kernel alone. Percentages reported on seed coat or pericarp are subject to error because of the large loss of nitrogen (16 to 40%) in the insoluble humin formed during acid hydrolysis of this material.

Discussion

The authors' data show the widespread occurrence of hydroxyproline in seed meals containing seed coat or seed coat and pericarp. Its presence in a number of separated seed coat and pericarp preparations, and its isolation from seed coat of *Iris germanica*, from which it could not be extracted with trichloroacetic acid, support the conclusion that hydroxyproline is a part of normal plant protein. Its presence in plants is not restricted to formation under plant tissue culture conditions.

Since hydroxyproline is found in collagens (structural proteins) of animals, its presence in the seed coat and pericarp of plants suggests that these parts of plants contain structural protein. The protective nature of seed coverings may be partly due to presence of protein of the structural type.

Available evidence (*9*, *13*) indicates that hydroxyproline formation in protein synthesis is different from that of most, if not all, remaining amino acids. It is formed from proline after proline becomes part of the proteinlike material. For this reason, information presented here might aid in such areas of plant physiological research as the study of processes associated with seed coat and surrounding tissues in both seed formation and germination, and in the isolation of their constituent proteins.

Presence of the compound in the large numbers and varieties of seed meals examined indicates that hydroxyproline is common in food and feed derived from plant sources. Practical use of these results might be made in testing meals and similar products for contamination by "hull material" that contains hydroxyproline.

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THIAMINE IN SOYBEAN MEAL

The Alleged "Thiamine-Destroying Factor" in Soybeans

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For 15 years, the literature has contained an unrefuted postulation that soybeans contain a thiamine-destroying factor. This postulation is based on loss of thiamine added to aqueous slurries of soybean meal, and an analogous observation with oriental millet meal which caused thiamine deficiency symptoms on bioassay. Data presented here show that the reported loss of thiamine is based on an unreliable thiochrome assay procedure, and that the analogy with millet meal is untenable. Thiamine in soybean meal exists as 40% free and 60% bound, presumably as cocarboxylase. Enzymes in unheated meal readily convert cocarboxylase to thiamine. Thiamine stability at neutral pH is decreased by addition of phenolic compounds, and increased by absence of air or addition of (ethylenedinitrilo)tetraacetic acid.

Many investigations of thiamine destruction in biological materials have been prompted either by poor recovery of thiamine added to assay samples or through evidence of thiamine deficiency symptoms when these materials are used in a diet. Thiaminase activity of bracken fern (*Pteris aquilina*) has been associated with the poisoning of cattle (*15*), horses (*18*), and rats (*5*) because steaming the fern destroyed activity and abolished toxicity for all species (*21*).

In previous studies (*11*) involving the measurement of thiamine in trichloroethylene-extracted soybean meals, we found no apparent discrepancy in thiamine content of different meals as determined by chemical analysis using the accepted thiochrome method described by Johnson (*10*). Known amounts of thiamine added to all assay samples were quantitatively recovered, and results were confirmed by microbiological assay. These findings led us to question the report by Bhagvat and Devi (*3*) that soybeans contain a factor that destroys thiamine.

Bhagvat and Devi used a thiochrome method in which thiamine was extracted

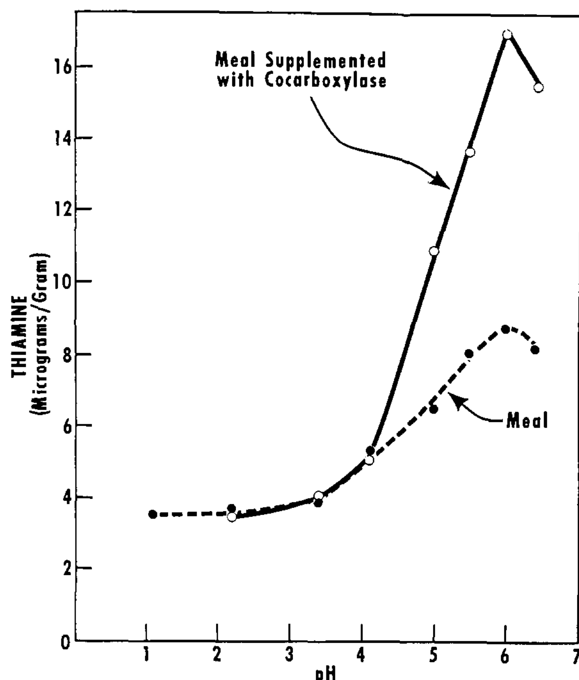

Figure 1. Effect of pH on liberation of free thiamine in unsupplemented and cocarboxylase-supplemented unheated, hexane-extracted soybean meal 1 hour at 25° C.

in the presence of a pig intestinal mucosa preparation at pH 6 to 7 resulting in loss of thiamine added to samples of soybeans. Loss of thiamine was likewise demonstrated with oriental millet or ragi (*Eleusine coracana*), which produced thiamine deficiency symptoms when fed to rats and pigeons. These findings led Indian workers to postulate that soybeans contained a thiamine-destroying factor, although feeding studies were not conducted with soybeans.

The purpose of this study was to determine the presence or absence of thiamine-destroying activity in soybeans.

Experimental and Results

Methods. BHAGVAT METHOD (*2*). Finely powdered meal was mixed with added pig mucosa in phosphate buffer at pH 6 to 7. Toluene was added and the samples were digested overnight at 37° C. To the centrifugate was added basic lead acetate, and the precipitated interfering substances were removed. The excess lead was removed with H_2SO_4 and the pH of the supernatant adjusted to 4.0 with NaOH. Thiamine was oxidized with alkaline ferricyanide and the thiochrome extracted with isoamyl alcohol and measured in a photofluorometer.

JOHNSON METHOD (*10*). Finely powdered meal was mixed with Clarase and papain in acetate buffer at pH 4.5. Following digestion at 37° C. overnight, the liberated thiamine was absorbed on Decalso, washed with water, and eluted with 25% of KCl in 0.1*N* HCl. Aliquots of the eluate were oxidized to thiochrome with alkaline ferricyanide and extracted with isobutyl alcohol. Fluorescence was measured in a photofluorometer.

The primary difference between the methods is the pH at which thiamine is extracted from assay materials.

Analysis of hexane-extracted, unheated soybean meal by the Johnson method showed 11.7 μg. of thiamine per gram of meal with 102% recovery of added thiamine. This recovery of added thiamine checked favorably with microbiological thiamine assay results previously reported (*11*). Using the Bhagvat method, except that Clarase and papain were substituted for pig mucosa enzyme preparations, a thiamine value of 8.0 μg. per gram of meal with a recovery of only 50% of the added thiamine was obtained; thus, loss of the meal thiamine in the modified Bhagvat method was less than that of the added thiamine.

Bhagvat reported that the major loss of added thiamine occurred in the first few minutes following its addition to the meal. However, the difference in response of meal thiamine and of added thiamine could have resulted from slow liberation of free thiamine from a combined form. Another factor could be interference of pseudo - thiochrome fluorescent materials in the assay sample.

Enzyme Preparations. A phosphatase-containing extract was prepared from pig mucosa as described by Bhagvat (*2*). The extract was checked for enzyme activity and compared with a commercial phosphatase preparation, Clarase, using cocarboxylase (thiamine pyrophosphate) as substrate: Cocarboxylase (15 μg.) was converted to the free form of thiamine either by a 2-ml. aliquot of the freshly prepared pig mucosa enzyme preparation (*2*), or by 0.3 gram of Clarase—the usual amount used with each meal thiamine assay sample in the Johnson method. Absence of thiamine in the pig enzyme preparation and recovery of added thiamine confirmed the results reported by Bhagvat (*2*). Use of pig mucosa or Clarase in the thiamine assay extraction procedure at pH 6 to 7 as carried out by the Bhagvat method, gave meal thiamine values ranging from 8.0 to 11.6 μg. per gram, and poor recovery of added thiamine ranging from 50 to 80%. When 50 mg. of wheat germ phosphatase (Mann Research Laboratories) was substituted for the pig phosphatase preparation or for Clarase in meal digestions at pH 6 to 7, 97 to 100% of the meal and added thiamine were recovered.

Evaluation of Interfering Meal Fluorescence. Meal thiamine was destroyed with sulfite (*12*) and with alkali (*16*) to determine whether nonthiamine materials in the meal were contributing to the fluorescence of the alkaline ferricyanide-oxidized samples in the thiochrome methods of Johnson and Bhagvat. In a typical sulfite destruction of meal thiamine, 25 mg. of sodium sulfite was added to the acidic eluate from the Decalso column in the Johnson method, after the sample was adjusted to pH 4 in the Bhagvat method. The sulfite-treated samples were heated 30 minutes at 100° C., cooled, and measured for increase in fluorescence following addition of alkaline ferricyanide. This treatment showed no increase in fluorescence above that obtained with the blank. Thus thiamine alone was responsible for the increase of fluorescence in the sample when assayed by the thiochrome procedures. Likewise, no increase in fluorescence was observed when potassium ferricyanide was added to a sample that had been heated in alkaline solution at 45° C. for 30 minutes.

Thiochrome produced from the alkaline ferricyanide oxidation of thiamine has been reported to be labile to continued ultraviolet irradiation (*12*). Therefore, following estimation of meal thiamine by both thiochrome methods, the fluorescent material presumed to be thiochrome was exposed to continued ultraviolet irradiation. Complete lability was observed. This destructive phenomenon is characteristic of thiochrome produced by chemical oxidation of the free form of thiamine and provides further evidence of the absence of interfering substances in the meal thiamine assay.

pH Effect on Thiamine Extraction. Extraction of thiamine from unheated soybean meal in the absence of added

enzyme preparations was conducted over the pH range 3 to 6.5 using the acetate buffer series described by Rubin (*20*), and at pH 1.1 with 0.1*N* sulfuric acid. The fate of thiamine, and of cocarboxylase added to the meal, was determined.

The amount of free thiamine extracted in 1 hour at 25° C. showed considerable variation (Figure 1). In each acetate buffer extract, the pH was within 0.1 of the original value, except for the extraction at pH 3 which gave a final pH of 3.4. All extracts were assayed for free thiamine by conversion to thiochrome according to the Johnson procedure. The amount extracted between pH 1 and 3.4 remained fairly constant, and then increased rapidly, reaching a peak at pH 6. Meal supplemented with known amounts of cocarboxylase enhanced the peak of the thiamine curve at pH 6, but did not affect the total thiamine value of the meal below pH 4.1. Because cocarboxylase is not assayable by the thiochrome method until hydrolyzed to the free form of thiamine, the rapid increase was apparently caused by liberation of thiamine by enzymes in the untoasted meal.

Forms of Meal Thiamine. The possibility that thiamine in soybean meal exists in several forms is indicated since soybean meal incubated with enzymes overnight contained 11.7 μg. of thiamine per gram, but the 1-hour extraction yielded only 8.7 μg. (Figure 1).

Because only the free form is estimated directly by any thiochrome method, any other forms must be converted to the free vitamin for measurement. Meals in which enzymes were heat-inactivated at pH 1 and 4 were allowed to autolyze overnight. Thiamine values identical with the 1-hour extractions at 25° C. at these pH values were obtained. Raising the pH of the heat-treated samples to 6 for autolysis did not change the results. When wheat germ acid phosphatase was added to the heat-treated meal followed by incubation at 37° C. overnight, an increase in thiamine was obtained (11.0 μg. per gram). Free thiamine (4.4 μg. per gram) constituted 40% of the total in the meal. The remainder is apparently cocarboxylase based on the similarity of activity of cocarboxylase added to the meal. Treatment with crystalline trypsin and pepsin failed to liberate additional thiamine, which indicates absence of protein-bound thiamine.

Autolysis of Unheated Meal. Bhagvat reported (*2*) that added thiamine disappeared on autolysis of soybean and millet samples, as well as with samples incubated with the pig mucosa preparation. Therefore, recovery of thiamine added at the start of an autolysis of hexane-extracted soybean meal was investigated.

Unheated solvent-extracted meal was dispersed in water (1 to 75) and autolyzed overnight at 37° C. Toluene was used as a preservative. Similar autolyzates were supplemented with thiamine in amounts approximately equal to meal thiamine estimated in previous assays. Thiamine liberated in the autolysis, together with recovery of added thiamine, was determined by conversion to thiochrome by the Johnson procedure. Added thiamine was recovered quantitatively. Total thiamine compared well with that obtained by the use of enzymes in the normal Johnson procedure.

Thus disappearance of thiamine added to soybean meal samples at pH 6 to 7 in the presence of crude enzymes (Clarase and pig mucosa) differs from that reported by Bhagvat. The good recovery of thiamine added to autolyzed meal samples implicates the crude enzymes. Thiamine added to pig mucosa at pH 6.5 was quantitatively recovered, but not when meal was present. Similar results were obtained with Clarase (Table I).

Studies on Inhibition of Thiamine Loss. Various attempts were made to inhibit the loss of thiamine added to soybean meal incubated at pH 6 to 7 in the presence of Clarase. Meal extractions were performed under nitrogen using water previously swept with nitrogen. Also investigated was the addition of graded amounts of cysteine or the disodium salt of (ethylenedinitrilo)tetraacetic acid (EDTA) on the recovery of thiamine added to samples. Use of EDTA was prompted by the reported (*14*) equilibrium between the thiazole and thiol forms of thiamine in aqueous solution at pH 7.5 and by the reported (*6*) stabilizing effect of EDTA on the reduced (thiol) form of glutathione in aqueous solution.

Table II shows that the thiamine loss was inhibited in the presence of EDTA. A similar inhibition was obtained by keeping the assay sample under nitrogen during the extraction procedure. As shown in Table II, lower levels of cysteine added to soybean meal assay samples resulted in increased recovery of added thiamine, but higher levels gave lower recovery. Similar concentration effects have been reported (*5*) in which low recovery of thiamine was observed when high concentration of thioglycolic acid was used to reduce the disulfide form of thiamine.

Comparative Thiamine Studies of Millet and Soybean Meal. The effects of the following treatments on estimating the recovery of added thiamine from millet (*Eleusine coracana*) and from soybean meal assay samples were compared: boiling or autoclaving the assay sample before starting the assay procedure; extraction of samples at various pH values in the presence of crude enzyme preparations; and autolysis of samples at pH 6.5.

Table III compares the effects of pH and EDTA on the recovery of thiamine added to millet (*Eleusine coracana*) and to unheated soybean meal in the presence of added crude enzymes. Loss of thiamine in the millet assay is not dependent on pH of the extraction (*3*) in contrast to the soybean meal samples. Poor recovery (38%) of thiamine added to millet occurred at pH 4.5, as well as at pH 6.5 (55%). At pH 6.5 with added

Table I. Recovery of Thiamine at pH 6.5 in Absence of Soybean Meal[a]

Mixture	*Recovery of Thiamine, %*
Thiamine, as is	97–99
Thiamine and pig mucosa enzyme preparation[b]	95–97
Thiamine and Clarase (0.3 gram)[c]	95–99
Thiamine and Clarase (1.5 grams)	89
Thiamine, Clarase (1.5 grams), and EDTA (75 mg.)	97

[a] Thiamine (10 μg.) after 20 hours at 37° C. using toluene as preservative.
[b] 2 ml. of enzyme preparation.
[c] Usual amount of Clarase used per gram of soybean meal assayed.

Table II. Effect of EDTA and Cysteine on Meal Thiamine and Recovery of Added Thiamine[a] **in Presence of Unheated Hexane-Extracted Soybean Meal**

Reagent Added	*Mg./G. Meal*	*Meal Thiamine, μg./G.*	*Recovery of Added Thiamine, %*
None	0	11.5	60
EDTA	2	11.6	70
EDTA	30	11.7	98
Cysteine hydrochloride	25	12.6	82
Cysteine hydrochloride	50	12.4	85
Cysteine hydrochloride	100	11.9	77

[a] Estimation of thiamine as thiochrome in the Johnson thiochrome procedure, following incubation at pH 6 to 7, 37° C., overnight with Clarase and papain.

Table III. Recovery of Thiamine Added to Soybean Meal and to Millet (*Eleusine coracana* ragi)

Method or Treatment	*Thiamine Recovery, %*	
	Soybean meal	*Millet*
Johnson thiochrome[a]	105	38
Bhagvat thiochrome[b]	70	55
Autolysis[b]	102	30
EDTA (30 mg./gram sample)[c]	99	39
Boiled sample[d]	97	25
Autoclaved sample[e]	...	37
Sample extracted under N_2	101	..

[a] pH 4.5.
[b] pH 6.5.
[c] pH 6.5 in presence of Clarase.
[d] Boiled at pH 4 and digested at pH 6.5.
[e] 30 minutes at 15 p.s.i.

Table IV. Chromatographic Investigations of Synthetic Mixtures of Thiamine-Thiol Binding Reagents at Various pH Values[a]

Compound or Mixture	R_f of Ultraviolet Absorbing Spots, 250 Mμ		
	pH 4–4.5	pH 6–7	pH 9
Thiamine[b]	0.39	0.33–0.36	0.42
p-CMB	0.83	0.86	...
NEM[c]	...	0.84–0.86	0.83
p-CMB-thiamine mixture	0.39	0.37	...
	0.83	0.50	
		0.83	
NEM-thiamine mixture	0.39	0.33–0.35	0.40
	...	0.63–0.66	0.66[d]
		0.84–0.86	0.83

[a] Following the specified period at the given pH, compounds and mixtures were chromatogrammed on Whatman No. 1 paper using the upper layer from a mixture of alcohol, acetic acid, butanol, and water (1:1:4:10) (*14*).
[b] Detected with Dragendorff's reagent and also by intense fluorescence following conversion to thiochrome.
[c] Apparently hydrolyzes to *N*-ethylmaleamic acid.
[d] Pink color on white background when sprayed with alkali.

EDTA (30 mg. per gram), there was quantitative recovery of thiamine added to the soybean meal; only 39% was recovered from millet. Autolysis studies with the millet showed poor recovery (30%) of added thiamine. Samples incubated with crude phosphatase preparations gave recovery of 55%. Autoclaving the millet prior to assay did not increase the recovery of added thiamine and confirmed the previous results reported by Bhagvat. These comparative studies indicate that the mechanisms responsible for the disappearance of thiamine added to millet and soybean meal are unrelated. Hence, the analogy made by Bhagvat and Devi (*3*) of the instability of thiamine in the presence of these two seeds is not confirmed.

Thiamine Stability and Reactions. Equilibrium between the thiazole and thiol forms of thiamine at pH 7.5 has been reported (*9, 14*) by the formation of a mixed disulfide of cysteine and thiamine. These reports prompted investigations of mixtures of thiamine and thiol-binding agents, *p*-chloromercuribenzoic acid (*p*-CMB) and *N*-ethylmaleimide (NEM). Mixtures were made at pH 4 to 4.5, 6 to 7, and 9. The first two samples were allowed to stand for 24 hours, the third for only 4 hours. Mixtures were chromatographed on Whatman No. 1 paper, and results are shown in Table IV. No new ultraviolet-absorbing spots were observed at pH 4 to 4.5, but at pH 6 to 7 both mixtures revealed new ultraviolet-absorbing spots (R_f 0.50 and 0.66) which did not form thiochrome when sprayed with alkaline ferricyanide (*4*). The spot observed at R_f 0.63 to 0.66 with the pH 6 to 7 thiamine–*N*-ethylmaleimide mixture was also obtained when the alkaline mixture was chromatographed. This ultraviolet-absorbing spot gave a pink color on a white background when sprayed with alkali, which is characteristic of reaction products of *N*-ethylmaleimide and thiols (*1*).

The reported disappearance of thiamine in the presence of rutin (quercetin-3-rutinoside), quercetin (the aglucon of rutin) (*7, 8*), or other polyphenols (*13*), together with the isolation of genistin (genistein-7-glucoside) in 0.10% yield from hexane-extracted soybean flakes (*22*), prompted an investigation of thiamine lability at pH 6 to 7 in the presence of known polyphenols and Clarase. A high mole ratio of genistin to thiamine in solvent-extracted soybean flakes is indicated by the 0.10% isolated yield, whereas thiamine content is approximately 10 to 12 μg. per gram of flakes. Therefore, 10 μg. of thiamine was added to pH 6 to 7 solutions containing 0.3 gram of Clarase and 100 μg. of phenol, resorcinol, phloroglucinol, and pyrogallol. After incubating at 37° C. overnight, thiamine remaining in the mixtures was estimated by the thiochrome procedure of Johnson.

Recovery of thiamine in the presence of phenol, resorcinol, and phloroglucinol was 40 to 60%; the presence of pyrogallol allowed a recovery of 88%. A marked effect of *o*-diphenols on disappearance of thiamine has been reported by Hasegawa (*7, 8*), and was demonstrated with naturally occurring phenolic substances.

Discussion

Bhagvat and Devi (*3*) conducted feeding studies which show rather convincingly that incorporation of oriental millet (*Eleusine coracana*) into the diets of rats and pigeons produces thiamine deficiency symptoms. These symptoms developed, although excess thiamine was added to the diets. Fractionation studies implicated a heat-stable substance. Present studies confirm their work in that thiochrome procedures, which are reliable for measuring thiamine in soybean meal, show a loss of over 50% of the thiamine added to millet meal (Table III). This loss persisted even with boiled or autoclaved millet meal. The nature of a thiamine-destroying factor that satisfies these in vivo and in vitro data is not readily apparent; a thiaminase, an antimetabolite, and binding agent (biotin-type) are all contraindicated. Because this seed is a staple food grain in India, it is surprising that further studies of its thiamine-destroying properties have not been reported.

The authors did not find Bhagvat's thiochrome procedure (*2*) reliable for determination of thiamine in soybean meal or for thiamine added to the meal. The primary weakness of the procedure appears to be digestion of biological materials with enzymes overnight at pH 6 to 7 (natural pH) instead of pH 4.5 as is done with the accepted method developed by Johnson (*10*). Bhagvat's method consistently gave meal thiamine values 60 to 80% of those obtained by Johnson's method; while Johnson's method allowed 100% recovery of added thiamine, recoveries were 50 to 80% by Bhagvat's method. These differences in recovery coincide with the reported differences in stability of the vitamin at pH 4.5 and 6 to 7 (*19*). When Bhagvat and Devi applied their newly developed thiamine analysis to soybean and millet meals, they observed that a large portion of added thiamine was not recovered (*3*). Feeding studies noted above indicated that millet contained a thiamine-destroying factor. Because soybeans responded to chemical assay for thiamine in the same manner as millet did, the Indian workers postulated that soybeans also contained a thiamine-destroying factor. Present data (Table III) do not confirm this analogy.

The stability of thiamine at neutral pH is of concern. Losses of this vitamin in processing and cooking foods and feeds are well recognized. The high temperatures reached in the older screw-press oil extractors destroyed 80 to 90% of the thiamine in soybeans; even the modern desolventizer-toasters destroy about one half of the vitamin as determined by the Johnson method. Some insight was gained, during present studies, into this lability of thiamine at neutral pH. Evidence is presented that thiamine exists in equilibrium with thiazole and thiol forms at pH 6 to 7 (Table IV). Presence of the thiol form would allow interaction with other constituents present. Lowering the pH of the meal to slightly below 6 before cooking to avoid thiol formation could leave a greater portion of the vitamin in the feed. The stability of thiamine in meal slurries was increased by excluding oxygen and by adding a metal-chelating agent, EDTA. The presence of *o*-diphenols resulted in losses of thiamine. There could be a metal-catalyzed oxidation of phenols to quinones, which could then react with the thiol group (*17*) or possibly with the amino group of thiamine near neutrality, thus eliminating thiamine from the analysis.

Addition of Clarase or pig mucosa to soybean meal slurries digested in water (pH 6.5) caused loss of added thiamine. No loss was observed by addition of wheat germ phosphatase, or by autolysis of the raw meal. The cause of losses by Clarase and pig mucosa was not apparent. Likewise, the good recovery with wheat germ phosphatase is not understood.

About 60% of the thiamine in soybeans is present in bound form presumed to be cocarboxylase. Raw meal contains a phosphatase with optimum activity at pH 6.0 which liberates free thiamine from cocarboxylase on autolysis. In the Johnson analysis, soybean meal is digested at pH 4.5 with Clarase to liberate thiamine from its phosphate form, and with papain presumably to liberate protein-bound thiamine. No additional thiamine was liberated by papain, trypsin, or pepsin; hence, their presence in the digestion is apparently unnecessary. The use of Clarase or some other phosphatase is advisable because cooking soybean meal for feeds inactivates the natural phosphatase; also the natural phosphatase is not very active at pH 4.5.

Destruction of meal thiamine with sulfite and with alkali, followed by addition of ferricyanide, failed to yield fluorescing materials in excess of those in the blank. Also, fluorescent materials obtained from the meal acted like thiochrome, in that they were entirely destroyed by ultraviolet irradiation. Hence, there was no evidence that fluorescence was not a measure of thiamine in the meal.

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QUALITY AND FLAVOR OF DAIRY PRODUCTS

Review of Biochemical Properties of Milk and the Lipide Deterioration in Milk and Milk Products as Influenced by Natural Varietal Factors

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The flavors of milk and milk products and their behavior in normal use seriously affect their consumption and may make them totally unfit for human food. The psychic effect of smell and taste cannot be lightly dismissed (*40*), and oxidized fats in the diet may lower the utilization of vitamin A and actually be injurious to health (*38*, *44*). Consequently, it is very desirable to make milk products both palatable and relatively resistant to the causes of bad flavors and losses of nutritive value.

The prevailing tendency, however, is still to emphasize quantity production of feed and milk to reduce production costs, to promote consumption of milk by stressing its unique nutritive properties, and to lean largely on processing methods as a source of aid, rather than to improve production methods on the farm. Yet, fat-soluble vitamin content of milk and certain biochemical properties which control palatability and thus consumption are related not to quantity production of feed and milk, but to the type and quality of roughages and supplements fed, the physiological response of cows of a given breed to the feed consumed, and the handling of milk after withdrawal from the mammary gland.

Major Causes of Undesirable Flavors

Major causes of undesirable flavors in milk and milk products include: odorous principles or their metabolites which pass from the feed via the cow's blood to the milk, giving it a "feedy flavor"; splitting of fat by the milk enzyme lipase, products of which give milk a rancid odor and bitter taste and increase the acid degree of fat; and certain other chemical reactions, enhanced by specific substances or by exposure of milk and milk products to light, which result in metallic-to-fishy, oily, and chalky-to-soapy-tallowy (cardboard-like) flavors.

Lipolytic Rancidity in Raw Milk

Shortly before World War II, lipolytic rancidity caused by by-products of fat splitting was one of the greatest problems of the dairy industry. It was solved here at Cornell University by a careful study of the methods used for handling raw milk on the farm and in the milk plant.

The milk enzyme lipase was found to be activated by slight warming of cold milk and subsequent recooling; activity increased as the temperature was lowered