When varying amounts of PCA were added to samples of a normally flavored puree for off-flavor intensity evaluation by the triangle-intensity test, the panel selected the treated purees. The average panel scores from the triangle-intensity tests were then related in Figure 2 to the logarithm of the PCA concentration. In this method of taste-testing, the lower panel scores correspond to an increasing ability to detect flavor differences.

In concentrations approaching or exceeding 200 mg. per 100 grams of puree, PCA may cause an off-flavor in beet puree, and differences in PCA concentration of about 50 mg. per 100 grams of puree may cause a significant flavor difference.

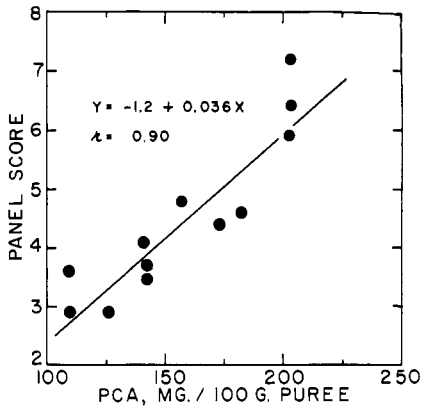

Figure 1. Relation between off-flavor scores for beet puree and concentration of PCA

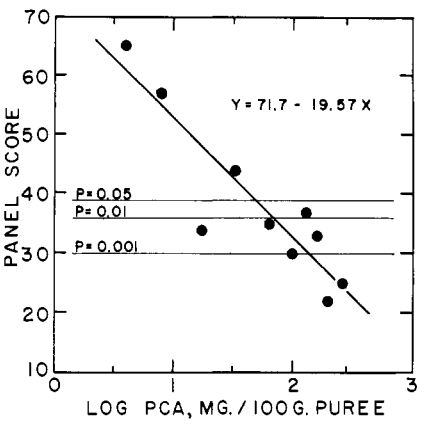

Figure 2. Effect of PCA, added to beet purees as the ammonium salt, upon the triangle-intensity score

Discussion

Taste tests made on aqueous solutions of ammonium, sodium, and potassium salts of PCA showed that they possess unpleasant flavors which might all be described in such terms as bitter and medicinal. Glutamine, the main probable precursor, is essentially tasteless.

Another source of PCA may be glutamic acid (*1, 7, 24*), but the relatively rigorous conditions required for the closing of the lactam ring make glutamic acid an unlikely source of PCA in processed foods. Moreover, this reaction provides no free ammonia. The free ammonia concentration (*13*) was found to parallel the occurrence of PCA in processed purees, and this may be considered as evidence that the probable precursor of PCA in processed beets is glutamine. The assumption that glutamine is broken down during processing to PCA and ammonia, with subsequent loss of buffering capacity, would explain the higher acidity initially observed in the off-flavored samples.

Other Products. The occurrence and contribution of PCA to off-flavor in processed foods may not be uncommon. Higher concentrations of PCA have been found, by the authors, in off-flavored dehydrated potato granules—510 mg. in the off-flavored sample in comparison with 210 mg. per 100 grams (dry-weight basis) of normal flavored granules. The PCA content of off-flavored carrot puree was found to be 50 mg. in contrast with 5 mg. per 100 grams of normal carrot puree. Pederson and Christensen (*10*) have also found an increase of from 70 to 150 mg. of PCA per 100 ml. of juice when comparing normal and off-flavored sauerkraut.

Acknowledgment

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MILK ANALYSIS

Determination of Manganese in Milk

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The low level of manganese—0.02 to 0.06 γ per ml.—in the presence of a high level of calcium—up to 0.1%—makes the determination of manganese in milk difficult because of turbidity interference by calcium sulfate.

Richards (*2*), using the periodate method with visual estimation, removed the calcium sulfate by precipitation in 33% (v./v.) sulfuric acid. The subsequent evaporation of the acid and the repeated filtrations which may be required to clarify the solution make the method inconvenient, especially with large samples. Fairly large samples, as high as 500-ml., may be required to give accurate results with the periodate method using most ab-

Accurate determination of manganese (down to 2 γ) in 100-ml. samples of milk, by the periodate method, can be made using 5-cm. light path cells of low volume. Calcium phosphate is removed in the presence of HEEDTA with little or no loss of manganese.

Table I. Recovery of 50-γ Amounts of Manganese Using Chelating Agents

Chelating Agent	Vol. of 10% Solution Used, Ml.	Oven-Dry Weight of $Ca(PO_3)_2$, G.	Mn Recovered, γ
	0	1.84	0
	0	1.87	0
EDTA	10	1.81	40
	10	1.62	40
	20	1.28	43
	20	1.19	45
	20	..	44
	20	..	43
	40	0.56	..
	40	0.52	..
DTPA	10	1.72	40
	10	1.74	40
	20	1.74	49
	20	1.63	50
	20	..	49
	20	..	49
HEEDTA	10	1.84	43
	10	1.76	40
	20	1.72	50
	20	1.74	50
	20	..	49
	20	..	50

Table II. Recovery of 10-γ Amounts of Manganese from 100-Ml. Milk Samples Using HEEDTA

Run	Mn in Milk, γ	Mn-Content after Addition of 10 γ Mn	Mean Recovery, γ
A	4.1	14.1	
	4.6	14.5	
	4.6	14.8	
Av.	4.4	14.45	10.05
B	4.3	14.3	
	4.3	14.1	
Av.	4.3	14.2	9.9
C	6.4	16.3	
	7.0	17.5	
	6.5	16.5	
	6.3	16.4	
Av.	6.55	16.7	10.15

sorptiometers. This paper presents a method for the determination of manganese in 100-ml. samples of milk. The bulk of the calcium is removed as calcium phosphate in the presence of HEEDTA with little or no loss of manganese and the permanganate density is measured in 5-cm. light path cells requiring only 6 ml. of solution, using a Beckman DU spectrophotometer.

Experimental

Selection of Chelating Agent. Preliminary studies indicated that losses of manganese, when removing calcium as calcium phosphate, could be much reduced by the use of EDTA [(ethylenedinitrilo)tetraacetic acid]. Complete recoveries of microgram amounts of manganese could not be obtained, for as the concentration of EDTA was increased in an attempt to retain manganese, precipitation of calcium phosphate was reduced. This led to a study of two other chelating agents HEEDTA (*N*-hydroxyethylethylenediaminetriacetic acid) and DTPA (diethylenetriaminepentaacetic acid) in the hope that they would retain manganese preferentially.

To 20 ml. of synthetic milk ash solution (equivalent to 500 ml. of milk with respect to major constituents), 50 γ of manganese was added, together with a given volume of 10% chelate solution and bromothymol blue indicator. The volume was increased to about 90 ml., and the solution was titrated slowly with approximately 2*N* ammonium hydroxide to the blue-green (pH 6.8 to 7.0) color of the indicator. After centrifugation, the supernatant liquid was poured off into a beaker, the precipitate was suspended in distilled water, and recentrifuged, and the second supernatant was added to the first. This was taken to dryness (an infrared lamp is preferred as it reduces the danger of spattering) and, after addition of 25 ml. of nitric acid, taken to dryness on the hot plate to remove most of the ammonium ion. The residue was treated with 10 ml. of nitric acid, 5 ml. of perchloric acid, and 5 ml. of sulfuric acid, and taken to dryness once more. The permanganate color was developed in 10% sulfuric acid using potassium periodate, the volume was made up to 25 ml., and the absorption was measured with a Fisher electrophotometer, using a 2-cm. light path cell with a 23-ml. volume. The results are given in Table I.

EDTA is not a sufficient selective chelating agent under these conditions to prevent loss of manganese. Raising its concentration improves recovery of manganese but interferes with the precipitation of calcium phosphate. Both HEEDTA and DTPA enable manganese to be recovered satisfactorily with little reduction in the amount of calcium phosphate precipitated. This appears to agree with the rating of Kroll, Kuykendall, and Powers (*1*) of the stabilities of the iron chelates, DTPA > HEEDTA > EDTA, assuming that manganese behaves in much the same way as iron. Under these conditions, the ratio (Mn/Ca) of stabilities of manganese and calcium chelates is greater in the case of HEEDTA and DTPA, than for EDTA.

In the above investigation, 50 γ of manganese were used in the presence of ash equivalent to 500 ml. of milk. This quantity of milk would normally contain 10 to 30 γ of manganese. Absorption values for 50 γ were low, and small amounts would have made it more difficult to evaluate the chelating agents. Determinations of manganese at the 2- to 6-γ level to be expected in 100-ml. samples of milk require absorption cells of long light path associated with low volume. For a closer investigation of HEEDTA, cells of 5-cm. light path requiring 6 ml. of solution were used—these being a modification of the type described by Vallee (*3*). The standard curve (0.25 to 2.0 γ manganese per ml. in the final solution), using these cells and a Beckman Model DU spectrophotometer with an extended cell compartment, is a straight-line function at 525 mμ. Under these conditions, the concentration of manganese (micrograms per milliliter) is five times the density reading.

Recovery of Manganese Using HEEDTA. Although a single precipitation appeared satisfactory when 50 γ were used, a double precipitation of calcium phosphate at the 10-γ level was necessary. Little extra time is involved in this operation, as it replaces the suspension and washing of the precipitate. As synthetic ash solution equivalent to 100 ml. of milk was used, the ratio of manganese to calcium phosphate is unchanged and the necessity for a double precipitation probably reflects the greater sensitivity obtainable with the longer light path cells. Recoveries of manganese from synthetic milk ash solution, equivalent to 100 ml. of milk, varied between 95 to 101%, averaging 98.2%. Recoveries from 100 ml. of natural milk are given in Table II. The procedure followed the recommended method; the 10 γ of manganese was added just prior to precipitation of calcium phosphate.

Recommended Method

Special Apparatus and Reagents. Beckman Model DU spectrophotometer with extended cell compartment.

Absorption cells with 5-cm. light path and requiring 6 ml. of solution.

HEEDTA, 10% solution of trisodium salt of *N*-hydroxyethylethylenediaminetriacetic acid.

Phosphoric acid–potassium periodate solution:

7.0 grams KIO_4 + 800 ml. H_2O + 200 ml. H_3PO_4.

Acid mixture. HNO_3 + $HClO_4$ + H_2SO_4 (2 + 1 + 1).

All acids, reagent grade.

Procedure. Using infrared lamps, take 100-ml. samples of milk to dryness, ash at 500° to 550° C., digest on water bath with 5 ml. of approximately 6*N* hydrochloric acid, together with a little water, and filter. Take the filtrate to dryness to remove most of the hydrochloric acid, dissolve in about 5 ml. of water, transfer to a 50-ml. centrifuge tube, and wash with about 10 ml. of water. Add 3 ml. of 10% HEEDTA and two drops of bromothymol blue, and titrate slowly with approximately 2*N* ammonium hydroxide to the blue-green color. (The volume prior to addition of ammonium hydroxide is about 20 ml.) Centrifuge and transfer the supernatant to a 50-ml. beaker and set aside.

Dissolve the precipitate in about 10 drops of nitric acid, add another 3 ml. of 10% HEEDTA, and repeat the precipitation of calcium phosphate. Centrifuge, add the second supernatant to the first, and take the whole to dryness under an infrared lamp. To the residue, add 15 ml. of nitric acid, cover with a Speedyvap, and take to dryness on the hot plate to remove ammonia. Add 10 ml. of the acid mixture and, when most of the nitric acid has been removed, raise the hot plate temperature to ensure efficient oxidation by the perchloric acid. When the vigorous oxidation reaction is complete, allow the beaker to cool, wash down the Speedyvap and the sides of the beaker with distilled water and return to the hot plate at low temperature to evaporate off the water.

When fuming starts, raise the temperature to fume off the bulk of the sulfuric acid, and finally flame the beaker. Cool beaker contents, add about 2 ml. of water, bring to near boiling on the hot plate, and transfer to a 10-ml. graduated centrifuge tube. Add 3 ml. of the phosphoric-periodate solution, mix, immerse in boiling water, and keep just boiling for about 2 hours. The initial volume of about 10 ml. is reduced to about 6 ml. over this period. After cooling, centrifuge to remove water from the upper parts of the tube and to bring down the small amount of calcium sulfate, which usually occupies about 0.3 ml. Make the volume up to 6.3 ml.—i.e., 6 ml. over the volume occupied by the calcium sulfate, mix thoroughly, recentrifuge, and transfer to a 5-cm. cell for density determinations at 525 mμ.

Conclusions

Recoveries of manganese by this method appear satisfactory, taking into account the levels involved. Although not investigated, DTPA would probably behave in much the same way as HEEDTA. This method may well have application to other materials high in calcium and/or phosphorus, such as bone, teeth, phosphate rock, and limestone.

Acknowledgment

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POTATO EXTRACTION

Determination of End Point in Extraction of Free Amino Acids from Potatoes

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A satisfactory batchwise procedure for the extraction of the free amino acids from potatoes has been developed. Lysine and arginine are the last of the amino acids to be extracted by 70% aqueous ethyl alcohol using this procedure. The order of extraction of the amino acids in potatoes was very different from that reported in young corn shoots. Methods used for the extraction of plant constituents should be tested on each plant material for which they are to be used.

Aqueous ethyl alcohol, 70 to 80%, has been recommended and used (*1–6, 10, 13*) to extract the free amino acids from plant materials; however, the workers were not very specific as to when the extraction was complete. Woodward and Rabideau (*17*) have reported on the completeness of extraction of amino acids and other components in corn. They used hot 80% ethyl alcohol in a Soxhlet apparatus, which is known to cause destruction of glutamine (*14*), and as the authors wished to obtain this compound essentially unchanged, their procedure was unsatisfactory.

In the case of sugars, Williams and Potter (*15*) found, "that the sugar solution entrapped in the spongy plant material is of the same concentration as the remainder of the solution" and "that the alcohol-insoluble material does not occupy a significant volume." This procedure did not work in extracting amino acids from potatoes.

This study agrees with the Woodward and Rabideau (*17*) and Oland and Yemm (*9*) findings that the amino acids are extracted at different rates. Hence, the extraction must be complete or the relative amounts of the different amino acids in an extract will depend on the extent of extraction. Woodward and Rabideau found that the last amino acids to be removed from young corn shoots were aspartic and glutamic acids. Oland and Yemm found arginine to be the last amino acid to be extracted from apple twigs. Arginine and lysine—with a slight emphasis on the latter—were the last amino acids to be removed from potatoes, according to the present work. Methods used for the extraction of constituents from materials derived from different plant species should be tested on each plant material for which they are used.

Experimental

The equilibrium procedure of Williams and Potter (*15*) was compared with a batchwise procedure using Wisconsin Russet potatoes of specific gravity 1.076, which corresponds to a solids content of approximately 19%. The potatoes were hand-peeled and slurried in ethyl alcohol in an electric blender at high speed (10,000 r.p.m.) for 3 minutes. The slurry was transferred to a sampling blender with sufficient alcohol to make the final concentration 70% by weight, taking into consideration the water originally in the potatoes. Samples were taken for the extraction procedures and for nitrogen analyses with the blender running at low speed. The actual amount taken was determined by weight.