laboratory. Not all commercial pectic enzymes contain this naringin hydrolytic activity. A sample of Takamine pectic enzyme was tried and was ineffective, but three sources of diastase—viz., Parke-Davis Takadiastase, Wallerstein Laboratory pharmaceutical Mylase, and Clarase diastic enzyme, produced by Takamine Laboratory—showed some degree of activity.

β-Glycosidase from sweet almonds was reported to have an optimum activity at pH 4.4 (*11*). The optimum action of the glycosidase reported in the present paper is about pH 4 with only small differences within the range of pH 3.5 to 4.5. This enzyme has very little action at pH 2. Hall (*4*) used the enzyme from celery seed at pH 7. He did not report the activity at any other pH and failed to ascertain the optimum hydrogen ion concentration for the enzyme. He also reported that the end products contained a disaccharide. Chromatographic studies in this laboratory showed only rhamnose and glucose separately. In general, the end products of an enzyme-catalyzed reaction exert a mass action effect which was shown by the experiment on the decreasing of enzymic activity with increasing concentrations of glucose. Although naringenin is insoluble in water, a 0.02% suspension had inhibitory action, while rhamnose at that concentration had little effect.

The enzymic activity was definitely reduced at 70° C. Complete inactivation of the enzyme was obtained in 3 minutes at 80° C. and in 15 seconds at 90° C. The time recorded in Table II did not take into consideration the time required for the samples to reach the desired temperature—for 80° C., about 1 minute and for 90° C., 30 seconds. As the experiments on heat inactivation were carried out at pH 4, varying the pH may change the critical temperatures.

The effect of citrate ion on the enzymic activity was studied as, in any practical applications, citrate will be the main buffering medium. The concentration of the citrate ions had no critical significance on the enzymic activity.

Enzyme Efficiency. The efficiency of the naringin-hydrolyzing enzyme in the commercial pectinase preparations was not uniform in every lot, and a more or less arbitrary unit of efficiency must be established in order to measure the activity of various preparations. The activity was measured by determining the velocity constant, k, for hydrolysis of a 0.05% naringin (melting point, 82° C.) by 1 gram of an enzyme preparation in 100 ml. of the reaction solution in the first 60 minutes.

$$k = \frac{2.303}{t_2 - t_1} \log \frac{C_1}{C_2}$$

where k is the velocity constant, t_1 and t_2 are time, and C_1 and C_2 are concentrations at time t_1 and t_2, respectively. The use of a time limit and a definite initial substrate concentration is necessary as k varies with these factors.

Possible Applications of Enzymic Hydrolysis of Naringin. The discovery of a commercially available enzyme capable of hydrolyzing naringin may have various applications. Although a slight bitterness is characteristic of all grapefruit products, excessive bitterness is objectionable. The application of this enzymic hydrolysis when used in appropriate conditions can reduce the naringin content of grapefruit products. Of special interest is bland sirup. During the concentration of the expressed "peel juice" the bitter principle, naringin, tends to concentrate and to render the products excessively bitter. By treating the liquor with Pectinol or with another source of this enzyme before or during concentration, it is possible to remove all the naringin.

The utilization of this process in the "debittering" of grapefruit juice is hindered by the association of this enzyme with large quantities of pectic enzymes which readily clarify the juice. The isolation of the naringin-hydrolyzing enzyme from the bulk of pectic enzymes is a prerequisite to the practical application of enzymic hydrolysis for the "debittering" of grapefruit juice. However, the loss of colloidal suspension of the juice may be remedied by the use of various commercially available protective colloids.

Also, as naringin can be quantitatively removed by this enzyme, the determination of total flavanone glycoside with the Davis procedure, before and after hydrolysis, shows the amount of naringin in the sample.

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VITAMIN A IN EGGS

Seasonal Variations in the Vitamin A Content of Hens' Eggs

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THE VITAMIN A CONTENT of fresh and stored shell eggs was determined by means of techniques not previously used with eggs. A study of stored shell eggs indicated there might be seasonal changes in the vitamin A content of eggs from any one hen. Data from eggs from a group of hens over a 10-month period and the method used in the determinations are presented.

Determination of Vitamin A

Reagents. Skellysolve B. Petroleum naphtha with a boiling point of 60–71° C. obtained from Skelly Oil Co., Chicago, Ill.

Florisil. 60/100 mesh obtained from The Floridin Co., Tallahassee, Fla.

Activated glycerol dichlorohydrin. Preparation and recovery of used glycerol

The vitamin A content of eggs was determined from the yolks of hard-cooked eggs by extraction, separation, and spectrophotometric measurement of the vitamin. These techniques, not previously used on eggs, gave 100% recovery of vitamin A when freshly extracted egg oil was used as the solvent. Further tests with the method presented showed that hens on the same ration differ slightly in their ability to put vitamin A into their eggs. Much greater variation is obtained with eggs from any one hen during the laying period.

dichlorohydrin as recommended by Sobel and Snow (*33*).

Procedure. Weighed shell eggs were cooked in boiling water for 20 minutes and cooled in running water. Each yolk was removed and weighed. A 5.0-gram sample of the well mixed yolk from each egg was weighed into a tared alundum extraction thimble and extracted with 50 ml. of Skellysolve B in a Goldfisch extractor until practically all the yolk color was removed. The time required depended upon the age of the egg (longer for older eggs) and the porosity of the alundum thimble. Ignition of the thimble in a muffle furnace overnight at 650° C. after each run gradually deposited iron salts in the pores, making it necessary to boil the thimble periodically in 20% hydrochloric acid to restore porosity.

The cooled extraction liquid was made to a volume of 50 ml. with Skellysolve B in the previously marked extraction beakers. Twenty milliliters of extraction solution was pipetted into a Hennessey tube containing Florisil prewetted with Skellysolve B, and rinsed down onto the chromatographic column with two 3-ml. portions of Skellysolve B. The vitamin A was eluted with 20 ml. of 3% acetone in Skellysolve B followed by 20 ml. of 1% ethyl alcohol in Skellysolve B. The eluate and washings were collected in a 250-ml. glass-stoppered Erlenmeyer flask and evaporated with a stream of nitrogen on a thermostatically controlled embedding table with a temperature of 60° to 65° C. until no solvent odor was detectable. A residue of approximately 1 ml. of a very light yellow, odorless oil remained.

The sides of the flask were washed down with 2 ml. of chloroform, 7 ml. of activated glycerol dichlorohydrin was added, and the flask was shaken for exactly 1 minute. A portion of the solution was poured into a cuvette, and the absorbance was determined at 555 mμ in the Beckman Model B spectrophotometer, exactly 2 minutes after the addition of the glycerol dichlorohydrin. Thirty minutes later the absorbance at 830 mμ was obtained in order to correct for carotenoids. The solutions were read against a blank composed of 2 ml. of chloroform and 8 ml. of glycerol dichlorohydrin. Standard curves, tables, and corrections for carotenoids were made as described by Sobel and Snow (*33*). All corrections were small.

Discussion. Three steps are necessary in the determination of vitamin A in eggs: extraction of the vitamin A from the eggs, separation of vitamin A from interfering materials, and measurement of the quantity of vitamin A present. Neff, Parrish, Hughes, and Payne (*23*) prepared an egg yolk–water emulsion and extracted that with Skellysolve B. Hard-cooked eggs have been used in earlier work from this station on the vitamin content of eggs, because of the easy separation of yolks from whites, and, as vitamin A is relatively stable to heat in the absence of air, (*15*) hard-cooked eggs were used for this study. Saponification was found unnecessary for the extraction, separation, and determination of total vitamin A.

Vitamin A is held very tenaciously on Florisil. However, when the solution of vitamin A and egg oil in Skellysolve B was passed through a Florisil column, the vitamin A was readily eluted. A light yellow band moved down the column during washing with 3% acetone in Skellysolve B. With the 1% alcohol in Skellysolve B eluent, a deep yellow band, separated from the pale yellow one by a clear zone, was carried down the column. The dark yellow band—mainly xanthophylls—was never eluted from the column in the determination of vitamin A. Further elution with 1% or higher alcohol in Skellysolve B did not remove any additional vitamin A. Part of the vitamin A passed right through the column with the Skellysolve B, but part remained on the column for elution with the acetone and alcohol solutions in Skellysolve. The determination of vitamin A was generally made the following day on the residue left in the flask after evaporation. Vitamin A was surprisingly stable in the oil that passed through the florisil column, particularly when kept in the refrigerator.

Sobel and Werbin (*34*) first described the reaction of vitamin A with activated glycerol dichlorohydrin to give a colored solution with maximum absorption at 625 mμ. Sobel and Snow (*33*) adapted the reaction to the determination of vitamin A in blood serum and showed that the method does not suffer from many of the disadvantages of the Carr-Price antimony trichloride reaction (*5*) for vitamin A. At 555 mμ, much of the carotenoid interference was eliminated, and the rest was corrected for by reference to calibration charts.

Good agreement between duplicate samples was obtained either when separate samples of one yolk were extracted and chromatographed or when duplicate aliquots from one extract were run through Florisil columns (Table I).

Recovery of vitamin A added to yolk material was dependent upon the solvent. Table II shows that when freshly extracted egg oil was used as the solvent for the vitamin A, which was added either to the yolk before extraction and chromatography or to the yolk extract before chromatography, the vitamin A recovery was 100%. Other experiments in which the vitamin A was dissolved in Skellysolve B and added to these same yolk materials showed recoveries of 50% or less.

Seasonal Changes in Vitamin A Content of Eggs

White Leghorn pullets hatched in May of 1955 were placed in laying cages at about the time they started egg laying in September. Five consecutive eggs from each hen were taken during the third week of each month starting in October. Each egg was weighed and hard cooked, the yolk was separated and weighed, and vitamin A in the yolk was determined as described above. The experiment ran for 10 months, and was discontinued after the July collection.

The ration fed was an all-mash laying ration which has been used at this station for many years for feeding hens kept in laying cages. It was composed of ground corn, 34.5%; ground oats, 20.0%; wheat bran, 15.0%; flour middlings, 10.0%; dehydrated alfalfa, 3.0%; meat scraps, 3.0%; dried milk, 2.0%; fish meal, 2.5%; soybean oil meal, 2.5%; ground oyster shell flour, 5.0%; steamed bone meal, 1.5%; salt, 0.6%; and fish oil (400 AOAC chick units of vitamin D and 2000 USP units of vitamin A per gram), 0.4%. It was received fresh every month during the experiment in triple-thickness heavy paper bags, containing 50 pounds each. The vitamin A concentration was determined by extraction from the feed with Skellysolve B (*21*) separation from interfering substances by chromatography on aluminum oxide (*24*), and treatment of the eluate residue with glycerol dichlorohydrin as described above. The concentration was found to be 3000 I.U. per pound for several different lots, and remained at this value for at least 4 months of storage.

The experiment was started with six hens, but one of them soon went out of

Table I. Vitamin A Content of Duplicate Samples

	γ Vitamin A per Gram of Yolk			
	Same yolk extracted and chromatographed		Same egg extract chromatographed	
	3.54	3.59	3.29	3.33
	2.80	3.10	3.84	3.80
	3.81	3.69	3.63	3.59
	3.21	3.20	3.09	3.00
	3.46	3.40	3.86	3.70
	3.58	3.49	3.58	3.62
	3.08	3.0[illegible]	3.10	3.06
	3.18	3.16	2.85	2.81
	2.54	2.58	3.34	3.26
	2.49	2.63		
	1.90	1.90		
	1.74	1.79		
Av.	2.94	2.96	3.40	3.35

Table II. Recovery of Vitamin A Added in Egg Oil

	Vitamin A Present in Egg Oil, γ	Vitamin A Added to Egg Oil, γ	Total Vitamin A Determined, γ	Added Vitamin A Recovered γ	Added Vitamin A Recovered %
Extraction + chromatography[a]	20.40	5.58	26.07	5.67	101.6
	19.72	5.58	25.39	5.67	101.6
	22.44	5.58	28.11	5.67	101.6
	16.78	61.21	77.13	60.35	98.6
	17.91	61.21	79.38	61.47	100.4
	18.82	61.21	78.25	59.43	97.1
				Av.	100.15
Chromatography[b]	9.91	3.08	12.93	3.02	98.05
	9.20	3.08	12.25	3.05	99.02
	9.61	6.17	15.88	6.27	101.62
	9.79	6.17	16.10	6.31	102.26
	13.38	13.42	26.75	13.37	99.62
	12.92	13.42	26.30	13.38	99.70
				Av.	100.05

[a] Added to yolk before extraction and chromatography.
[b] Added to egg extract before chromatography.

Table III. Change in Weight of Shell Eggs and Egg Yolks with Season

(Average values of five consecutive eggs used from each hen per month; superscript denotes number of eggs other than five used in average)

	Hen Number													
	1		3		4		5		9		11		15	
	Wt., G.													
Month	Egg	Yolk	Egg	Yolk	Egg	Yolk	Egg	Yolk	Egg	Yolk	Egg	Yolk	Egg	Yolk
Oct.	55.4	13.1	51.2	13.1	49.8	12.7	49.9	12.2	...	...	...	...	51.3	12.3
Nov.	59.0	14.9	53.5	14.6	52.4	14.2	53.6[4]	13.6[4]	...	...	...	...	54.6	13.7
Dec.	63.4	16.4	57.2	16.3	54.4	15.1	60.1	16.1	61.1	16.2	...	...	58.0	14.8
Jan.	62.3	16.7	55.9	15.9	53.7	15.3	62.4	17.3	58.8	15.7	63.8	16.4	57.4	14.3
Feb.	66.0	18.8	59.9	16.9	59.6	17.3	61.3	17.3	62.2	17.6	61.9	16.5	58.1	14.7
Mar.	64.0	18.1	58.9[1]	18.2[1]	54.7[2]	15.1[2]	59.8	17.3	62.5	17.7	62.2	16.9	55.0[2]	15.0[2]
Apr.	66.9	19.4	60.6	17.8	...	...	62.8	18.0	61.6	17.8	63.9	18.1	...	...
May	65.1	18.8	60.8	18.2	60.0	17.3	63.6	18.3	61.6	17.0	64.0	18.0	...	...
June	64.6	19.0	59.3	17.4	59.6	17.6	62.7	18.1	58.9	16.4	61.8	17.0	46.4[4]	11.0[4]
July	65.0	17.3	...	...	57.2[2]	16.8[2]	...	...	61.0	15.9	...	...	49.2	10.7

Table IV. Change in Vitamin A Content and Concentration in Yolks of Shell Eggs with Season

(Average of values for five consecutive eggs from each hen per month; superscript indicates number of eggs other than five used in average)

	Hen Number													
	1		3		4		5		9		11		15	
Month	γ/g.	γ/yolk	γ/g.	γ/yolk	γ/g.	γ/yolk	γ/g.	γ/yolk	γ/g.	γ/yolk	γ/g.	γ/yolk	γ/g.	γ/yolk
Oct.	3.11	40.85	3.43	44.75	3.41	43.18	3.92	48.20	...	...	...	...	3.41	41.39
Nov.	3.64	54.00	3.50	51.05	3.31	47.16	3.99[4]	57.72[4]	...	...	...	...	3.59	49.19
Dec.	3.38	55.55	3.44	56.10	3.41	51.56	3.75	60.24	3.56	57.50	...	...	3.78	55.84
Jan.	3.74	62.50	3.93	62.35	3.66	56.90	3.84	66.43	3.86	60.53	3.83	62.80	3.83	54.64
Feb.	4.10	77.09	4.25	71.90	3.81	65.98	4.28	74.19	4.26	74.74	4.20	69.21	4.32	61.90
Mar.	4.37	79.31	4.08[1]	74.26[1]	4.20[2]	63.40[2]	4.37	75.63	4.61	81.52	4.64	78.54	4.60[2]	69.06[2]
Apr.	4.61	89.36	4.41	78.44	...	...	4.29	77.40	4.30	76.55	3.97	71.96	...	...
May	4.24	79.91	4.25	77.25	4.16	72.06	4.24	77.62	4.10	69.89	3.99	71.63	...	...
June	3.33	63.13	3.17	55.35	3.22	56.79	3.76[3]	60.70[3]	3.03	50.76	3.19	54.32	2.58[4]	31.27[4]
July	3.12	53.99	...	...	3.22[2]	54.12[2]	...	...	2.92	46.36	...	...	2.73	24.19

production and died. She was replaced by hen No. 9 in December and to protect against loss of any more hens, hen No. 11 was added in January. There were periods while the eggs were being used when one or more hens were not in production. At other times, it was difficult to obtain the five eggs desired. However, those eggs obtained were analyzed, and the average data are included in the tables with a notation of the number of eggs used. Only hen No. 1 laid eggs in every period.

Average weights of whole shell eggs before cooking and of cooked yolks for each of the hens are presented in Table III. Maximum egg weights occurred in March, April, or May for all except hen No. 15, which stopped laying during the March collection period and did not resume production until the June collection period. Maximum yolk size came at the same time as maximum egg size in most cases or where there was very little difference in weight between 2 month's eggs.

Hens on the same ration differed but slightly in their ability to put vitamin A into their eggs (Table IV). Greater variation was obtained with the eggs from any one hen. There was about 50% more vitamin A in the eggs produced February through April than in the October and July ones, even though the July egg yolks were larger (Table III).

Eggs contained the most micrograms of vitamin A per egg during March or April (Table IV), but maximum vitamin A content of eggs did not always come at the same time as maximum egg or yolk weight. Vitamin A concentration (microgram per gram) in the eggs was not constant but changed throughout the entire laying period increasing from October to April and starting to decrease in May. By July, the vitamin A concentration was as low as, or lower than, that of the October eggs.

The data are not in agreement with those of Sherwood and Fraps (*29–31*). They observed that the vitamin A content of eggs from hens receiving 224 or 444 I.U. of vitamin A per day decreased steadily from the start of produc-

tion to the time the experiments were discontinued. The higher value is considerably less than the recommended nutrient requirement for poultry (*22*). Furthermore, their data were obtained by biological assay (*28*), a difficult method for the determination of vitamin A in eggs. During an 8-week period, Grimbleby and Black (*14*) likewise found a similar decrease in the vitamin A content of eggs from hens whose basal ration, devoid of cartenoid pigments and vitamin A, was supplemented with good pasture. They determined vitamin A by the Carr-Price reaction (*5*).

Many investigators have conclusively demonstrated the effect of the hen's diet on the vitamin A content of the egg (*2, 3, 7, 9, 12, 16, 19, 23, 29–32, 36, 37*). The higher the vitamin A or carotene in the diet the more vitamin A is deposited in the egg. The ration fed to hens in this experiment contained by analysis 3000 I.U. of vitamin A per pound, which agrees very closely with the nutrient requirements for poultry of 2000 I.U. with the 66% safety margin (*22*). On the basis of 9 pounds of feed per hen per 30-day month, each hen had a daily intake of 900 I.U. of vitamin A sufficient to ensure against a drop in egg production with healthy hens (*35*).

Values for the vitamin A content of eggs obtained by a number of investigators between 1932 and 1952 are presented in Table V. Several different methods of assay for vitamin A were used, the vitamin A contents of the rations differed greatly, and the hens were different types. Except for the data of Vermes, Meunier, and Raoul (*37*), all values were between 0.5 and 17.2 γ per gram of yolk, or between 3 and 320 per whole egg [assuming an egg yolk weight of 18.7 grams (*25*) for the yolk containing 17.2 γ per gram]. For some reason, a very high value for vitamin A was obtained by Vermes *et al.* (*37*) who used Meunier and Raoul's (*20*) modified Carr-Price method (*5*). The data presented in Table IV agree reasonably well with the literature data summarized in Table V, especially if the values—17.2 γ per gram of yolk obtained by De Vaney, Titus, and Nestler (*9*) who fed 8% cod liver oil and Cruickshank and Moore (*7*) who gave each hen 100 mg. of vitamin A daily—are not used for comparison.

Table V. Vitamin A Content of Fresh Shell Eggs

Reference	Procedure for Vitamin A	γ Vitamin A/ Egg	γ Vitamin A/G. Yolk
	Data in chronological order		
(*10*)	USP rat assay	150	
(*29*)	Sherman-Munsell rat assay		1.1–4.3
(*11*)	Carr-Price		9.0
(*4*)	Sherman-Munsell rat assay		1.5–6.0
(*30*)	Sherman-Munsell rat assay		1.3–5.4
(*31*)	Sherman-Munsell rat assay		1.3–3.9
(*9*)	Sherman-Munsell rat assay		4.3–17.2
(*17*)	Sherman-Munsell rat assay		4.3–7.1
(*27*)	Sherman-Munsell rat assay	150–240	
(*6*)	Rat assay		9.0
(*13*)	Carr-Price (chromatographic)		0.5–1.0
(*16*)	USP rat assay	64–96	
(*7*)	Carr-Price		3.4–17.0
(*3*)	USP rat assay		3.2–8.4
(*26*)	Sherman-Munsell rat assay		15.0
(*2*)	Spectrophotometric	112–170	6.6–9.2
(*19*)	USP rat assay		3.6–8.4
(*37*)	Carr-Price	750–1500	
(*8*)	Spectrophotometric		14.0
(*32*)	Carr-Price	18–90	
(*1*)	Chick growth assay	38–141	
(*12*)	Carr-Price	3–12	
(*23*)	Carr-Price (chromatographic)		2.0–13.4
(*35*)	Carr-Price		3.0–5.0
(*14*)	Carr-Price	25–116	
(*18*)	Carr-Price (chromatographic)		2.2
	Glycerol dichlorohydrin (chromatographic)	29–89	2.7–4.6

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