

# Vitamin A Added to Fats as Related to Stability During Baking

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## Introduction

In connection with the vitamin fortification of foods, the addition of vitamin A to fats has attracted considerable attention. The processing required to refine shortening to a quality acceptable to the present-day consumer destroys large percentages of this vitamin naturally occurring in raw fats and oils. It has been suggested that the amount of vitamin A originally present in the crude fats be replaced and that even larger quantities be incorporated to insure an adequate supply in the diet. Such quantities would presumably be in line with the recommended minimum of 9,000 units of A per pound of margarine. However, since most fats with the exception of butter, table margarine, and salad oils, are consumed largely in the form of baked goods, it was necessary to know the stability of added vitamin A during baking procedures. Various investigations have indicated that at least a portion of the vitamin A naturally occurring in fats survives baking processes (1) (2).

## Methods of Analysis

In order to ascertain the stability of vitamin A to baking it was necessary to determine the amount of vitamin A in the fat used in the baked goods and the amount of fat recovered from such baked goods. Three methods are available for the assay of vitamin A. Each has received considerable attention and one, the biological method, is well established. It is recognized as an official method by the U. S. Pharmacopoeia. However, because of the time and expense associated with this method much effort has been expended on physical and chemical methods of analysis. The literature on these methods is relatively voluminous and somewhat contradictory. Wilkie (3) gave a good review of the possibilities and limitations of existing methods.

In brief, there are two non-biological methods of vitamin A assay: (1) the Carr-Price method, in which the reaction of antimony trichloride with vitamin A results in the formation of a blue colored compound, the amount of color being proportional to the amount of vitamin A present; and (2) measurement of the absorption of ultraviolet light by vitamin A at 3280 Angstroms. In applying both methods, it is necessary to concentrate the vitamin A into the non-saponifiable fraction of the fatty carrier; in the case of baked goods, extraction of the fat is first required.

In this investigation the Carr-Price technique was used and the results were checked by U.S.P. biological assays.

## Results

The fat employed in each case was fortified with a known amount of the alcohol form of vitamin A added in the form of a concentrate. The baked goods were prepared, attention being given to the com-

position so that the per cent of fat in the final baked product was known. Table I shows the per cent of

TABLE I

Bakery Product	Calculated Fat Content (basis finished goods)	Baking Conditions	
		Time	Temperature
	Per cent	Minutes	°F.
Bread.....	6.0	40	425
Biscuits.....	14.4	20	450
White Pound Cake.....	13.7	60	360
Pie Crust.....	37.4	30	425

fat and the baking conditions for the types of baked goods used. These types were selected because they represent wide variations in fat content and baking conditions. Fresh baked products were prepared weekly, and after being crumbled were extracted in Soxhlets with analytical reagent peroxide-free ether for 2 to 4 hours. After the solvent had been evaporated under nitrogen the remaining fats were saponified and the unsaponifiable fraction extracted with ether. The ether solutions were washed free of alkali.

Unsaponifiable fractions of the fats were assayed for vitamin A by the Carr-Price technique and by the U.S.P. biological method. The vitamin content of the original fortified fat was determined by the Carr-Price reaction. The Carr-Price values on the baked goods were corrected by determining the vitamin A in the unfortified baked goods. For the bio-assays the unsaponifiable fractions were diluted with the required amount of cottonseed oil to produce a concentration of 30 U.S.P. units per milliliter, based on 100 per cent recovery of vitamin originally present in the shortening. Then dilutions were fed in 0.1 milliliter quantities daily. Table II gives the results of the assays and the percentage of survival.

TABLE II

Recovery of Vitamin A From Baked Goods Containing Fortified Fats

Product	Units Vitamin A Per Pound of Fat Used	Units Vitamin A Recovered Per Pound Fat		Percentage Survival	
		Bio-assay	Blue Color	Bio-assay	Blue Color
				Per cent	Per cent
Bread.....	10,700	10,700	9,310-11,770	100	87-110
Biscuits.....	10,400	9,048	9,670- 9,780	87	93- 94
Cake.....	10,400	9,670	8,200- 9,880	93	79- 95
Pie Crust.....	10,700	Less than 3,000	750- 9,310	Less than 30	7- 87

Attention is called to the results on pie crust. The fat extracted from one sample showed a very low potency by the blue color assay and did not promote growth. Since this sample was slightly overbaked we suspected that the baking time might influence the stability of the vitamin. Hence, three shells made from the same dough were baked for different lengths of time and assayed by the Carr-Price method. Table III shows that the destruction is proportioned to the baking time. No appreciable variation was noted in

the recovery of the vitamin from the other baked goods.

TABLE III  
Destruction of Vitamin A During Baking of Pie Shells

Baking Time	Color of Crust	Vitamin A in Fat Used	Vitamin A Recovered	Recovery
Minutes		Units/Lb.	Units/Lb.	Per cent
35	Light brown	9,760	6,790	70
40	Medium brown	Same	3,535	36
45	Dark brown	Same	1,365	14

In products of low fat content, such as bread, biscuits, and cake, which are baked under moderate conditions, vitamin A appears to survive the baking process to the extent of 80 to 100 per cent. In pie crust, which undergoes more severe baking conditions, there is appreciable destruction, the amount depending on the extent of the baking.

These data confirm preliminary results obtained by spectrophotometric assays of various original fortified fats and fats recovered from baked goods.

### Summary

Various types of baked goods prepared from fats fortified with vitamin A have been assayed for the vitamin by the Carr-Price color reaction and by U.S.P. bio-assays.

In products such as bread, biscuits, and cake, which are baked under moderate conditions, it appears that 80 to 100 per cent of the vitamin survives the baking process.

In pie crust, which undergoes more severe baking conditions, considerable destruction, depending on the extent of the baking, is likely to occur.

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## Use of Ground Glass Covers in Glycerol Oxidation

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The standard methods of analysis of glycerol of the American Oil Chemists' Society require that the bichromate oxidation of glycerol be continued for two hours in a boiling water bath (1). It is further specified that the oxidation mixture be kept from dust and organic vapors, such as alcohol and ether, until the titration is completed.

In this laboratory considerable difficulty has been experienced with vibration from the boiling water causing the cover glasses to chatter and creep off the top of the beakers thus exposing the contents to danger of evaporation and objectionable fumes of ether, alcohol, etc., which are usually present in the atmosphere of a laboratory devoted to work on fats and oils.

To avoid this source of error use has been made for some time of 500-ml. beakers without lip and provided with a ground glass rim. The underside (convex surface) of the watch glass used to cover the beaker is ground in a circular segment so that when placed over the beaker the ground surface of the beaker rim and the ground surface of the cover glass is in contact. Steam from the solution in the beaker wets the two contacting ground surfaces causing them to adhere and effectually preventing the

watch glass from "riding" off the beaker.

Before introducing this modification in the glycerol procedure it was necessary to ascertain whether sufficient CO<sub>2</sub> is trapped in the beaker to influence the course of oxidation.

Assuming 0.2 grams of 100 per cent glycerol is oxidized, a volume of approximately 145 ml. of CO<sub>2</sub> at 0° and 760 mm. is liberated. The volume of the beaker above the surface of the contents is approximately 440 ml. It is apparent that at no time is a very great concentration of CO<sub>2</sub> present, and it is highly probable that it is quantitatively dissipated during the two-hour oxidation period.

Experiments run in this laboratory several years ago showed that a continually renewed atmosphere of pure CO<sub>2</sub> at 760 mm. pressure above the surface of the oxidation mixture for a period of 2 hours reduced the value for a known glycerol content (97.82 per cent) by only 0.62 per cent. Since 1938 several thousand glycerol determinations have been run with complete satisfaction as to accuracy using the above described procedure.

### LITERATURE CITED

- (1) Official and tentative methods of the American Oil Chemists' Society, p. D-6 (1941).

## Abstracts

### Oils and Fats

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