Determination of Nitrate and Nitrite in Whey Powder

Nrisinha P. Sen* and Young C. Lee

Two methods are described for the determination of nitrate and nitrite in whey powder. Both the methods are based on diazotization and coupling reactions, and final quantitation by colorimetric measurement. One of the methods worked well with the majority of the samples with the exception of a few which produced turbid or colored extracts. The other method, which uses an extra ion-exchange cleanup step, was useful in analyzing these troublesome samples. The methods were applied to the analysis of 15 whey powder samples, some of which (those prepared from cheese milk containing nitrate as an additive) contained high (up to 1760 ppm) levels of nitrate. The average percentage recovery of nitrate added to various whey powders at levels of 30–50 ppm was 96.4 and that for nitrite at levels of 10–30 ppm was 89.6. The minimum detection limit is about 2–10 ppm depending on the sample size taken for the analysis. The second method has the potential for the analysis of nitrate and nitrite in other difficult to analyze samples such as animal feed.

Because of the concern over the toxicity of nitrate and nitrite to humans, especially to infants, and the role these chemicals play in the formation of nitrosamines, which are highly carcinogenic, considerable attention has been paid to developing suitable analytical methodology for nitrates and nitrites in foods. Although there are numerous methods available for this purpose, many of them present difficulties when applied to the analysis of whey powder. Since whey powder is becoming increasingly important as a supplementary food item and since it is incorporated in certain types of baby foods, the International Dairy Federation in its 1975 annual meeting recommended that a suitable method be developed for the quantitative determination of nitrate and nitrite in whey powder (International Dairy Federation Report, 1975). Gray et al. (1979) have also pointed out the need to monitor the levels of nitrate, nitrite, and nitrosamines in whey powders made with the addition of nitrate. As a result of these recommendations we applied our own method (Sen and Donaldson, 1978) to whey powder and also encountered some difficulties. Our method worked reasonably well with most of the samples analyzed, but some samples produced turbid or colored extracts which interfered in the final colorimetric estimation step. In this paper we wish to report an ion-exchange cleanup technique which solved the above-mentioned problems.

EXPERIMENTAL SECTION

Anion-Exchange Column. About 100 g of the resin (Dowex 1-X1, 50–100 mesh, chloride form, strongly basic anion-exchange resin, J. T. Baker Chemical Co., Phillipsberg, NJ) was allowed to soak in water overnight. A 25-mL glass buret, 1 cm i.d., was used to prepare the column, and the dimensions of the resin bed were about 3 cm high × 1 cm diameter. The resin column was first washed with 25 mL of 1 N sodium hydroxide and then with water until the pH of the washings was 7–8. Care was taken to keep the resin bed filled with liquid at all stages of the operation. [Note: A fresh column must be used for each analysis. A blank run should be carried out with each new batch of resin to ensure that it is not contaminated with nitrate or nitrite.]

Analysis of Whey Powder for Nitrate and Nitrite by the Normal Method. Method A. The details of the method have already been published (Sen and Donaldson, 1978). It can be briefly outlined as follows. (a) Extraction of whey powder (2-, 5-, or 10-g aliquots) by blending with a weakly alkaline aqueous solution and digestion at 55 °C for about 15–20 min. (b) Precipitation of the protein and other interfering materials by the addition of $ZnSO_4$ solution and removal of the precipitate by filtration. (c) Colorimetric determination of nitrite after diazotization and coupling with a solution containing sulfanilic acid and *N*-(1-naphthyl)ethylenediamine dihydrochloride. (d) Determination of total nitrate and nitrite as above after reduction of the nitrate to nitrite on a spongy Cd column. (e) Calculation of the nitrate content by difference of the two values obtained under steps d and c.

For routine analysis of an unknown whey powder, a 10-g aliquot was analyzed first. If the result was higher than 50 ppm of sodium nitrate, the analysis was repeated with a smaller (2 or 5 g) sample size. In cases where the nitrate contents were very high (>500 ppm), the filtrate from step b above was appropriately diluted with water before passing the solution through the Cd column.

Analysis by the Method Involving the Ion-Exchange Cleanup Step. Method B. The initial extraction and digestion steps were same as in method A. The filtrate obtained from step b above was used for further clean-up on the ion-exchange column.

Ion-Exchange Cleanup. A 2–25-mL aliquot (depending on the sensitivity desired and the expected concentration of nitrate in the sample) of the filtrate was adjusted to pH 7–8 by the addition of a few drops of 0.2 or 1 N NaOH, and the solution was passed through the resin column at a flow rate of 2–4 mL/min. The column was then washed with 50 mL of water and the washing discarded. Finally, the nitrate and nitrite from the column were eluted with 20 mL of 5% sodium chloride solution, and the eluate was collected and made up to 25.0 mL in a volumetric flask. The solution was mixed well and used for the colorimetric estimation of nitrate and nitrite. [Note: The resin column was discarded after each analysis. Care should be taken not to overload the column as it results in a greater loss of nitrate and nitrite in the effluent and washing.]

Determination of Nitrite. A 10-mL aliquot of the solution from the step above was analyzed for nitrite as described previously (Sen and Donaldson, 1978) with one additional change. During color development, 2.0 mL of 5% sodium acetate solution was added to each flask in addition to the reagents described in the method. The resin column treatment changed the pH of the eluate and

Food Research Division, Food Directorate, Health Protection Branch, Ottawa, Canada K1A 0L2.

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Table I. Percentage Recoveries of Added Nitrite and Nitrate from Various Samples of Whey Powder as Obtained by Method A

sample no.	spiking level, ppm		% recovery[a]		
	$NaNO_2$	$NaNO_3$	$NaNO_2$	$NaNO_3$	$NaNO_3$[b]
without any whey powder	30	37	96.4	93.4	104
1 (turbid extract)	10	30	124	170	189
1	40	100	100	98	109
2 (strongly colored extract)	10	30	64	78	87
3	10	30	74.4	82	91
3	40	100	83	82	91
3	20	50	87	88	98
3	20	50	97	91	101
4 (turbid and colored extract)	10	30	103	137	152
4	20	50	82	93	103
4	40	100	89	93	103
5	30	37	96	86.6	97
6	20	50	85.5	87.5	97
		av[c]	90.4	87.9	97.6

[a] Replicate analyses were carried out on different days. [b] Corrected for 90% Cd column efficiency. [c] Excluding the extremely high recovery data for nitrate obtained with the two turbid extracts and those of the control sample (without any whey powder).

Table II. Percentage Recoveries of Added Nitrite and Nitrate from Various Samples of Whey Powder as Obtained by the Ion-Exchange Cleanup Method (Method B)

sample no.	spiking level, ppm		% recovery		
	$NaNO_2$	$NaNO_3$	$NaNO_2$	$NaNO_3$	$NaNO_3$[a]
without any whey powder	10	30	97.8	89.4	99.3
1	10	30	67	95.7	106
2	10	30	85	105.9	118
3	20	50	92.5	80.3	89
4	20	50	98	77.4	86
5	20	50	90	75.8	84
6	20	50	96	74.8	83
		av[b]	88.0	85.0	94.3

[a] Corrected for 90% Cd column efficiency. [b] Excluding the control sample (without any whey powder).

hence it was necessary to add the sodium acetate reagent to bring the final pH of the solution (during color development) close to 3.1. [Note: Sodium acetate reagent should not be used while determining the standard curve for nitrite because the standards are not taken through resin columns and hence there is no change in the pH.]

Determination of Nitrate and Nitrite after Reduction on a Spongy Cd Column. Another 10-mL aliquot of the solution obtained after the ion-exchange cleanup step was analyzed for total nitrate and nitrite as described previously (Sen and Donaldson, 1978). As in the case for nitrite estimation, 2.0 mL of 5% sodium acetate solution was added to each flask during the final color development. The difference between the two results (nitrite values obtained before and after reduction on Cd column) gave a measure of the nitrate content. Since all calibrations and measurements were made as sodium nitrite, this result (nitrate content) was multiplied by the factor 1.23 to convert it in terms of sodium nitrate.

RESULTS AND DISCUSSION

Percentage recoveries of nitrite and nitrate added to various whey powders as obtained by the methods A and B are presented in Tables I and II, respectively. All the samples used for the recovery studies were of Canadian origin and all gave negative results for nitrate and nitrite. As can be seen from the data, the normal method A worked quite well with all the samples except three which produced turbid or colored extracts, and hence the results in these three cases were inconsistent and erratic. Although on repeat analysis two of these troublesome samples (no. 1 and 4) produced clear extracts and gave good recovery results at higher levels of spiking, the turbidity and the color were still a problem at the lower level. All of these samples could, however, be easily analyzed by the method B. With the method B the final extracts in all cases appeared very clear and nearly colorless. Because of the added cleanup step, small losses of nitrite and nitrate

Table III. Nitrate and Nitrite Levels in Some Samples of Commercial Whey Powder

sample no.	method used	level, ppm		
		$NaNO_2$	$NaNO_3$	$NaNO_3$[a]
1	A	0	0	0
2	A	0	0	0
3	A	0	0	0
4	A	0	0	0
5	A	0	0	0
6	A	0	0	0
7	A	0	0	0
8	A	0	16	18
9	A	0	990	1100
	B	0	940	1050
10	A	0	1590	1760
	B	0	1500	1660
11	A	0	1020	1130
	B	0	940	1040
12	A	0	1390	1540
	B	0	1450	1610
13	A	0	840	930
	B	0	982	1090
14	A	0	895	994
	B	0	833	925
15	A	Tr	847	941
	B	Tr	873	970

[a] Corrected for 90% Cd column efficiency.

occurred during the resin cleanup (mainly in the effluent and the washing). The average recoveries obtained by the method B were, therefore, slightly lower than those obtained by the method A.

Nitrate and nitrite levels detected in some 15 whey powder samples are given in Table III. Of these, the first eight samples were of Canadian origin and they were known to have been manufactured without the addition of any nitrate. The remaining samples were of foreign origin, and they were made by a process which involved the addition of nitrate as an additive to the cheese milk. As expected, all the Canadian samples were negative except one which contained very low levels of nitrate. All the samples prepared by the nitrate addition process contained high levels of nitrate. Some of the samples were analyzed by both the methods (A and B), and the two sets of results agreed quite well, mostly within ±10%. No foreign normal (made without nitrate additive) whey powders were analyzed in this study. They would be expected to contain similar levels of nitrate and nitrite as the domestic varieties.

It must be emphasized that these whey powders containing excessively high levels of nitrate are exclusively used as animal feeds and not meant to be used for human consumption. Therefore, no health hazards to humans are expected from such products.

Since both the nitrate-free and nitrate-rich whey powders look alike, inadvertent mixing or mislabeling is possible. It would be advisable, therefore, to mark or stain such nitrate-rich whey powders with some acceptable food colors, thereby reducing the chance of such accidental mix-ups and eliminating any possible health hazard to infants consuming whey powder containing baby food.

Preliminary results suggest that this technique would be extremely useful in analyzing laboratory animal diets (e.g., rat chow) for nitrate and nitrite. These animal feeds also produce strongly colored extracts which make them difficult to analyze by the method A.

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Composition of Polar Lipids in Carrot Roots

Juhani Soimajärvi*[1] and Reino R. Linko

Lipids extracted from carrot roots have been fractionated on a silicic acid column into neutral lipids (NL), glycolipids (GL), and phospholipids (PL), and the composition of the polar lipids have been studied by chemical analysis after thin-layer chromatographic (TLC) development. In root samples of different origin the total lipid content at harvest varied from 225 to 340 mg/100 g of fresh root, but the percent composition of the three main lipid fractions was nearly similar: NL represented over 60% of the total lipid and the percentages of GL and PL were respectively 13–21% and 16–21%. The most abundant lipid classes in the PL fraction were phosphatidylcholines and phosphatidylethanolamines which together amounted to 65% of this fraction. The PL fraction contained 1.0% aldehydogenic lipids. The main part of GL consisted of digalactosyl and monogalactosyl diglycerides. The quantitative composition of polar lipids was in general very similar to that in many other nonphotosynthetic plant storage tissues.

Lipids in carrot root have received little attention, although their composition and changes are included among possible quality factors in carrot products. On the other hand, environmental conditions, which have an influence on yield, and different constituents of carrot have been studied extensively. Most research on carrot lipids has been applied to carotenes because of their importance as previtamins. Since the early work of Hanahan and Chaikoff (1947), only occasional published information has been available on the polar lipids of carrot (Hölzl, 1965). Quite recently, lipid composition from carrot root tissue cultures has been reported (Kleinig and Kopp, 1978) and it has been also compared with certain major lipids from the root material (Gregor, 1977).

In the present paper the authors present the composition of polar lipid classes in carrot roots, to supplement their earlier report, which was mostly concerned with the composition of neutral lipids (Soimajärvi and Linko, 1973).

EXPERIMENTAL SECTION

Materials. Five different samples of carrot, *Daucus carota* cv. Feonia Hunderup S 64 LH, roots were used. Samples A and B originated from a farm in Köyliö, southwestern Finland, sample C from another farm in southwestern Finland, and samples D and E from the Agricultural Research Station of Laukaa, central Finland. The mean fresh weights of roots are given in Table I. The roots were harvested at the normal time in September or October and stored at 4 °C for 1–2 weeks before use. The growth season of carrots was limited by natural environmental factors as the temperature, and it was 19 weeks in samples A–C and 16 weeks in samples D and E.

Department of Biology, University of Jyväskylä SF 40100 Jyväskylä, Finland (J.S.) and the Department of Chemistry and Biochemistry, University of Turku, SF 20500 Turku, Finland (R.R.L.).

[1]Present address: Regional Laboratory for Food Research, Rautatienkatu 11, SF-44100 Aänekoski, Finland.

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