075 mm	0.100 mm	0.125 mm
A	0.1	60.5	85.8	94.4
B	1.1	19.0	70.4	87.2
C	38.7	76.3	91.2	95.3

After homogenisation in the Turbula mixer the powder was put into 250 ml well cleaned bottles on a clean bench. The quantity of powder put into each bottle was determined and recorded on the label. The bottles were closed with an aluminium protected insert and a srew cap.

3.2 Homogeneity of samples

According to the protocol the homogeneity was verified by determining the PCB content, the fat content and the Kjeldahl-N content in the freeze dried and spray dried milkpowders.

The results of the PCB analysis for the congeners PCB 118, 138, 153 and 180 in the spray dried and freeze dried milkpowders are summarized in table 5. In spite of the low content of the PCB congeners (around the limit of detection of 1.0 μg/kg fat) it is shown that the materials are homogeneous.

The water content was determined according to IDF standard 26: 1964 to express the Kjeldahl-N content and the fat content also on dry matter basis.

The results of the fat, water and Kjeldahl-N content are summarized for the spray dried milkpowders in table 6 and for the freeze dried milkpowders in table 7. The results show that the materials are homogeneous.

Table 5: Homogeneity test - PCB content in freeze dried and spray dried milkpowder (μg/kg milkfat)

		Batch A	Batch B	Batch C	Batch A	Batch B	Batch C
		Freeze dried milkpowder			Spray dried milkpowder		
PCB118	$\bar{x}$	4.2	4.5	4.2	4.4	4.0	4.6
	st.dev.	0.34	0.19	0.24	0.52	1.2	0.74
	CV(%)	8.2	4.3	5.6	11.9	29.3	16.0
PCB153	$\bar{x}$	5.3	5.9	5.6	4.8	4.8	5.1
	st.dev.	0.41	0.27	0.37	0.40	0.68	0.60
	CV(%)	7.8	4.6	6.6	8.4	14.2	11.7
PCB138	$\bar{x}$	5.7	6.4	6.3	5.9	6.1	7.0
	st.dev.	0.31	0.40	0.42	0.49	1.0	0.98
	CV(%)	5.5	6.2	6.6	8.4	17.1	14.0
PCB180	$\bar{x}$	2.4	2.7	2.6	2.6	2.5	3.1
	st.dev.	0.19	0.13	0.29	0.12	0.12	0.51
	CV(%)	8.1	4.9	11.3	4.8	4.8	16.4
Number of samples		5	5	5	5	4	5

Table 6: Homogeneity test - Fat, water and Kjeldahl-N content in spray dried milkpowder (%)

		Batch A (n=5)	Batch B (n=5)	Batch C (n=5)
Fat[1)]	$\bar{x}$	29.60	29.75	29.67
	st.dev.	0.044	0.056	0.047
	CV	0.15	0.19	0.16
Water	$\bar{x}$	2.67	2.55	2.84
	st.dev.	0.115	0.131	0.159
	CV	4.32	5.15	5.59
Kjeldahl-N[1)]	$\bar{x}$	27.84	27.73	27.68
	st.dev.	0.073	0.086	0.088
	CV	0.26	0.31	0.32

1) expressed on dry mass basis

Table 7: Homogeneity test - Fat, water and Kjeldahl-N content in freeze dried milkpowder (%)

		Batch A (n=5)	Batch B (n=5)	Batch C (n=5)
Fat[1)]	$\bar{x}$	29.44	29.61	29.82
	st.dev.	0.044	0.030	0.107
	CV	0.15	0.10	0.36
Water	$\bar{x}$	2.40	1.87	1.76
	st.dev.	0.105	0.036	0.086
	CV	4.38	1.92	4.89
Kjeldahl-N[1)]	$\bar{x}$	27.81	27.75	27.68
	st.dev.	0.033	0.086	0.052
	CV	0.12	0.31	0.19

1) expressed on dry mass basis

The theoretical final dioxin content expressed on dry mass in the spray dried and freeze dried milkpowders of batch B and C is summarized in table 8. For the calculation the theoretical final dose reported in table 3 and the fat content expressed on dry mass in table 6 and 7 is used.

Table 8: Theoretical final dioxin content in spray dried and freeze dried milkpowders (pg/g dry mass)

Isomer		Spray dried		Freeze dried	
		Batch B	Batch C	Batch B	Batch C
2378	TCDD	0.2844	0.5265	0.2830	0.5292
12378	PCDD	0.6755	1.2507	0.6723	1.2571
123478	HCDD	0.3621	0.6702	0.3604	0.6736
123678	HCDD	1.0118	1.8731	1.0070	1.8825
123789	HCDD	0.3558	0.6587	0.3541	0.6620
2378	TCDF	0.3344	0.6189	0.3328	0.6220
12378	PCDF	0.0375	0.0688	0.0373	0.0692
23478	PCDF	1.4692	2.7203	1.4623	2.7341
123478	HCDF	0.8550	1.5829	0.8510	1.5909
123678	HCDF	0.7684	1.4227	0.7648	1.4299
123789	HCDF	0.0202	0.0374	0.0201	0.0376
234678	HCDF	0.9799	1.8146	0.9754	1.8238

Table 9: Stability test - Fat, water and Kjeldahl-N content in freeze dried milkpowder at different temperatures (%)

		Temperature			
		-20°C	+20°C	+20°C	+37°C
		February 1992	March 1991	February 1992	February 1992
		(n=3)	(n=3)	(n=3)	(n=3)
Batch A					
Fat[1)]	$\bar{x}$	29.46	29.30	29.44	29.38
	st.dev.	0.041	0.144	0.074	0.044
	CV(%)	0.14	0.49	0.25	0.15
Water	$\bar{x}$	2.46	2.34	2.50	2.37
	st.dev.	0.057	0.076	0.038	0.031
	CV(%)	2.32	3.25	1.52	1.31
Kjeldahl-N[1)]	$\bar{x}$	27.70	27.75	27.77	27.77
	st.dev.	0.053	0.082	0.029	0.062
	CV(%)	0.19	0.29	0.10	0.22
Batch B					
Fat[1)]	$\bar{x}$	29.65	29.47	29.67	29.63
	st.dev.	0.077	0.109	0.071	0.047
	CV(%)	0.26	0.37	0.24	0.16
Water	$\bar{x}$	1.82	1.79	2.07	1.85
	st.dev.	0.053	0.018	0.057	0.032
	CV(%)	2.91	1.01	2.75	1.73
Kjeldahl-N[1)]	$\bar{x}$	27.67	27.58	27.66	27.70
	st.dev.	0.079	0.053	0.060	0.029
	CV(%)	0.29	0.19	0.22	0.10
Batch C					
Fat[1)]	$\bar{x}$	29.69	29.64	29.69	29.60
	st.dev.	0.024	0.068	0.018	0.038
	CV(%)	0.082	0.23	0.062	0.13
Water	$\bar{x}$	1.73	1.72	1.80	1.72
	st.dev.	0.041	0.124	0.083	0.089
	CV(%)	2.37	7.21	4.61	5.17
Kjeldahl-N[1)]	$\bar{x}$	27.73	27.59	27.71	27.73
	st.dev.	0.019	0.093	0.072	0.071
	CV(%)	0.068	0.33	0.26	0.26

1) expressed on dry matter

3.3 Stability study

The stability study was carried out only with the freeze dried milkpowders by determining the water, fat and Kjeldahl-N content at the moment of receipt of the samples in March 1991 and after storage during 11 months at a temperature of -20°C, +20°C and +37°C in February 1992.
The results of the stability study are summarized in table 9 and show for each batch (A, B and C) that the fat and Kjeldahl-N content at the different temperatures is within the repeatability of the method of analysis.

4 CONCLUSION

The homogeneity of the spray dried and freeze dried milkpowders determined by fat, Kjeldahl-N and PCB analyses showed that the different batches milkpowder are homogeneous.
The stability tested over a period of about one year at three temperatures (-20°C, +20°C and +37°C) showed that the different batches milkpowder, based on analysis of the fat and Kjeldahl-N content, are stable.

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