Stability of a Long Chain Polyphosphate in Aqueous Cheese Extracts

L. G. Scharpf, Jr., and T. P. Kichline

The stability of a long chain sodium polyphosphate (Graham's salt) added to aqueous cheese extracts was investigated. The concentration and distribution of phosphate species as P_2O_5 remained unchanged in extracts stored at 3–7° C. for four weeks. During four weeks of storage at 20° C., long chain species decreased from 89 to 64% while orthophosphate species increased from 4 to 27%. Two predominant genera of microorganisms were isolated from cheese extracts, and natural alkaline phosphatase activity of extracts was high.

Increasing quantities of condensed phosphates are being used with considerable success in the manufacture of pasteurized process cheese, cheese foods, and cheese spreads. European countries have reported that these compounds have a definite advantage over conventional orthophosphates in producing certain desired functional properties in cheese. Polyphosphates, for example, do not produce certain flavors often encountered with orthophosphates (Becker and Ney, 1965). Process cheeses with superior consistencies, emulsions, and organoleptic properties have been produced with the use of polyphosphates (Becker and Ney, 1965; Kiermeier and Mohler, 1960; Mair-Waldburg, 1958).

Stability of condensed phosphates in cheese is important, since any degradation of the compounds could seriously reduce the quality of the stored product. Degradation of emulsifier systems in process cheese generally results in the appearance of discolorations, development of a sandy or gritty texture, and possible destruction of the emulsion (Becker and Ney, 1965). Since these changes also may be the result of chemical and/or microbiological decomposition of the cheese itself, they are probably not adequate criteria in studying the chemical decomposition of the phosphates. Studies on extracts of stored cheeses indicate that condensed phosphates undergo little, if any, degradation in cheese on storage at refrigerator temperatures (Becker and Ney, 1965). These data, however, do not preclude the possibility that phosphates may be degraded during storage at various temperatures in cheese extracts containing microbiological and enzymatic activity.

In this study, Graham's salt was chosen as a typical representative of the family of linear chain polyphosphates. The compound is an amorphous polyphosphate having the empirical composition $NaPO_3$ (molar ratio Na_2O/P_2O_5 = 1.0) and consisting of 40–90 PO_3 units (Kichline and McCullough, 1957). Factors such as temperature, pH, ionic environment of the media, and concentration of complexing cations affect the rate of hydrolytic degradation of Graham's salt (McCullough *et al.*, 1956). At neutral pH and room temperature, chain phosphates hydrolyze extremely slowly, their half-lives being of the order of years (Van Wazer *et al.*, 1955). On the other hand, enzymatic hydrolysis of condensed phosphates can be quite rapid (McElroy and Glass, 1951). Various microorganisms are indigenous to natural cheeses used in the manufacture of process cheese (Kosikowski, 1966). These microorganisms may be able to degrade long chain phosphates.

Since casein components (Pyne, 1962; Zittle, 1966) and, perhaps, paracasein have an affinity for phosphate compounds, the latter are difficult to extract quantitatively from cheese (Chojnicka, 1963). Furthermore, when protein precipitants are used in the extraction process, a portion of the phosphate probably is adsorbed on the protein precipitate (Chojnicka, 1964). Finally, the chemical instability of several of the phosphate anions precludes the use of acids, alkalis, and many organic solvents as cheese extractants. In light of these difficulties, the stability of a long chain phosphate in aqueous cheese extracts was investigated. The extracts provide quantitative addition and recovery of the phosphate, a medium in which the individual phosphate species are stable, and contain enzyme systems and microorganisms indigenous to cheese. The results show that a long chain phosphate (Graham's salt) is stable at refrigerator temperatures and is degraded partially at 20° C. during four weeks of storage.

MATERIALS AND METHODS

Phosphates. Graham's salt (50 PO_3 units) of food grade quality was supplied by the Monsanto Co., St. Louis, Mo.

Preparation of Cheese Extracts. Raw barrel cheddar cheese, 21 days old and stored at 3–7° C., was used to prepare the cheese extracts. The cheese (100 grams) was chopped and blended with 700 ml. of deionized water for 5 minutes at 25° C. at low speeds in a Waring Blendor. The slurries were filtered through two layers of cheese cloth and through two pieces of filter paper (Whatman No. 3) under vacuum. The pH of the cheese itself was 5.7 and pH of the extracts ranged from 6.7–6.9.

Storage of Extract-Phosphate Mixtures. Storage tests were carried out in 118-ml. screw top vials in a refrigerator at 3–7° C. to simulate commercial conditions, and in a constant temperature box maintained at 20° ± 1° C. Mixtures were prepared by adding a known amount of the phosphate (about 2 grams) to exactly 100 ml. of the cheese extract, and were shaken twice daily. The control phosphate solution was prepared by adding 2.0 grams of phosphate to exactly 100 ml. of sterile deionized water. The control solution had a pH of 6.0. Containers were shaken before sampling to ensure homogeneity of the

Inorganic Chemicals Division, Research Department, Monsanto Co., St. Louis, Mo. 63166.

mixtures. Sample volumes of 10 ml. were removed at the selected time intervals and were clarified by filtering through a millipore filter (0.45 micron) under vacuum.

Phosphate Analyses. Phosphate species in cheese extracts were determined by paper chromatography using a modified Karl-Kroupa technique as described in ASTM Standards, 1963. The filtered samples (0.5 ml.) were spotted in bands on sheets of chromatographic paper (Schleicher and Schuell No. 589). Mixtures were separated after 1.0 to 1.5 hours into ortho, pyro, tripoly, trimeta (cyclic), and long chain species which were cut out and measured quantitatively by colorimetry. Total phosphorus in extracts was expressed as P_2O_5 and was determined according to ASTM Standards (1963).

Microbiological Analysis of Cheese. Cheese extracts for microbial counts were prepared by blending 10 grams of cheese with 90 ml. of sterile saline solution. These extracts differ from extracts for phosphate stability tests in that saline was used in place of distilled water and extracts were not filtered. A 10-fold dilution of this solution was made with sterile saline, and 1.0-ml. aliquots were plated onto the surface of agar for counts. Bacterial counts were made on nutrient agar (Difco), and fungal counts were made on Sabouraud's agar and cheese agar. Cheese agar contained 1.0% peptone, 10% cheese, and 1.5% agar and was buffered to pH 7.0. The incubation temperature, rather than the medium, was the most important factor in determining predominating species. Viable microorganisms were isolated and identified according to standard microbiological procedures.

Alkaline Phosphatase Determination. Alkaline phosphatase activity of cheese extracts was determined using a modified King-Armstrong method (King and Armstrong, 1934). *p*-Nitro phenyl phosphate was used as the substrate, and liberation of phenol was determined colorimetrically in an AutoAnalyzer using 4-amino-*anti*-pyrine as a color reagent.

RESULTS AND DISCUSSION

The total amounts of P_2O_5 and individual phosphate species as P_2O_5 at the selected time intervals are summarized in Table I. Essentially no change within experimental error was observed in the concentration and distribution of phosphate species in the control solution. These results are consistent with those previously reported in which the half-life of Graham's salt in water at pH = 7.0 and 30° C. is 5.4 years (Kichline and McCullough, 1957). The cheese extracts contained a small amount of phosphorus which is inherently associated with the paracasein component. Cheddar cheese normally contains about 478 mg. of P per 100 grams of cheese (Kosikowski, 1966).

The phosphate distribution in the extract stored at refrigerator temperatures remained unchanged, while considerable changes in distribution were observed in 20° C. extracts. During storage, long chain species decreased from 89 to 64%, while trimeta and ortho species increased from 4 to 7 and from 4 to 27%, respectively. Considerable microbial growth was observed in the 20° C. extracts and microbiological analyses showed that *Penicillium* species predominated at about that temperature (Table II). The growth of microorganisms in the 20° C. extract undoubtedly catalyzed the breakdown of the phosphate. Various organisms such as bacteria, fungi, and algae accelerate the decomposition of pyro- and tripolyphosphates a thousandfold (Karl-Kroupa *et al.*, 1957). At least five enzyme systems have been isolated from microbial cells that degrade inorganic polyphosphates (Harold and Harold, 1965).

Since considerable alkaline phosphatase activity was present in the barrel cheese as compared to fresh pasteurized cheddar cheese, it was concluded that the barrel cheese was ineffectively pasteurized (Table II). Therefore, other enzyme systems indigenous to the cheese were probably active in the extracts and may have contributed to the degradation of the polyphosphate. It is doubtful

Table I. Stability of Graham's Salt in Aqueous Cheese Extracts

Extract	Total P_2O_5 Added, Mg.	Temp. of Storage, ° C.	Time of Storage, Hr.	Total P_2O_5 Recovered, Mg.	% of Total P_2O_5					pH Range of Extracts
					Ortho ± 1%	Pyro ± 1%	Tripoly ± 1%	Trimeta ± 1%	Long chain ± 2%	
Cheese extract alone	...	20	0	27.5	98.02	1.98	...	...	...	6.7-6.9
			168	27.8	99.02	0.98	...	...	...	
			336	27.5	98.90	1.10	...	...	...	
			504	27.8	98.95	1.05	...	...	...	
			672	27.8	98.95	1.05	...	...	...	
Phosphate control	1345.1	20	0	1335.2	1.26	2.12	0.42	6.74	89.46	6.0
			168	1336.2	1.80	2.83	0.64	6.44	88.29	
			336	1336.2	1.79	2.68	0.90	6.01	88.62	
			504	1336.2	1.97	2.95	0.95	6.08	88.15	
			672	1336.2	2.10	2.52	0.50	7.30	87.58	
Cheese extract with phosphate	1345.2	3-7	0	1390.2	4.26	2.20	0.28	4.13	89.13	6.4-6.6
			168	1391.9	3.85	1.80	0.39	4.62	89.34	
			336	1398.1	4.14	2.00	0.53	4.00	89.33	
			504	1398.1	4.48	1.74	0.50	5.23	89.05	
			672	1398.0	4.35	1.87	0.45	4.20	89.13	
Cheese extract with phosphate	1348.3	20	0	1394.6	4.28	2.07	0.28	4.14	89.23	6.4-6.6
			168	1407.4	8.54	0.17	0.34	5.03	85.92	
			336	1407.5	14.73	0.13	0.26	5.87	79.01	
			504	1407.4	20.86	0.22	0.22	6.95	71.75	
			672	1407.5	27.68	0.10	0.10	7.83	64.29	

Table II. Microbiological and Phosphatase Analyses of Cheese Extracts

Microorganisms Isolated	Plate Count
Bacillus species	100,000/g. at 37° C.
Penicillium species	100,000/g. at 25° C.
Micrococcus species	1,000/g. at 37° C.
Alkaline Phosphatase Activity	**μg. Phenol/G. Cheese**
Raw barrel cheese	100[a]
Fresh, pasteurized cheddar cheese	20

[a] Mean of triplicate analyses.

that the alkaline phosphatase contributed to the breakdown of the Graham's salt. Nonspecific alkaline phosphatases of animal origin are monoesterases and are devoid of any diesterase activity on such substrates as inorganic pyro- and polyphosphates (Boyer *et al.*, 1961).

Although the polyphosphate undergoes degradation at elevated temperatures in raw barrel cheese extracts, these compounds probably do not undergo any significant degradation in pasteurized process cheese even when held at room temperature. The moisture content of process cheese is approximately 40% which would reduce enzymatic and bacterial action as compared with the action of these components in aqueous solution. Moreover, the processing temperature (75–80° C.) used in the manufacture of process cheese would destroy most of the microflora and enzyme systems of the cheese.

ACKNOWLEDGMENT

The authors thank William Rorie for phosphate determinations and R. F. Gray for microbiological analyses.

LITERATURE CITED

American Society for Testing and Materials, Philadelphia, ASTM Standards, Part 22, p. 388, 1963.
Becker, E., Ney, K. H., *Z. Lebensm. Untersuch. Forsch.* **127,** 206 (1965).
Boyer, P. D., Lardy, H., Myrback, K., "The Enzymes," T. Stadtman, Ed., Vol. **5,** Chap. 4, pp. 55–71, Academic Press, New York, 1961.
Chojnicka, B., *Roczniki Panstwowego Zakladu Hig.* **14,** 415 (1963).
Chojnicka, B., *Roczniki Panstwowego Zakladu Hig.* **15,** 421 (1964).
Harold, F. M., Harold, R. L., *J. Bact.* **89,** 1261 (1965).
Karl-Kroupa, E., Callis, C. F., Seifter, E., *Ind. Eng. Chem.* **49,** 2061 (1957).
Kichline, T. P., McCullough, J. F., Monsanto Co., St. Louis, Mo., unpublished report, 1957.
Kiermeier, F., Mohler, K., *Z. Lebensm. Untersuch. Forsch.* **112,** 175 (1960).
King, E. J., Armstrong, A. R., *Can. Med. Assoc. J.* **31,** 376 (1934).
Kosikowski, F., "Cheese and Fermented Milk Foods," pp. 199, 403, Edwards Bros., Inc., Ann Arbor, Mich., 1966.
Mair-Waldburg, H., Kondensierte Phosphate Lebensmitteln Symposium, Mainz, p. 104, 1958.
McCullough, J. F., Van Wazer, J. R., Griffith, E. J., *J. Am. Chem. Soc.* **78,** 4528 (1956).
McElroy, W. D., Glass, B., "Phosphorus Metabolism," G. Schmidt, Ed., Vol. **I,** pp. 462–9, The Johns Hopkins Press, Baltimore, 1951.
Pyne, G. T., *J. Dairy Res.* **29,** 101 (1962).
Van Wazer, J. R., Griffith, E. J., McCullough, J F., *J. Am. Chem. Soc.* **77,** 287 (1955).
Zittle, C. A., *J. Dairy Sci.* **49,** 361 (1966).

Received for review February 6, 1967. Accepted May 1, 1967.