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CHEMISTRY OF DRY BEAN PROTEINS

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INTRODUCTION

The importance of dry bean proteins to human nutrition does not need to be restated. Detailed biochemical information on these proteins may provide a basis for improved utilization or for potential improvement of the nutritional quality of the proteins. The quality of a protein is influenced by its amino acid profile, digestibility, amino acid availability and the presence of other substances possessing biological activity, e.g., protease inhibitors and lectins. Research on this topic has been complicated by the diversity of the dry bean varieties and the complexity of their proteins. Recent developments in the protein chemistry and nutritional quality of dry beans are reviewed in this paper. Unless particularly pointed out, the bean varieties discussed are of *Phaseolus vulgaris* cultivars.

Physicochemical Properties of Dry Bean Proteins

Dry beans generally contain from 20 to 30% protein, which is 55-80% globulins, 10-20% albumins and minor amounts of glutelins and prolamins. Proteins which have been isolated and characterized include protease inhibitors, hemagglutinins (lectins) and major storage proteins.

Protease inhibitors have attracted the attention of nutritional scientists because of their function in inhibiting proteolytic enzymes during protein digestion. Yet, it has been shown that little correlation exists between trypsin and chymotrypsin inhibitory activities and digestibility of dry bean proteins (Jaffé 1975). Trypsin inhibitors

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Table 1. Physicochemical properties of protease inhibitors

Bean	MW (Daltons)	I.P.	Cys (Mole)	Enzyme Inhibited	Number of Reactive Sites	Reactive Site	Reference
Garden	8,100-9,000	—	14	Try, Chy Elas	2	Lys, Arg	Wilson and Laskowski (1973, 1975)
Kintoki	13,000	4.7	17	Try, Chy	—	—	Miyoshi *et al.* (1978)
Navy	23,000	—	30	Try	—	—	Wagner and Riehm (1967)
Kidney	10,000	—	11	Try, Chy, Elas	2	—	Pusztai (1968)
Black Gram	7,892	—	2	Try, Chy	2	Lys, Arg	Padhye and Salunkhe (1980)
Soy (Kunitz)	23,000	4.5	4	Try, Chy	1	Arg-Ile	Odani and Ikenaka (1973)
Soy (Bowman-Birk)	7,861	4.2	14	Try, Chy	2	Lys-Ser Leu-Ser	Koide *et al.* (1972)

isolated from dry beans are quite similar chemically to one another (Table 1). All are low molecular weight proteins in the range of 8000-13,000 with the exception of Navy bean trypsin inhibitor (M.W. 23,000) (Wagner and Riehm 1967). All are characterized as having a high aspartic acid, serine and half-cystine content and a low content of valine, methionine, phenylalanine, tyrosine and tryptophan. Most of the trypsin inhibitors are able to inhibit both trypsin and chymotrypsin simultaneously.

Large quantities of intramolecular disulfide bonds play an important role in maintaining a compact protein structure. The protease inhibitor from the Kintoki bean, which has an isoelectric point of 4.7, is very resistant to heat, acid and enzymatic attack (Miyoshi *et al.* 1978). This inhibitor still possessed 50% of its inhibitory activity after cooking at pH 5.0 for 10 h; however, it was easily denatured at pH > 6.0 (Fig. 1). These properties are similar to those displayed by the Bowman Birk inhibitor from soybean (*Glycine max*). The soybean Bowman Birk trypsin inhibitor (M.W. 7800) is stabilized by seven disulfide bonds and has been implicated as being responsible for the residual trypsin inhibitory activity in heated soy products.

Among the trypsin inhibitors isolated from dry beans, only the one from Navy beans was found to be a glycoprotein (Wagner and Riehm

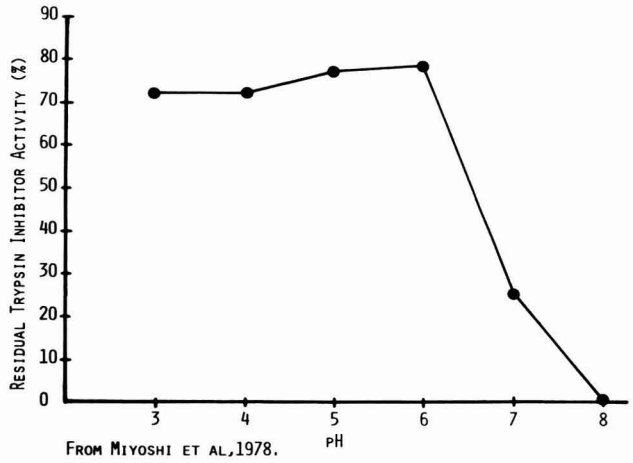

FIG. 1. EFFECT OF PH ON THE TRYPSIN INHIBITORY ACTIVITY OF A TRYPSIN INHIBITOR HEATED AT 100° FOR 1 H

(from Miyoshi *et al.* 1978)

1967). Amino acid sequence of the dry bean protease inhibitors has not been fully investigated; partial amino acid sequence homologies have been observed between isoinhibitor II (from Garden beans), Lima bean (*Phaseolus lunatus*) inhibitor IV and soybean Bowman Birk inhibitor (Wilson and Laskowski 1975). Lysine and/or arginine residues have been found to be the reactive sites for the inhibitor-enzyme reaction (Wilson and Laskowski 1973). Contrary to the stabilizing effect exerted by the high cystine content in the Bowman Birk inhibitor, a trypsin inhibitor (M.W. 7892) isolated from Black Gram (*Phaseolus mungo*) has been found to be stabilized by a single disulfide bond (Padhyde and Salunkhe 1980).

Lectins are proteins that possess the ability to agglutinate red and/or white blood cells. Like protease inhibitors, lectins are diversified and widely distributed in nature. They are common to the seeds of legumes, e.g., 10% of the total Kidney bean proteins (Pusztai and Watt 1974). It is known that dry bean lectins are quite toxic to experimental animals and partially responsible for the poor nutritive value of raw beans (Jaffé 1969; Pusztai *et al.* 1975, 1979), while soybean lectin has been found to contribute very little to the growth depressing effect on the rat (Turner and Liener 1975).

Homogeneous dry bean lectins have been isolated and characterized in the past decade (Table 2) (Liener 1976a). The most extensively studied dry bean lectins are those isolated from red Kidney beans (Allen *et al.* 1969; Miller *et al.* 1973, 1975). Five isolectins have been isolated from the red Kidney bean, each also possessing mitogenic properties. These Kidney bean isolectins are tetrameric glycoproteins consisting of varying quantities of two different subunits, designated L and R. The L and R subunits have identical molecular weight (approximately 34,000) but differ in isoelectric points. The isolectin having four L subunits is a potent mitogen and leukoagglutinin with low hemagglutinating activity, while the isolectin having four R subunits is not a mitogen but is a potent hemagglutinin. The two subunits have identical amino acid sequences from the 8th to 24th residue, beginning at the N-terminal end. The twelfth residue in each subunit is a glycosylated asparagine. A glycopeptide has been isolated from the L-subunit by Hsu *et al.* (1979). The carbohydrate moiety of that glycopeptide is branched, consisting of five mannose and two glucosamine.

The role of the carbohydrate in the structure and function of plant lectins is not understood, since some plant lectins do not contain carbohydrates. The results from the studies of white Kidney beans (Pusztai and Watt 1974) indicate the presence of a range of closely

Table 2. Physicochemical properties of lectins

Bean	M.W. (Daltons)	I.P.	Met (g/100g)	Cys (g/100g)	Carbohydrate (%)	Reference
Navy	114,000	—	0	0	8.1	Andrews (1974)
Brazillian	100,250	5.1	0	0.22	10.5	Moreira and Perrone (1977)
White Kidney	125,000	4.5-6.9	0.3-0.5	0.4-0.5	10-26	Pusztai and Watt (1974)
Kintoki	104,000	5.2	0	0	8.1	Hamaguchi *et al.* (1977)
Red Kidney (L-PHAP)	115,000	5.25	0	0	4.1	Allen *et al.* (1969)
Red Kidney (H-PHAP)		5.25, 5.95	0	0	8.9	Miller *et al.* (1973)
Tora-mame	120,000	5.5	0	0	7.89	Itoh *et al.* (1980)
Wax Bean	132,000	5.5	0	0	10.4	Takahashi *et al.* (1967)
Jack bean[A]	71,000	5.5	0.88	0	0	Olson and Liener (1967)
Soy bean	89,000-110,000	5.85-6.20	0.79	0	10-13.0	Wolf (1972)

[A]Jack Bean *(conavalia ensiformis)* concanavalin A

Table 3. Physicochemical properties of major storage proteins

Bean	MW (Daltons)	I.P.	Cys (g/100g)	Met (g/100g)	CH_2O (%)	Reference
(Kidney) "Haricot"						
Glycoprotein I	—	—	0.06	1.16	11.7	Pusztai (1966)
Glycoprotein II	140,000	—	0.28	0.69	5.5	Pusztai and Watt (1974)
Seafarer	147,000-197,000	—	0.42*	1.03*	—	Barker *et al.* (1976)
Tendergreen (G1)	143,000	4.4-5.6	—	—	—	Sun and Hall (1975)
Great Northern	186,000	5.6-6.4	0.07	1.21	6.5	Chang and Satterlee (1981)
Negro Macentral (α-Globulin)	170,000	—	0.22*	0.92*	5.1	Ishino and Ortega D. (1976)
Soybean (7S)	180,000-210,000	4.9	0.22	0.22	4.9	(Wolf 1972)
Soybean (11S)	330,000-350,000	5.0	1.36	1.26	0	(Wolf 1972)

*Calculated from the original mole % values

related isolectins having IP between 4.5 and 6.9 in the albumin fraction. These isolectins are composed of 2 types of subunits 30,000 and 35,000 in a ratio of about 3:1. They are all glycoproteins and have similar chemical composition. The isolectin having an IP of 5.7 was found to be the most effective agglutinin for both red and white cells. The other isolectins having lower IP had more than 90% of their cystine residues in the form of cysteic acid. The other two isolectins with higher IP contained higher amounts of the basic amino acids. A lectin made up of the subunit of 30,000 was also found in the globulin fraction, its amino acid composition is different from the albumin lectins. The agglutinating activity of all isolectins from white Kidney bean was inhibited by N-acetyl-D-galactosamine and by glycopeptides containing N-acetyl-D-glucosamine, mannose and galactose.

Several other lectins have been isolated from dry beans (Andrews 1974; Hamaguchi *et al.* 1977; Moreira and Perrone 1977; Itoh *et al.* 1980). These lectins generally have molecular weights in the range from 100,000 to 120,00 consisting of 3 or 4 subunits with subunit M.W. of about 30,000 to 34,000. The amino acid composition of these lectins is characterized by the high contents of aspartic acid, serine, threonine and leucine, and either the complete absence or very low contents of methionine and cystine. The glutamic acid content of these lectins is low as compared to other major storage proteins. These lectins are also glycoproteins containing various amounts of mannose, glucosamine and other types of sugar. As indicated by Hsu *et al.* (1979), more detailed structural studies of lectins within or between species are needed to explain the biological function of their carbohydate moiety. Heat denaturation studies on purified lectins have not yet been performed.

Since the largest quantity of proteins in dry beans is the storage proteins, then their amino acid composition and structural feature play a major role in determining the nutritive quality of the total seed protein. Nonstorage proteins such as enzymes are present in smaller amounts, but they are usually rich in the essential amino acids. A few homogeneous storage proteins from dry beans have been isolated and characterized (Table 3). Glycoprotein I and II have been isolated from Kidney beans (Pusztai 1966; Pusztai and Watt 1970). Glycoprotein I contains very little cysteine but is relatively rich in the aromatic amino acids. Glycoprotein II, the major protein constituent of Kidney beans, also contains very small quantities of methionine and cystine. Glycoprotein II was found to be a strong antigen against rabbit antisera; however, it had no hemagglutinating activity for rabbit erythrocytes. The protein has a molecular weight of 140,000 at

slightly alkaline pH and consists of subunits with molecular weights varying from 35,000 to 43,000. Glycoprotein II associates into a polymer (M.W. 560,000) at pH between 3.4 and 6.6. Sun and Hall (1975) were able to separate G1 and G2 proteins from Tendergreen bean, based on the differential solubility of these proteins in salt solutions. G1 is a strong-salt soluble protein, while G2 is a dilute-salt soluble protein. G1 is the major globulin (76% of the total globulins) found in the Tendergreen bean and consists of 3 subunits with molecular weights of 53,000, 47,000 and 43,000. The IP of the G1 protein was found to be between pH 4.4 and 5.6; G2 was 3.7.

Ishino and Ortega D. (1975) isolated four major globulins from Negro Mecentral bean. The largest protein found in this bean is called the α-globulin, a 7.4S glycoprotein having a molecular weight of 170,000. The α-globulin is also deficient in the sulfur-containing amino acids. Barker *et al.* (1976) isolated the major storage protein from Seafarer bean. The major storage protein was composed of 2 types of subunits with molecular weights 50,000 and 47,000 in the approximate ratio of 2 to 3:1. The major protein in the Seafarer bean again is a glycoprotein which is soluble at pH 4.7 and like soybean 11S protein can be cryoprecipitated. The protein has a sedimentation coefficient of 7S at pH 7.0 and associates into an 18S polymer at pH 6.2.

This protein is quite similar to Glycoprotein II isolated from Kidney beans by Pusztai and Watt (1970). These two proteins are alike in electrophoretic mobility, amino acid composition and subunit association behavior. Native storage proteins of dry beans have been found to be very compact and resistant to enzymatic hydrolysis. Ishino and Ortega D. (1975) reported that the α-globulin of the Negro Mecentral bean could not be dissociated by 8 M urea. Vaintraub *et al.* (1976) reported that only 10 to 20% of 7S proteins from various dry bean varieties were susceptible to enzymatic hydrolysis. Romero and Ryan (1978) and Chang and Satterlee (1981) also reported on the resistant nature of native dry bean storage proteins. Seidl *et al.* (1969) reported that a globulin fraction of the Black bean (30% of the total bean protein) was resistant to a variety of proteolytic enzymes even after heat denaturation. Unlike trypsin inhibitors which are stabilized by high numbers of disulfide cross-links, the stability of the various storage proteins is most likely attributed to their tertiary and quaternary structure which is stabilized by numerous hydrogen bonds, hydrophobic interactions and electrostatic forces. Disulfide bonds do not seem to exist in the major storage proteins of Great Northern beans, since only one cysteine residue has been found in the

protein (Chang and Satterlee 1981). It has been observed that when using an in vitro digestion assay, only about 10% of the native protein is hydrolyzed to small molecular weight peptides. The low digestibility of the protein may also be associated with the presence of a bound carbohydrate moiety which serves to block enzyme-specific peptide bonds on the surface of the protein molecule from attack by proteases.

Nutritional Quality of Dry Bean Proteins

The nutritional quality of legume derived proteins has been reviewed by Bressani (1975), Tobin and Carpenter (1978) and Carpenter (1981). Factors influencing the nutritive quality of dry bean proteins include the amino acid pattern and degree of digestibility, as well as the quantity and quality of the other food proteins consumed along with the bean proteins. The digestibility of a protein is dependent upon factors such as protein structure and the presence of protease inhibitors and lectins.

The amino acid pattern of dry bean proteins is generally characterized by their deficiency in the sulfur amino acids and tryptophan. Some of the methionine and cystine have been found to be present in the oxidized forms, e.g., methionine sulfone, methionine sulfoxide (Kasai *et al.* 1971, 1972; Otoul *et al.* 1975), S-methyl cysteine sulfoxide, S-methyl cysteine (Baldi and Salamini 1973) and cysteic acid (Pusztai and Watt 1974) in the native state and in processed bean products. Methionine sulfone, cysteic acid and S-methyl cysteine are known to be unavailable to rats (Anderson *et al.* 1976; Block and Jackson 1932). The ability of humans to utilize methionine sulfoxide is uncertain, but is has been reported to be from 60 to 100% utilizable by rats (Anderson *et al.* 1976; Sjoberg and Bostrom 1977; Slump and Shreuder 1973). Wide variations from the studies on availability of methionine sulfoxide indicate that further studies need to be done to describe the degree of utilization of methionine sulfoxide by humans and rats. The presence of unavailable forms of methionine and cysteine makes these amino acids more limiting in the bean proteins. Extreme processing (high pH and/or heat) can lessen the availability of the sulfur amino acids and lysine. Evans and Bauer (1978) determined the availability of methionine and cysteine using rat and microbiological assays and reported that only 50% of the methionine and 59% of cysteine present in autoclaved Sanilac beans were available. Sgarbieri and co-workers (1979) also reported the low availability of methionine (30 ~ 40% available) of 4 varieties of dry beans.

The digestibility of raw bean proteins generally ranges from 25 to 60%. Cooked beans have an apparent protein digestibility ranging from 65 to 85%, depending on bean variety and cooking process used. It is known that many of the trypsin inhibitors found in dry beans are high in cysteine and very resistant to enzymatic hydrolysis in their native state. Trypsin inhibitors, therefore, would pose a double detrimental effect to the utilization of bean proteins, i.e., they inhibit trypsin and/or chymotrypsin and thereby decrease protein digestibility and also carry away a significant portion of cystine (Kakade *et al.* 1969) which is excreted in the feces in the form of an inhibitor-enzyme complex. Both the trypsin inhibitor and the enzymes in the complex are rich in the sulfur-amino acids. The inhibitor-enzyme complex also lowers the free trypsin concentration in the intestine; therefore, the trypsin synthesis feedback mechanism is inhibited and causes the pancreas to rapidly secrete more enzymes into the intestinal tract and resulting in pancreatic hypertrophy. These series of reactions rendered sulfur-amino acid more limiting and the growth of rats was thereby depressed (Liener 1976b).

The role of dry bean protease inhibitors in human nutrition is yet unclear. Studies have shown that human pancreatic cationic trypsin (80-90% of the total pancreatic trypsin) is not inhibited by the soybean Kunitz trypsin inhibitors (Mallory and Travis 1973; Travis and Roberts 1969). However, a Lima bean trypsin inhibitor and dry bean protease inhibitors were shown to inhibit human cationic trypsin (Feeney *et al.* 1969). More research is needed to examine the mode of inhibition of human trypsin and chymotrypsin by the various forms of dry bean protease inhibitors.

Lectins from several varieties (e.g., Kidney beans) have been proven to be very toxic to rats (Pusztai and Palmer 1977), while those from some varieties are practically nontoxic, e.g., Pinto III and Great Northern beans (Pusztai *et al.* 1979). Two theories have been proposed to explain the mechanism that causes the toxic effect in animals when lectins are ingested orally (Liener 1976a). Theory 1 states that lectins bind to specific receptor sites on the surface of the intestinal epithelial cells and impair the ability of the cells to absorb nutrients from the intestinal tract. The second theory states that lectins impair the defense mechanism of the intestine which leads to tissue invasion by normally innocuous intestinal microflora. Recently, Pusztai *et al.* (1979) observed that lectins disrupt the brush borders of duodenal and jejunal enterocytes, thus decreasing the absorption of nutrients and causing the absorption of potential harmful substances through the damaged intestinal lining. Immediately following these systemat-

ically harmful effects, an abnormally high rate of tissue protein catabolism makes it impossible for the rats to remain healthy. The toxicity of purified lectins in humans has not been investigated.

The compactness of storage proteins has been suggested to be partially responsible for the poor protein digestibility of the raw soybeans (Liener 1976b). The compactness of major storage proteins is important in determining the digestibility of bean proteins which do not possess protease inhibitory and lectin activities. Two varieties without trypsin and chymotrypsin inhibitory activities had lower protein digestiblity than other varieties having trypsin and chymotrypsin inhibitory activities (Jaffé 1975). We do not yet know the degree of the poor utilization of raw dry bean proteins that is due to protein structure, protease inhibitors and lectins, respectively. Varietal differences within dry beans could also exist. Fortunately, the compact storage proteins, protease inhibitors and lectins can be denatured by heat treatment. We do not yet have data to show the sequence of protein denaturation of any particular dry beans. Uncooked dry beans generally cannot support the protein requirement for the maintenance and growth of the rat. Cooked dry beans generally have PER values between 1.0 and 2.0, with the majority between 1 and 1.5, depending on bean variety and cooking method (Tobin and Carpenter 1978). Intrinsic factors, aside from protease inhibitors, lectins and the compact structural characteristics of major proteins, such as phytic acid, tannins, saponins and other toxic substances, may exist in raw beans and contribute to the low degree of protein utilization. Extrinsic factors affecting protein quality include storage and processing conditions, the latter of which will be discussed further.

Approaches for Improving Protein Quality

Breeding programs for improving the protein quality of dry beans are costly and time consuming, since it can be a decade or more from the beginning of the program to the release of an improved bean variety. So far, success in the breeding of a high sulfur-amino acid bean variety analogous to the development of the high lysine/tryptophan opaque-2 in corn breeding has not been reported. Currently, the identification of the nutritional attributes through detailed compositional and structural studies is of first importance. Rapid and sensitive analytical methods for the qualification and quantification of the perspective nutritional attributes are required to screen thou-

sands of samples. Modern electrophoretic and chromatographic methods have been used for these purposes.

Romero *et al.* (1975) employed an electophoretic procedure to study the heritable variation in the polypeptide subunits of the dry bean storage protein (G1 protein) and found the protein subunit banding patterns for two strains high in methionine were typically three-banded. The strains lower in methionine were two-banded. Among various types of dry bean storage proteins, the globulins in particular were reported to be the proteins which could be manipulated to improve nutritional quality, specifically because they are deficient in the sulfur-amino acids (Boulter 1977). Nonstorage proteins (e.g., enzymes) are usually abundant in the essential amino acids. Selection for varieties with low heat-labile antinutritional factors does not seem to be urgent, since they can be inactivated by the cooking process. The possibility of inducing a high sulfur-amino acid content in a particular protein is unlikely (Boulter 1977). A change in the proportion of some storage proteins may lead to the improvement of protein quality. For instance, selection of a variety with higher 11S globulins could substantially improve the nutritive value of dry beans. The 11S globulins which resemble soybean 11S proteins possess a higher content of the sulfur-amino acids than do the 7S globulins which are the main storage proteins of dry beans (Derbyshire and Boulter 1976). It has also been suggested that proteins in the albumin fraction contribute significantly to the nutritional value of the total bean proteins. Peas, which contain a higher level of albumin, have higher PER values (Bajaj *et al.* 1971). Selection of a variety with higher content of albumins could be as important as selecting a variety with globulins of higher nutritional values.

It has long been known that the supplementation of limiting amino acids to the cooked bean diets could remarkably improve protein quality. Kakade and Evans (1965) reported autoclaved Navy beans, when supplemented either with methionine alone or with all the limiting essential amino acids, had PER as good as that of the casein diet. Bressani *et al.* (1963) reported an improved PER and biological value of Black beans when supplemented with 0.2% methionine, even though the digestibility of the protein was not improved by the addition of the methionine. There have been many other studies that show improvement of bean protein quality by supplementation of limiting essential amino acids (Jaffé 1975; Bressani, 1975; Yadav and Liener 1977; Antunes and Sgarbieri 1979; Chang and Satterlee 1979; Antunes *et al.* 1979; Hernandez-Infante *et al.* 1979). Supplementation of limiting amino acids can be done by direct addition to the diets,

infusion into the beans or covalent attachment of the amino acids to the protein sources enzymatically or chemically. Monti and Jost (1979) were able to prepare a plastein using a peptic soy protein hydrolyzate and ^{14}C-labeled methionine ethyl ester as substrates and papain as a catalyst. The resulting water-soluble plastein contained 14.3% of covalently bonded methionine and less than 5% of free methionine. Puigserver *et al.* (1979) chemically attached methionine to casein and evaluated the bioavailability of casein derivative.They found that the isopeptide bond formed between the ξ-amino group of the lysyl residues of casein and methionine or N-acetyl methionine was readily hydrolyzed by an intestinal amino peptidase and proteolytic enzymes. Isonitrogenous diets containing the derivatized casein had PER higher than the control casein diet. Therefore, covalent attachment of limiting essential amino acid to proteins could be a good approach for improving the nutritional quality of food proteins. Puigserver *et al.* (1979) stated that such modification of proteins would have a number of advantages over the direct addition of free amino acids to the diet. In addition to the greater biological availability of protein bound amino acids, they are not as easily lost during processing as are free amino acids. Finally, the off-flavor and off-color, which can be induced through Maillard reaction and Strecker degradation, would be essentially eliminated when the amino acid is covalently bound to casein.

A vast amount of research has been reported on the complementation of cooked beans with various other protein sources (e.g., corn, rice, wheat, barley, oats, sesame). Yadav and Liener (1977) showed that roasted Navy beans, when combined with various cereal grains in proportions predicted to give a maximum chemical score, had PER values which were not significantly different from that of casein. Navy beans, when complemented with rice, had a PER significantly higher than that of casein. Bressani and Elias (1979) found that the optimum efficiency for protein utilization of corn and bean mixtures was with a mixture having 1:1 ratio of protein contribution. When Navy beans were complemented with an equal quantity of sesame, the PER was improved from the 1.56 of the beans and 1.19 of the sesame to 2.30 for the mixture (Boloorforooshan and Maskakis 1979). Like cereal grains, sesame is relatively rich in the sulfur-amino acids but low in lysine. Sesame easily complements the Navy bean proteins which are limited in the sulfur-amino acids and rich in lysine. The upgrading of legume protein quality through complementation with cereals and other protein sources has also been discussed by Bressani (1975), Tobin and Carpenter (1978) and Rockland and Radke (1981).

Processing and Its Effects on Protein Quality

The purpose of heat treatment is to make raw beans soft and edible through hydration and denaturation of both the heat labile antinutritional factors and the compact structure of the storage proteins. Undercooking results in the presence of significant quantities of antinutritional factors in the final bean product (Kote 1972). Overcooking reduces the availability of several select essential amino acids, e.g., lysine and the sulfur-amino acids, and results in a lowering of the nutritive quality of the protein in the final product. The complete denaturation of protease inhibitors and lectins is not required in order to improve protein nutritional quality. Rackis and McGhee (1975) described the biological threshold for soybean trypsin inhibitor (TI) activity and noted that optimal PER values were obtained whe 79 to 87% of the trypsin inhibitors had bene inactivated. The residual inhibitor activity was only 10 to 20 trypsin inhibitor units (TIU)/mg protein. Pancreatic hypertrophy did not occur in rats when fed soy which had from 55 to 69% of its initial TI activity destroyed.

Similar studies have not been performed using dry bean trypsin inhibitor. Kakade and Evans (1965) observed that autoclaving Navy beans at 121°C for longer than 5 min had adverse effects on their nutritive value. The autoclaving of raw beans for 5 min destroys 80% of the TI activity and 100% of the hemagglutinating activity. Studies on the biological threshold for dry bean lectins have not been conducted, but in the case of Navy beans, the lectins are more heat labile than are the trypsin inhibitors. Bressani *et al.* (1963) also reported pressure cooking of Black beans for longer than 30 min decreased their protein nutritional value. Molina and coworkers (1975) reported Black beans cooked for 10 min had PER values higher than those cooked for longer times. Soaking has been shown to have no significant effect on bean protein digestibility. Varriano-Marston and Omana (1979) observed that nitrogen was solubilized during the soaking of beans and found that beans soaked in the combined salt solution, which was used in Rockland's process for the preparation of quick cooking beans, lost significantly more nitrogen into the liquor than did any other soaking treatment. Varriano-Marston and Omana (1979) attributed this result to the combined effect of both pH and salt. It has been observed that the major storage protein of Great Northern beans (30% of the total bean protein) was easily denatured at pH above and below its isoelectric point range (pH 4-6) (Chang and Satterlee 1981) (Fig. 2). The pH of the water solution of soaked beans was in the range of 5.7 ~ 6.2, which was in the isoelectric point range

of the bean proteins. Longer cooking was needed to denature the major storage proteins in that pH range. Similarly, the trypsin inhibitors, when within their isoelectric point range (pH 4-6), are very stable (Miyoshi *et al.* 1978). Isolated lectins generally have an IP range from 4.5 to 7.0. If the beans could have their pH shifted away from the IP range of 4-6, the cooking time and energy requirements could be greatly reduced. Further work being performed in our laboratory has shown significant improvements in protein digestibility when dry beans are rehydrated in an alkali solution. Raw beans have a protein digestibility generally lower than 50%, while alkali soaked beans, even without heat treatment, have an in vitro protein digestibility of 82-83%. Additional soaking in an acid solution (pH 1.0) immediately after the alkali treatment, followed by a short heat treatment (autoclaving 3 min at 80°C), caused the in vitro protein digestibility to rise to 87%.

Methodology for the Study of Bean Protein Nutritional Quality

Numerous reviews have been written on the measurement of food protein digestibility and nutritional quality (Satterlee *et al.* 1979, 1981). In vivo protein digestibility measures the total effect protein

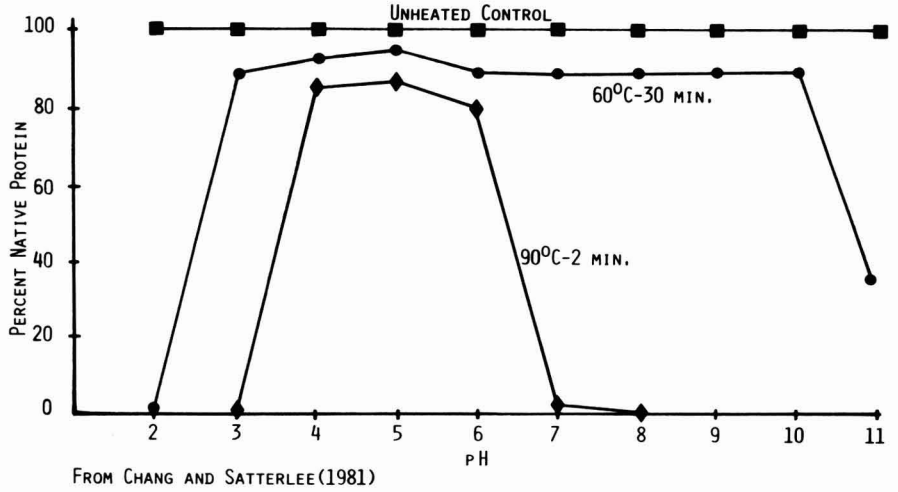

FIG. 2. EFFECT OF PH ON THE STABILITY OF A MAJOR STORAGE PROTEIN HEATED AT 60°C FOR 30 MIN AND 90°C FOR 2 MIN

(from Chang and Satterlee 1981)

structure, protein inhibitors, lectins and other substances have on protein digestibility. In vivo assays for both protein digestibility and quality using several species of animals may give differing results due to the different responses each species has to the various antinutritional factors. In vitro assays reflect the effect protein structure protease inhibitors have on protein digestibility and quality but do not measure any response to lectins present in the sample. Since lectins and protease inhibitors are destroyed during cooking process at almost equal rates, lectins do not pose serious problems for in vitro digestibility assays. Human , porcine and rat pancreatins have been found to possess similar abilities to digest food proteins (Marshall *et al.* 1979). A four-enzyme protease assay has been described to accurately estimate rat digestibility for a wide variety of food proteins (Satterlee *et al.* 1979). A commonly used procedure to estimate protein quality is the PER assay. PER was defined as the ratio of weight gain of rats to protein intake during a 28-day test period. The PER assay has several shortfalls: (1) it does not take into account protein requirements for maintenance; (2) weight gain does not exactly represent nitrogen retained in the body; and (3) the dietary protein level used in the PER assay was arbitrarily set at 10%, a level which is suboptimal for some proteins and above optimal for others. The PER assay is believed to underestimate the nutritional quality of bean protein, as compared to estimates obtained from human assay. Several factors may be responsible: (1) low food intake due to beany flavor; (2) a higher growth requirement for lysine and the sulfur-amino acids by the rat, the latter of which is a limiting amino acid in bean proteins; and (3) a different species response to antinutritional factors by the rat and the human, e.g., rat's pancreatic proteolytic enzymes are more sensitive to protease inhibitors. The slope ratio and NRP assays have been described to be better than PER in estimating protein quality (McLaughlan 1979). Despite the critiques of the PER assay, it is the current official method for use in the U.S.A. Recently, a rapid and accurate C-PER assay has been described (Satterlee *et al.* 1979, 1981). The C-PER values of a wide range of food proteins were determined using both in vitro digestibility and essential amino acid pattern data and correlated very well with PER values determined by the standard rat bioassay. A DC-PER method (Jewell *et al.* 1980), which utilizes only the amino acid pattern to predict protein digestibility and PER, has been found to be as accurate as the C-PER procedure for most common food proteins. Both C-PER and DC-PER assays are suitable for a wide range of foods, i.e., meat, egg, dairy and

plant proteins, but not suitable for raw beans which possess large amounts of the antinutritional factors. The C-PER procedure gives slightly higher values for cooked bean products, when compared to the rat based PER assay. The reasons for this difference are probably due to (1) low food intake by the rat will affect the PER; (2) presence of unknown heat-stable antinutritional factors will affect both PER and C-PER assay; and (3) lowered bioavailability of certain amino acids, e.g., lysine and the sulfur-amino acids could be partially in the unavailable forms that could not be detected by routine amino acid analysis and will affect the C-PER assay. The C-PER or DC-PER assays cannot reflect the bioavailability of the amino acids and the presence of antinutritional factors other than the protease inhibitors.

Since lysine and the sulfur-amino acids can be present in unavailable forms, rapid procedures to measure their availability are desired. The direct FDNB method of Carpenter (1960), as modified by Booth (1971), is widely used to measure available lysine. The FDNB procedure can detect early Maillard reaction products but has the disadvantages of carbohydrate interference and low speed of analysis. An O-methyl isourea method (Creamer *et al.* 1976) was found to be preferable to the FDNB method but is not commonly used because of a long analysis time. Hurrell *et al.* (1979) found that the dye-binding lysine values were in good agreement with FDNB lysine values for the legume products. However, the dye-binding method has been found in our laboratory to overestimate available lysine in several legume products.

Methionine can be oxidized into methionine sulfoxide and methionine sulfone. Methionine sulfoxide can be determined by first an alkaline hydrolysis and then analysis on an amino acid analyzer. Inherent methionine sulfone is determined by acid hydrolysis followed by amino acid analysis. Available methionine can be determined by substracting the amounts of methionine sulfoxide and sulfone from total methionine as determined by the procedure of Moore (1963). Many analytical procedures have been described which measure available cystine and cysteine in purified proteins (Friedman 1975). The procedure of Felker and Waines (1978) has been modified in our lab (Marshall *et al.* 1981) to measure available cystine and cysteine in legume proteins. Cystine residues are reduced with $HaBH_4$ in a solution of 8 M urea, the reduced sulfhydryl groups (cysteine) are measured using a colorimetric method. Oxidized products of cysteine, i.e., cysteic acid and cysteine sulfinic acid, are not reduced by the procedure; therefore, only available cystine plus cysteine are deter-

mined. The procedure is not applicable to food protein samples which are highly pigmented or colored, since these components may interfere with the colorimetric step in the procedure.

Recommendation for Further Research

Suggestions for needed research to improve bean protein quality in addition to an extensive breeding program are listed below:

(1) An overall comparative study of chemical and structural properties of various proteins both within and amongst various bean species.
(2) A study to determine the toxicity of various bean lectins and protease inhibitors in humans.
(3) Heat denaturation studies on various bean proteins under varying conditions should be performed to describe optimal conditions for the processing of beans in order to save fuel, conserve essential amino acids, and yet destroy unwanted heat-labile antinutritional factors.
(4) Studies on the effects carbohydrate and other constituents have on the physicochemical properties of the proteins in the whole beans.
(5) Studies on the bioavailability of the essential amino acids, with particular interest in the sulfur-amino acids, for both conventionally processed beans and those processed by newer energy-efficient methods.
(6) Studies on the effects the supplementation of select limiting amino acids would have on final bean products following processing and in-home reconstitution.

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KINETICS OF BETA-CAROTENE DEGRADATION AT TEMPERATURES TYPICAL OF AIR DRYING OF FOODS

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ABSTRACT

Degradation of β-carotene was studied in a model system at temperatures between 60 and 80°C in order to formulate a kinetic model useful in the optimization of air drying processes. Two previously reported kinetic analyses were used: a first order model and a simplified free radical reaction model. In both cases the models fit the experimental data but neither is entirely satisfactory since the kinetic parameters reflected an initial concentration dependence. This dependence disappeared at high carotene initial concentrations and also when the thickness of the lipid layer was increased either by using a support with a lower specific surface area or by adding a saturated fatty acid. These results indicate that at high concentrations oxygen diffusion becomes a limiting factor.

When using food systems, a first order model seemed a better choice for the simulation of the carotene degradation. Results could not be directly related to those of the model system, probably because the model was too simple, and did not contain the pro- and anti-oxidant components naturally occurring in foods.

INTRODUCTION

Quality deterioration in the form of decrease in nutritional value, color change, development of off-flavor and/or textural change may occur during processing and storage of foods. In order to optimize nutrient retention in processes in which environmental factors and/or composition are controllable variables, the relation between

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the nutrient destruction rate and these factors must be known. While most are specific and have to be dealt with separately for each individual situation, the effect of some of these factors can be expressed in mathematical terms in a form that is similar for very diverse nutrients and food systems (Karel 1979).

Recent studies have shown that it is feasible to predict changes during processing and storage of foods by combining equations expressing the dependence of nutrient deterioration on environmental factors, with equations describing how the environmental factors themselves change with time during processing and storage (Karel 1979). The capability of making nutrient retention predictions depends on the availability of kinetic models reflecting the nutrient degradation under different environmental factors. The present work attempts to find such a kinetic model for degradation of a nutrient at temperatures used in drying. Such models are a necessary basis for the optimization of the dehydration process. Carotenoids, found in most plants, play an important role as pigments and provitamins. Their highly unsaturated chemical structure makes them very susceptible to thermal degradation and oxidation. When carotenoids are spray-dried or freeze-dried, the possibility of degradation is increased considerably. It was considered appropriate, therefore, to use β-carotene to demonstrate the potential for development of nutrient degradation models.

The oxidation of carotene is generally considered to be autocatalytic, beginning only after an induction period in which radicals are built up and antioxidants depleted. In the analysis of the kinetics of carotene degradation, two approaches have been used: (1) the standard treatment of autoxidation mechanisms (Alekseev *et al.* 1968; Finkel'stein *et al.* 1972, 1974; Gagarina *et al.* 1970; Goldman *et al.* 1982) and (2) a simplified first order reaction approach (Arya *et al.* 1979; Chou and Breene 1972; Ramakrishnan and Francis 1979).

In the first case the oxidation of β-carotene in thin films was considered to be an unbranched-chain process with a nonstationary or acceleration period associated with the increase in concentration of free radicals, and a period in which the oxidation of β-carotene takes place at a constant rate (Finkel'stein *et al.* 1972). During the oxidation of carotene, the intermediate products formed are also consumed by chain reactions (Gagarina *et al.* 1970). It is assumed that all these intermediate products have the same reactivity. The degradation of β-carotene in the constant rate period is then given (Finkel'stein *et al.* 1974):

$$2\,RH \xrightarrow{k_i} 2R\cdot \qquad \text{(I)}$$

$$R\cdot + O_2 \underset{}{\overset{K}{\rightleftharpoons}} RO_2\cdot \qquad \text{(II)}$$

$$RO_2\cdot + RH \xrightarrow{k_p} R\cdot + ROOH \qquad \text{(III)}$$

$$R\cdot + R'H \xrightarrow{k'_p} R'\cdot + RH \qquad \text{(IV)}$$

$$R\cdot + RO_2\cdot \xrightarrow{k_t} products \qquad \text{(V)}$$

$$R\cdot + R\cdot \xrightarrow{k'_t} products \qquad \text{(VI)}$$

The rate of consumption of a hydrocarbon in a chain process with a second order chain termination is:

$$-\frac{dC}{dt} = BC\sqrt{r_i} \qquad (1)$$

where C is the β-carotene concentration, r_i the initiation rate, t the time and B a constant. According to a derivation presented by Finkel'stein *et al.* (1974),

$$B = \frac{k_p}{\sqrt{k_t}}\sqrt{K\,R_s\,pO_2} \qquad (2)$$

where k_p and k_t are the rate constants of reactions III and IV, K is the equilibrium constant of reaction II, k_s is the solubility coefficient of oxygen in carotene and pO_2 is the partial pressure of oxygen. The initiation rate is the sum of the initiation rates of radicals formed by the unreacted carotene and by the intermediate products. Therefore,

$$-\frac{dC}{dt} = BC\sqrt{b_oC + b(C_o - C)} \qquad (3)$$

where b_o is the initiation rate constant for the carotene, b is the initiation rate constant for the intermediate products and C_o is the initial β-carotene concentration.

Some researchers have simplified this analysis, considering that after an induction period which decreases with increasing temperature, the rate of decoloration follows first order kinetics (Chou and Breene 1972). First order kinetics is expected if β-carotene and oxygen

are the only reactants and if a large excess of oxygen is present (Ramakrishnan and Francis 1979).

In this study we analyzed the applicability of these two models to the degradation of β-carotene at temperatures typical of air drying of foods. Results in a simple model system were compared with those in foods where the degradation of carotenoids had generally been considered a first order reaction (Baloch *et al.* 1977; Kanner *et al.* 1978; Martinez and Labuza 1968; Quakenbush 1963; Walter and Purcell 1974).

EXPERIMENTAL METHODS

Preparation of the Model System

Synthetic β-carotene (Merck) dissolved in chloroform was dispersed on microcrystalline cellulose (Avicel PH 105, FMC) as in the model systems of Ramakrishnan and Francis (1979), and Chou and Breene (1972). The mixture was blended by hand until the color appeared homogeneous. It was then put in a dessicator and the solvent was evaporated under vacuum for approximately 8-10 h. The mixture was blended again. Dessicant ($CaSO_4$) was added to the dessicator and the model system was allowed to equilibrate under vacuum for 20-24 h. The model system was now considered to be "dry". This was further confirmed by the analysis of the moisture content (around 1% corresponding to a water activity very close to zero) according to the isotherm of Chou and Breene (1972). The model system was stored under these "dry" conditions at -15°C until needed, usually within one or two days. All steps were carried out under subdued light.

In some experiments microcrystalline cellulose Avicel PH 102 was used instead of Avicel PH 105. In other experiments, methyl palmitate (Nu-Chek-Prep, Inc.) was added to the model system. It was dissolved in choloroform and added to the cellulose at the same stage as the addition of β-carotene.

The β-carotene initial concentrations chosen were in the range of concentrations in foods (0.1-1.0 mg β-carotene/g solid).

Kinetic Studies

One gram samples of the model system were weighed into 25 ml Erlenmeyer flasks and sealed with rubber caps. The flasks were immersed in a constant temperature glycerine bath and experiments were carrried out at 60, 70 and 80°C. The temperature of the model

system was monitored by a thermal probe connected to a data logger (Instrulab model 2020) which also served as a timer. Time zero was established as the time when the temperature of the sample was within one degree of the temperature of the bath. All flasks were vented to atmospheric pressure to assure uniform oxygen pressure by inserting an hypodermic syringe in the rubber cap and removing it as soon as the pressures were equilibrated. Periodically, duplicate samples were removed from the bath and immediately quenched in ice to stop the reaction.

The degradation of β-carotene was followed spectrophotometrically. Hexane (25 ml) was added to the sample. The flasks were shaken for about 2 min and the solids were allowed to settle. With the appropriate dilutions, the β-carotene dissolved in the hexane was read at 450 nm. Earlier studies using an HPLC separation (Hsieh 1980) showed that at 450 nm only the undegraded carotene was measured. The absorbance readings were converted to milligrams of β-carotene using an extinction coefficient of 232 $(mg/ml)^{-1}$.

Preparation and Analysis of Food Systems

Three food systems were studied: yellow corn, butternut squash and sweet potato. Corn kernels were separated from the corn cob and both squash and sweet potatoes were peeled and cut in relatively small pieces. The three systems were freeze-dried, ground in a food processor (Waring) and stored at 15°C under dry conditions in jars containing $CaSO_4$.

The kinetic studies were performed in the same way as with the model system with the following exceptions: (1) the carotene from corn was extracted with acetone instead of hexane as the extraction was more complete; and (2) the solids and carotenoid solution were separated by filtration under vacuum in corn.

Since, in the food systems, β-carotene is not the only carotenoid present, HPLC was used to determine β-carotene.

HPLC Method for β-carotene

Sample Extraction. Vegetables were homogenized in a blender or food processor. Samples (2–5 g) were immediately weighed out into 50-ml centrifuge tubes, and extracted with 25 ml of acetone-pet ether (50:50) mixture by shaking or vortexing. Once the two layers were allowed to separate, the top layer was transferred into a 500-ml round bottom flask. The botton layer was reextracted with acetone-petroleum ether mixture several times until the top layer became colorless.

The top layers collected in the 500-ml round bottom flask were evaporated to dryness at 30°C. The vacuum was broken with nitrogen and immediately stoppered. The sample was redissolved with pet ether and filtered (Fluoropore, Millipore). The sample was diluted with elution buffer (8:92, chloroform:acetonitrile) before injection into the HPLC column.

Apparatus. A Waters Model 6000 A solvent delivery system (Waters Associates, Milford, MA) equipped with aValco sample injection valve (Model CV-6UHPa-N60) was used. The column effluents were monitored by a UV-visible detector (Waters Model No. 440) operating at 436 nm for α and β carotenes. The chromatographic peaks were recorded on an Omniscribe recorder.

Column. Stainless-steel (30 cm × 3.9 mm I.D.) C_{18} μ-Bondapak (10-μ particle size, Waters Associates). A protective precolumn (C_{18}/Corasil, Waters Associates) was attached to the front of the C_{18} chromatographic column to protect it from interfering substances. The mobile phase used was a slight modification of that described previously by Zakaria *et al.* (1978). A mixture of chloroform:acetonitrile (8:92) was used as the mobile phase for the C_{18} reverse phase column. The flow rate was maintained at 1.0 ml/min.

The standard deviation of the method for β-carotene was 4.2%.

RESULTS AND DISCUSSION

Model Systems

The curves representing the degradation of β-carotene in a model system (Fig. 1) have a sigmoidal shape. At each of the three experimental temperatures, the oxidation of β-carotene followed a similar pattern.

When the data were analyzed as a first order reaction the following was observed:

1. There is an induction period the length of which varies with the initial concentration and temperature.
2. After the induction period the data fit a first order model at any one initial concentration.
3. The rate constant is dependent on the initial concentration (Table 1).

These kinetic parameters were compared with extrapolated values from literature data obtained at lower temperatures (Table 2). The results of Ramakrishnan and Francis (1979) give extrapolated rate

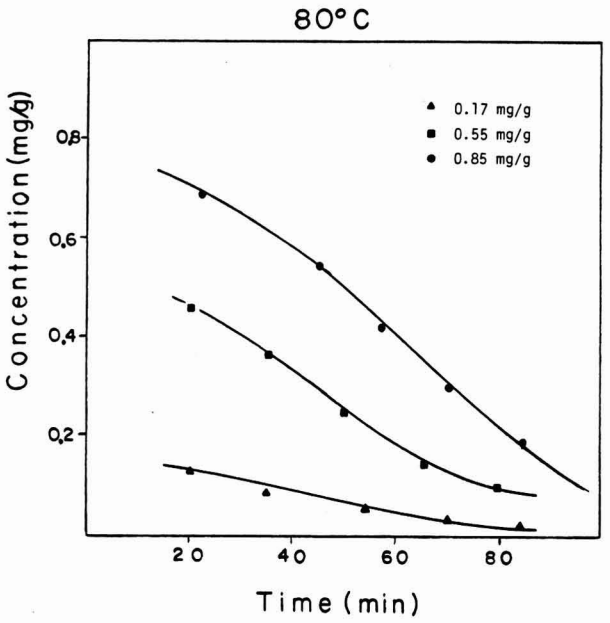

FIG. 1. DEGRADATION OF β-CAROTENE IN MODEL SYSTEMS AT 80°C

Table 1. First order kinetics rate constants derived from experimental data

Temperature (°C)	Initial Concentration (mg/g)	Rate Constant × 10^3 (min^{-1})	Correlation Coefficient (r)
60	0.14	3.7	0.996
	0.57	3.8	0.997
	0.89	3.5	0.995
70	0.16	11.9	1.000
	0.55	14.1	0.998
	0.91	13.8	0.997
80	0.17	23.9	0.999
	0.55	28.9	0.991
	0.85	25.6	0.997

constants similar to ours using the same initial concentration range as we used. The values obtained in two other papers (Chou and Breene 1972; Arya *et al.* 1979) are much lower.

The activation energies obtained in the experimental model system for a temperature range of 60-80°C are much larger than those reported in the literature for lower temperature ranges. Finkel'stein *et*

Table 2. Comparison between first order rate constants reported in literature and those observed in experimental model system

References	Experimental Conditions C_0 (mg/g)	T °C	$K \times 10^3$ (min^{-1})	Ea (Kcal/mol)	at 80°C $K \times 10^3$ (min^{-1})
Chou & Breene 1972	5	35	0.15	9.7	1.1
Arya *et al.* 1979	1.7	16-32	0.09	10.0*	1.2
Ramakrishnan & Francis 1979	1	25	1.58	10.0*	22.0
Experimental	0.17	60-80		21.7	23.9
	0.55			23.6	28.9
	0.85			23.0	25.6

*Assumed value

al. (1972), suggest that there could be two regions of temperature dependence due to a phase transition in the carotene. The experimental values of activation energies shown in Table 2 are similar to those of Finkel'stein *et al.* for carotene films: 9.9 Kcal/mol for a temperature range of 12-25°C and 20.0 Kcal/mol for 28-45°C.

Although the data does fit a first order model, the dependence of the rate constant on the initial concentration does not make it a very adequate model for the simulation of the β-carotene degradation. Equation 3, used as a model for the free radical recombination reaction, includes an initial concentration term. Thus it was expected that the kinetic parameters obtained would be constant.

To analyze the experimental data, an integrated form of Eq. 3 was used:

$$ln \frac{1 + \sqrt{1-C/Co}}{1 - \sqrt{1-C/Co}} = a \sqrt{b-b_o} \sqrt{Co}\, t \qquad (4)$$

It is important to note that the initiation rate constants of the unoxidized carotene (b_o) and of the products (b) cannot be considered separately, but only as the combined rate constant: ($a\sqrt{b-b_o}$). The experimental data fit this model at any one initial concentration after an induction period, but the kinetic parameters obtained still remain dependent on the initial concentration (Table 3).

This dependence of the kinetic parameters on the initial concentration was studied further at 80°C. Similar experiments were conducted using higher β-carotene initial concentrations (2.5, 4.9 and 7.1 mg/g).

Table 3. Free radical recombination kinetics parameters derived from experimental data

Temperature (°C)	Initial Concentration (mg/g)	Rate Constant × 10^3 (min^{-1})	Correlation Coefficient (r)
60	0.14	14.6	0.995
	0.57	7.3	0.982
	0.89	5.4	0.994
70	0.16	41.9	0.994
	0.55	18.7	0.993
	0.91	17.2	0.999
80	0.17	74.7	0.996
	0.55	49.9	0.998
	0.85	27.2	0.982

It was observed that the first order constant decreases until a certain value, where it becomes independent of the initial concentration (Fig. 2). This behavior was also observed with the kinetic parameters derived from the free radical recombination equation, and it could be related to the observations of Honn *et al.* (1951), Mead (1980), Mead *et al.* (1972) and Porter *et al.* (1972). These authors found that when linoleic acid was adsorbed on silica gel, oxidation occurred at a maximum value at the monolayer value. Only the top layer would oxidize at the maximum rate above this value, the rest requiring oxygen diffusion and thus having an overall slower rate of oxidation. Below the monolayer value the distance between molecules is such that the free radical propagation is not at its optimum rate. If this theory was to be applied to our model system, the same experiments performed with a different amount of carotene exposed on the surface would give different results.

Supports of different surface area do in fact affect the degradation of β-carotene. At lower surface areas, and therefore thicker layers of β-carotene, the degradation is slower (Fig. 3). Oxygen diffusion could be a limiting factor but the maximum rate of oxidation could not be related to the monolayer value of β-carotene on microcrystalline cellulose. On both supports at their respective monolayer values, the rates of oxidation should be the same. However, in this case, the maximum rates are very different for the two supports.

Adding other carotene-dissolving liquids such as fatty acids to the model system increases the total film thickness. When methyl palmitate, a saturated methyl ester, was added to the model system in a

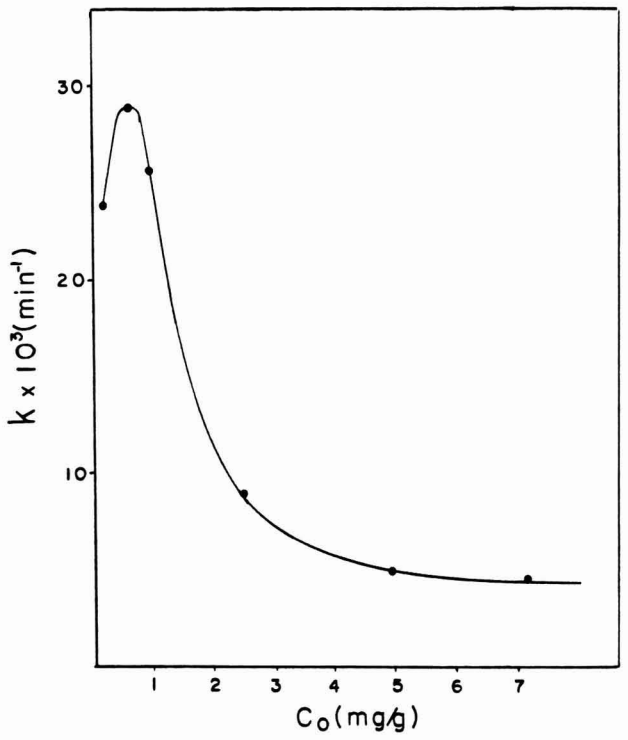

FIG. 2. DEPENDENCE OF FIRST ORDER RATE CONSTANT ON INITIAL CONCENTRATION OF CAROTENE

concentration much larger than that of the carotene (20 mg/g of solids), the rate of degradation of β-carotene became independent of its initial concentration (Fig. 4). Presumably the carotene is uniformly dispersed in the fatty acid layer through which oxygen has to diffuse before the oxidation can occur. These results support the conclusion that oxygen diffusion becomes a limiting factor in oxidation of β-carotene in the model system.

Food System

Although the results of the three food systems were studied are inconclusive regarding the degradation of carotene, some important observations have been made.

(1) Butternut squash—A separation of the lipid fraction of the butternut powder by HPLC showed that at 450 nm mainly α- and β-

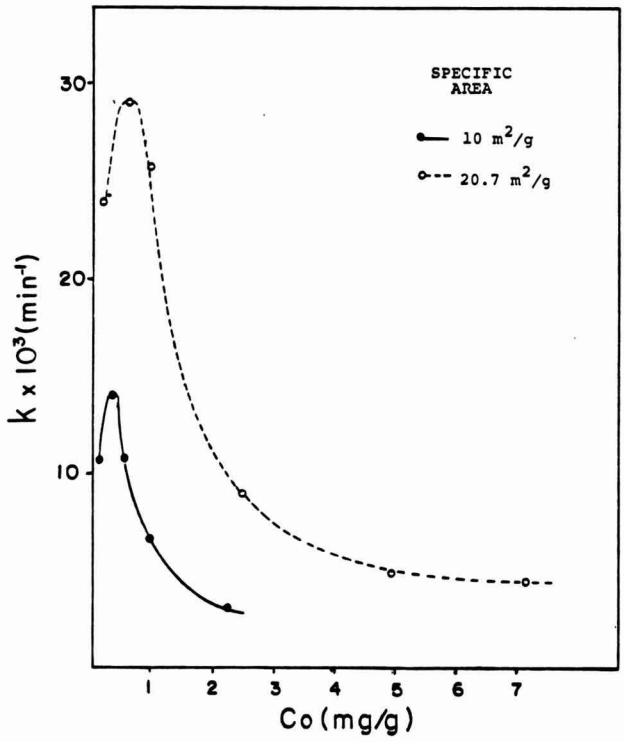

FIG. 3. DEPENDENCE OF FIRST ORDER RATE CONSTANT ON INITIAL CONCENTRATION OF CAROTENE ON SURFACES WITH DIFFERENT SPECIFIC AREAS

(•—•) Avicel pH 102; ○--- Avicel pH 105)

carotene are detected (20 and 65%, respectively). The degradation of the carotene which is initially present in a concentration of 0.63 mg per g of solids, does not show any induction period at any of the temperatures (Fig. 5), and it can be analyzed perfectly as a first order reaction.

(2) Sweet potato—α- and β-carotene are the main components of sweet potato fraction that absorbs at 450 nm. The degradation of the carotenes which are present in an initial concentration of 0.95 mg per g of solids does not show any induction period (Fig. 6) and can also be analyzed as a first order reaction.

(3) Yellow corn—Lutein is the predominant pigment in yellow corn (Quackenbush *et al.* 1963) and β-carotene is the main pro-vitamin A.

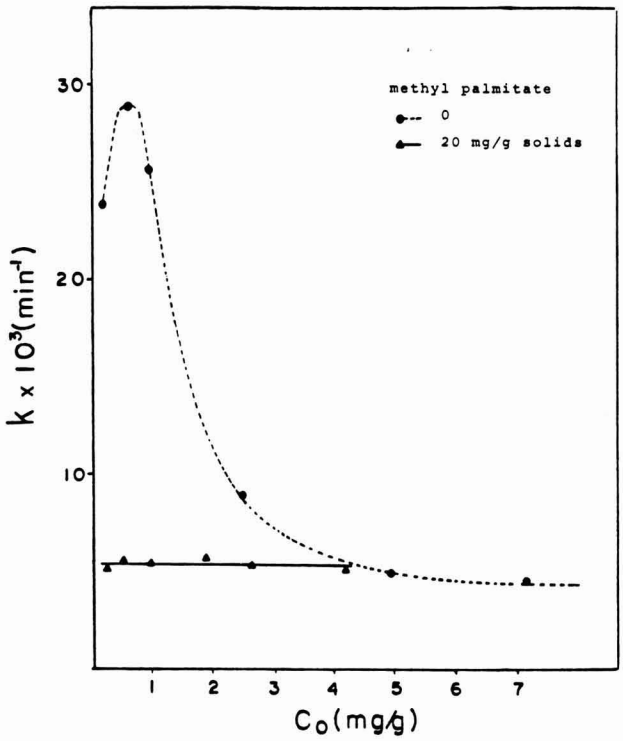

FIG. 4. EFFECT OF THE ADDITION OF METHYL PALMITATE ON THE β-CAROTENE DEGRADATION

They represent 31-35% and 2-5% of the pigments, respectively (Quackenbush *et al.* 1963). The carotene fraction has the lowest stability of all the fractions, and the zanthophylls, lutein and zeaxanthin disappear less rapidly than the carotenes (Quackenbush 1963). In our work the color loss was measured at 450 nm, a wavelength at which several pigments absorb (i.e., β-carotene, β-zeacarotene, zeinoxanthin, cryptoxanthin, zeaxanthin, lutein as well as some polyoxy pigments. The initial concentration of the pigments measured is 45 μg/g of solids. The color loss was well simulated by a first order kinetic model.

In the three cases, the kinetic parameters obtained from the analysis of the food systems are much smaller than those obtained from the model system (Table 4).

Apparently, there are factors in the food systems reducing the rate of oxidation and the activation energy. The composition of each

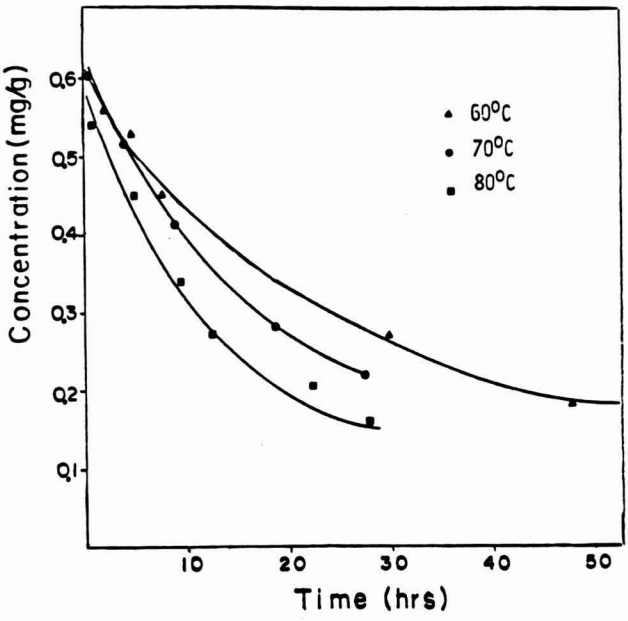

FIG. 5. CAROTENE DEGRADATION IN BUTTERNUT SQUASH

Table 4. Comparison of kinetic constant for carotene degradation in model system (using as support avicel pH 102), and decrease in absorption at 450 nm in foods

System	Rate Constant × 10^3 (min^{-1})			Activation energy (Kcal/mole)
	60°C	70°C	80°C	
Model System (c_0 = 0.14 mg/g)	3.7	11.9	23.9	21.7
Butternut Squash	0.4	0.7	1.1	13.5
Sweet Potato	0.47	0.61	1.16	10.6
Yellow Corn	0.23	0.23	1.35	4.8

individual system is probably important in the degree of this rate reduction.

Since the first order model fits the experimental data there is no need to use a more complicated model (e.g., the free radical recombination model) to simulate the degradation of carotene in food systems.

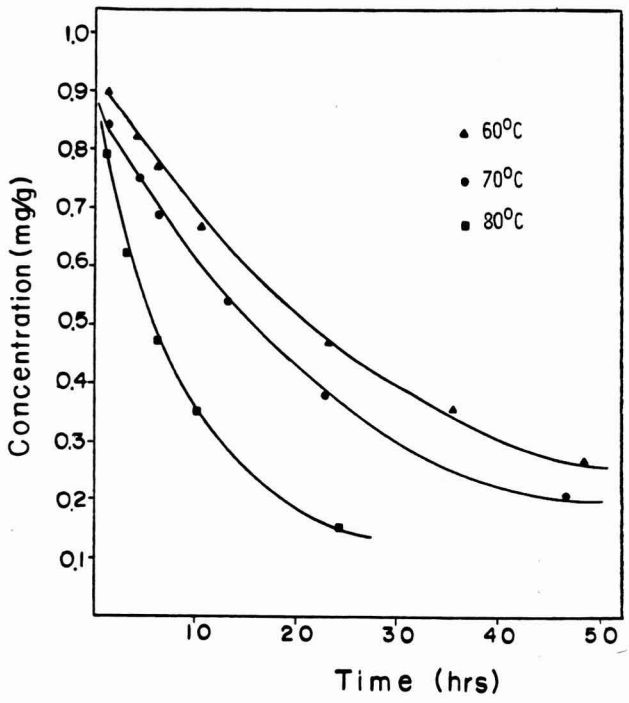

FIG. 6. CAROTENE DEGRADATION IN SWEET POTATO

CONCLUSIONS

The kinetics of the degradation of carotene at air-drying temperatures were studied using a model system composed of β-carotene dispersed on microcrystalline cellulose maintained in a dry state. The two approaches used previously to analyze the kinetics of the degradation were a first order reaction model and a simplified free radical recombination reaction model. Both models fit the experimental data, however the kinetic parameters obtained reflect a dependence on the initial concentration of carotene. This dependence is related to the thickness of the carotene layer where the diffusion of oxygen becomes a limiting factor in the oxidation rate. In the evaluation of the literature on the degradation of carotene in model systems, the initial concentration of carotene and the specific surface area of the support must be considered before comparisons and/or conclusive statements can be made.

Both models present the same problems; they do not consider the induction period, and they depend on the initial concentration of carotene. Neither one of them is really adequate to simulate the degradation of carotene. As for food systems, a first order model does simulate well the degradation of the carotene but the parameters vary in each individual system.

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EFFECTS OF HEAT TREATMENT AND κ-CARRAGEENAN ADDITION ON PROTEIN SOLUBILITY AND VISCOSITY OF MILK PROTEIN/PEANUT FLOUR BLENDS IN AN IONIC ENVIRONMENT SIMULATING COW'S MILK[1]

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ABSTRACT

Peanut flour (PF), whey protein concentrate (WPC), sodium caseinate (SC) and blends of WPC/PF or SC/PF at peanut protein concentrations of 25, 40 and 50% were dispersed in water at 7.0% protein. The dispersions were extensively dialyzed against 11.0% reconstituted nonfat dry milk followed by heating at 60 and 80°C for 1 h. κ-Carrageenan was added to dialyzed dispersions at levels of 0.1, 0.15 and 0.2% followed by heating at 80°C for 1 h. Soluble protein was estimated in supernatants following mid-speed (MS) centrifugation (40,000 × g for 20 min) and ultra-speed (US) centrifugation (200,000 × g for 1 h) at 25°C. Viscosity of protein/carrageenan dispersions was evaluated from Brookfield viscometer data fitted to the power law function. Soluble protein in PF dispersions was lower than that of the milk proteins examined. Increased centrifugation force from MS to US did not affect soluble protein of PF while supernatant protein in the milk proteins decreased with increased centrifugation force. Increased levels of PF in the milk protein/PF blends generally resulted in lowered soluble protein. Heat treatment increased PF soluble

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protein, decreased WPC soluble protein and had minimal effect on SC, SC/PF and WPC/PF soluble protein. Carrageenan addition generally increased PF and WPC/PF soluble protein, lowered WPC soluble protein and did not affect SC and SC/PF soluble protein. All heated protein/carrageenan systems exhibited pseudoplastic (shear-thinning) flow behavior. Highest viscosity and consistency index (k) values were observed for WPC and for blends containing WPC. k values for all protein/carrageenan mixture increased with increased levels of carrageenan.

INTRODUCTION

The calcium sensitivity of peanut protein preparations has been well documented. It has been suggested that the suitability of peanut protein preparations in the fortification of cow's milk or in the formulation of beverages simulating cow's milk is limited because of minimum solubility in the presence of calcium concentrations equivalent to that of milk (30 mM) (Mattil 1973). This conclusion, however, is based upon solubility data collected from dilute peanut protein dispersions at 30 mM $CaCl_2$ (Rhee *et al.* 1972) and, therefore, at considerably higher Ca (II) concentration than would exist in the complex milk environment. At similar levels of $CaCl_2$ (15 to 30 mM), milk proteins are also altered in solubility and heat stability (Schmidt and Mendelsohn 1978). However, peanut protein and peanut protein/milk protein blends were more dramatically affected by calcium than were milk proteins relative to solubility and heat stability. Data obtained from fortification of cow's milk with peanut protein preparations are difficult to interpret (Schmidt *et al.* 1980). Peanut flour (PF) fortified milk generally exhibited higher viscosity than similarly formulated controls fortified with nonfat dry milk (NDM). PF fortified milk also had visible sedimentation. While sedimentation decreased with increased heat treatment, the viscosity increased dramatically. Therefore, PF fortified milk may have limited acceptance without more optimum protein stabilization or prior modification of the peanut protein.

Polysaccharide hydrocolloids, such as carrageenan, are in widespread use in the food industry for their protein stabilizing ability at low protein concentrations or for their thickening ability at high protein concentrations. The majority of the research has been with milk proteins (especially casein) and dairy product applications. However, the interactive mechanisms are still not totally understood.

Stabilization of peanut protein against calcium precipitation by κ-carrageenan has been shown at low protein concentration (0.5%) (Chakraborty and Randolph 1972). Carrageenan effects on peanut protein or peanut/milk protein blends have not been investigated at higher protein concentrations.

The objective of this study was to evaluate heat treatment effects on protein stability of peanut protein dispersions blended with casein or whey protein in an ionic environment resembling cow's milk at moderately high protein concentration. The combined effects of carrageenan and protein blending on solubility and functionality also were investigated.

MATERIALS AND METHODS

Materials

The protein preparation used in this study included: peanut flour (PF) from Goldkist, Inc. (Atlanta, GA); Enrpro 50, a whey protein concentrate (WPC), from Stauffer Chemical Co. (Rochester, MN) and Savortone LF, a sodium caseinate (SC) from Western Dairy Product (San Francisco, CA). Compositional data for these preparations are presented in Table 1. The carrageenan used was Gelcharin GH (Marine Colloids, Inc., Springfield, NJ). This is a κ-carrageenan preparation recommended in cheese substitute formulation.

Sample Preparation

PF was dry blended with WPC or with SC to achieve peanut protein levels of 25, 40 and 50% of the total protein. Controls contained PF, WPC or SC individually. The proteins were dispersed in water at 7.0% total protein, adjusted to pH 8.0, homogenized at high speed for 3 min in a Virtis Model 45 homogenizer and equilibrated with stirring at 25°C for 30 min. The pH was adjusted to 7.0 and insoluble residue was removed by centrifugation (360 × g for 15 min). Final protein dispersions were dialyzed for 65 h at 4°C against three changes of three volumes of 11.0% reconstituted NDM. To prevent microbial growth, 0.05% sodium azide was added to the protein and NDM dispersions. Following dialysis the pH was readjusted to 7.0 and the dispersions were equilibrated with stirring for 1 h at 25°C.

Heat Treatment Effect on Soluble Protein

Samples were heated at 60°C and 80°C for 1 h in a water bath with intermittent mixing followed by immediate cooling to 25°C in an ice

Table 1. Compositional data of protein preparations

Protein Preparation	Composition (%)[1]			
	Fat	Carbohydrate	Protein	Ash
Peanut flour (PF)[2]	0.6	30.0	57.0	4.6
Whey protein conc (WPC)[3]	2.5	26.5	53.6	10.2
Sodium caseinate (SC)[4]	1.2	—	90.2	3.8

[1]Corporation data
[2]Goldnut II, Goldkist, Inc., Atlanta, GA
[3]Enrpro 50, Stauffer Chemical Co., Rochester, MN
[4]Savortone LF, Western Dairy, Inc., San Francisco, CA

bath. Heating was done in round-bottom flasks fitted with Snyder columns to prevent evaporation.

Soluble protein was estimated by measuring supernatant protein content of aliquots centrifuged under mid-speed (MS) conditions (40,000 × g for 20 min at 25°C) and under ultra-speed (US) conditions (200,000 × g for 1 h at 25°C) as previously described (Schmidt and Ahmed 1979). Tabulated results are expressed as % of total protein present.

Effect of Carrageenan Addition on Soluble Protein and Viscosity

Carrageenan was added at levels of 0.10, 0.15 and 0.20% to the dialyzed PF, WPC, SC and PF/WPC or PF/SC blends (at 40% peanut

Table 2. Heat treatment effect on soluble protein in peanut flour whey protein concentrate and sodium caseinate dispersions formulated at 7.0% protein and dialyzed against skim milk

Protein System	Heat Treatment[1] (°C)	Supernatant Protein[2]	
		40,000 × g	200,000 × g
Peanut Flour (PF)	Initial	38.8 xy	35.5 y
	60	45.4 vw	43.2 wx
	80	56.3 stu	45.8 vw
Whey Protein Conc (WPC)	Initial	93.1 a	83.8 c-f
	60	88.2 abc	84.6 cde
	80	79.7 e-j	51.3 uv
Sodium Caseinate (SC)	Initial	90.8 ab	83.0 c-h
	60	85.9 bcd	82.4 c-i
	80	91.0 ab	83.5 c-g

[1]One h at specified temperature
[2]Supernatant protein following centrifugation at force indicated; Expressed as % of total protein. Means of triplicate trials
Means followed by the same letter are not different ($p<0.05$)

protein). The protein/carrageenan mixtures were heated at 80°C for 1 h followed by cooling to 25°C. Supernatant protein was determined following MS and US centrifugation as described above.

Viscosity was determined at 25°C using a Brookfield RVT viscometer fitted with Spindle #1. Data were fitted to the power law flow model (Holdsworth 1971). The equation used was $\mu_{app}=k\gamma^{n-1}$ where μ_{app} is apparent viscosity (poise), γ is shear rate (s^{-1}), k is the consistency index or μ_{app} at unit shear rate (dyne sec^n cm^{-2}) and n is flow behavior index or coefficient of abnormality.

Statistical Analysis. Statistical Analysis System (SAS) programs at the Northeast Regional Data Center located at the Unversity of Florida were used for analysis of variance on the data. Tabulated data represents means of triplicate trials which were statistically tested using the Duncan Multiple range technique (Duncan 1955).

RESULTS AND DISCUSSION

Heat Treatment Effects on Protein Solubility

Results of heat treatment effects on protein solubility of PF, WPC and SC dispersions are summarized in Table 2. As expected, initial protein solubility (at 25°C) of PF was considerably lower than that of WPC or SC. Similar protein solubility levels were previously noted in PF dispersions formulated in water at pH 7.0 and at 1.0% protein (Schmidt and Mendelsohn 1978). Increased centrifugation force from MS to US did not significantly affect supernatant protein suggesting that the PF soluble proteins are not of large molecular weight. Similar trends have been previously observed for centrifugation affects on supernatant protein in highly denatured peanut protein concentrates (Schmidt and Ahmed 1979). Increased centrifugation from MS to US, however, resulted in decreased supernatant protein for the milk proteins examined.

Heating resulted in increased soluble protein for PF while that of WPC decreased with higher heat treatment. Soluble protein of SC was not affected by heat treatment. The dramatic decrease in US supernatant protein for WPC indicates formation of soluble aggregates under higher heat treatment conditions.

Data for heat treatment effects on soluble protein in PF/milk protein blends are shown in Table 3. As expected, increased levels of PF in the blend generally decreased the amount of soluble protein

Table 3. Heat treatment effect on soluble protein in whey protein conc/peanut flour (WPC/PF) and sodium caseinate/peanut flour (SC/PF) blended dispersions formulated at 7.0% protein and dialyzed against skim milk

Protein System	Peanut Protein (% of total)	Heat Treatment[1] (°C)	Supernatant Protein[2] 40,000 × g	Supernatant Protein[2] 200,000 × g
WPC/PF	25	Initial	72.9 k-o	71.5 l-o
		60	75.9 j-m	71.2 l-o
		80	72.2 k-o	70.6 mno
	40	Initial	68.9 op	60.6 qrs
		60	68.2 op	63.8 pq
		80	68.9 op	66.8 op
	50	Initial	57.6 rst	56.6 stu
		60	63.2 rst	56.6 stu
		80	62.1 qrs	61.3 q-t
SC/PF	25	Initial	77.3 h-l	72.4 l-o
		60	80.0 d-j	77.7 g-k
		80	81.8 d-j	78.9 e-k
	40	Initial	70.5 mno	63.2 pqr
		60	72.6 l-o	69.5 o
		80	76.5 i-m	71.2 mno
	50	Initial	63.5 pq	56.0 stu
		60	68.9 op	63.8 pq
			70.1 no	68.9 op

[1]One h at specified temperature
[2]Supernatant protein following centrifugation at force indicated; Expressed as % of total protein. Means of triplicate trials
Means followed by the same letter are not different ($p<0.05$)

measured. The blended systems, however, do not follow similar data trends relative to the heat treatment effects on soluble protein observed for PF alone (Table 2). Heat treatment had a minimal effect on soluble protein in the blended protein systems. The dramatic reduction in US supernatant protein observed for WPC heated at 80°C (Table 2) was not noted in the WPC/PF blends. It is conceivable that the presence of peanut protein inhibited aggregation of the whey protein. However, more definitive data are needed to confirm this hypothesis.

Carrageenan and Heat Treatment Effects on Protein Solubility and Flow Behavior

Addition of carrageenan followed by heat treatment generally resulted in an increase in MS supernatant protein for dialyzed PF and

Table 4. Carrageenan effect on soluble protein in peanut flour (PF), whey protein conc (WPC), sodium caseinate (SC), WPC/PF blends and SC/PF blended dispersions formulated at 7.0% protein and heated at 80°C for 1 h

Protein System	Carrageenan (%)	Supernatant Protein[1] 40,000 × g	Supernatant Protein[1] 200,000 × g
Peanut Flour (PF)	0	56.3 j	45.8 kl
	0.10	73.4 fgh	35.5 mn
	0.15	67.6 hi	34.0 mn
	0.20	65.8 hi	31.8 n
Whey protein conc (WPC)	0	79.7 cde	51.3 jk
	0.10	68.5 hi	39.1 lm
	0.15	50.8 jk	33.1 mn
Sodium caseinate (SC)	0	91.0 a	83.5 bcd
	0.10	95.3 a	84.5 bc
	0.15	93.3 a	87.7 ab
	0.20	93.1 a	84.3 bc
Blended Proteins[2]			
WPC/PF	0	68.9 hi	66.8 hi
	0.10	79.4 cde	66.8 hi
	0.15	71.7 f-i	65.5 i
	0.20	70.0 ghi	55.3 j
SC/PF	0	76.5 d-g	71.2 ghi
	0.10	78.9 c-f	70.7 ghi
	0.15	75.1 efg	73.0 e-h
	0.20	73.1 e-h	71.9 f-i

[1]Supernatant protein following centrifugation at force indicated; Expressed as % of total protein. Means of triplicate trials

[2]Blends formulated at a level of 40% peanut protein relative to total protein

Means followed by the same letter are not different ($p<0.05$)

WPC/PF blends while that of WPC dispersions decreased (Table 4). MS supernatant protein of SC and SC/PF blends was not significantly affected by carrageenan addition. Increased centrifugation force from MS to US resulted in a decrease in the amount of supernatant protein for all carrageenan/protein systems. This may suggest the presence of protein containing aggregates which were not sedimented at 40,000 × g but which sedimented at 200,000 × g. Increased levels of carrageenan generally resulted in lowered US supernatant protein for the PF, WPC and WPC/PF systems. Carrageen addition did not affect the level of US supernatant protein in SC and in SC/PF dispersions.

The relationships of shear rate and viscosity as affected by carrageen addition for PF, SC, WPC/PF and SC/PF systems are illustrated in Fig. 1. The WPC investigated was highly reactive with

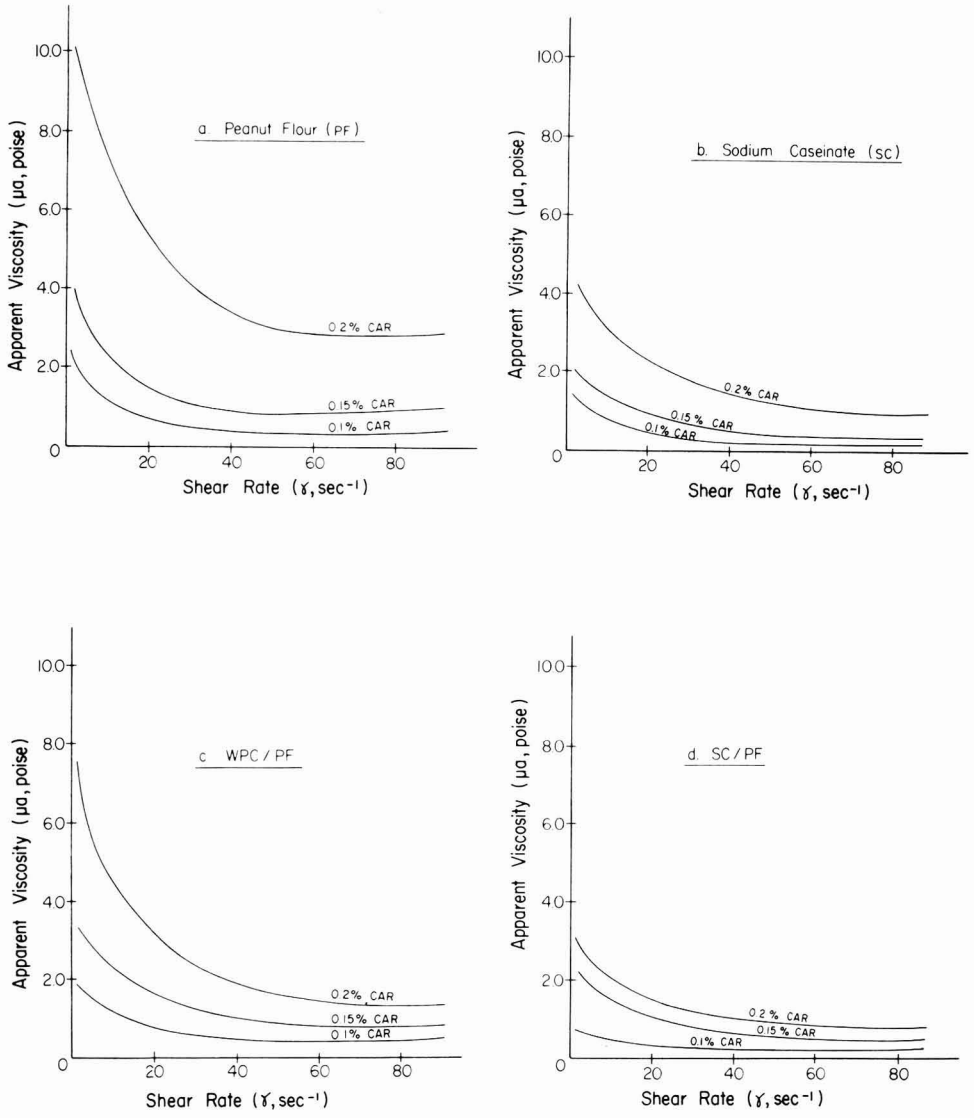

FIG. 1. EFFECT OF κ-CARRAGEENAN ADDITION ON THE RELATIONSHIP OF APPARENT VISCOSITY (μa) TO SHEAR RATE (s^{-1}) AT 25°C (BROOKFIELD RVT VISCOMETER AND SPINDLE #1) FOR 7.0% PROTEIN DISPERSIONS DIALYZED AGAINST 11.0% RECONSTITUTED NONFAT DRY MILK

a) peanut flour (PF), b) sodium caseinate (SC), c) whey protein concentrate (WPC)/PF blend and d) SC/PF blend (blends formulated at 40% peanut protein.)

Table 5. Flow behavior index (n) and consistency index (k) at 25°C for peanut flour (PF), sodium caseinate (SC), a whey protein concentrate (WPC)/PF blend and a SC/PF blend formulated at 7.0% protein, dialyzed against reconstituted nonfat dry milk, mixed with carrageenan and heated at 80°C for 1 h.

Protein System	Carrageenan (%)	Flow Behavior Index[1] (n)	Consistency Index[2] (k)
Peanut flour (PF)	0.10	0.43	3.12
	0.15	0.40	5.51
	0.20	0.38	13.30
Sodium caseinate (SC)	0.10	0.93	0.67
	0.15	0.68	3.17
	0.20	0.58	4.87
Blended Proteins[3]			
WPC/PF	0.10	0.56	3.41
	0.15	0.46	7.48
	0.20	0.40	21.82
SC/PF	0.10	0.67	2.12
	0.15	0.53	8.48
	0.20	0.47	10.53

[1]Calculated according to power law equation from data collected at 25°C with Brookfield RVT viscometer, spindle #1
[2]Apparent viscosity (μa) at shear rate of unity
[3]Blends formulated at a level of 40% peanut protein relative to total protein

carrageenan in that extremely firm gels formed upon heating. Therefore, accurate viscosity data could not be obtained for the WPC/carrageenan dispersions with the viscometer and spindle used. Flow behavior of the heated protein/carrageenan systems was generally characterized as pseudoplastic (or shear thinning). This is further indicated from flow behavior indices (n) of less than unity as shown in Table 5. Pseudoplastic behavior would be expected in highly solvated systems such as these. The shear-thinning behavior results since solvated layers are progressively sheared away with shearing resulting in apparent viscosity decreases (Holdsworth 1971). The n value data generally decreased (or pseudoplasticity increased) and k value data generally increased with increased carrageenan levels for all protein dispersions. The highest n value and lowest k value was observed in SC dispersions formulated at lower carrageenan levels. These dispersions were, in fact, almost Newtonian in behavior (n value of unity). Similar flow behavior and low viscosity has been previously observed for caseinate dispersions (Hermansson 1975). Increased levels of carrageenan dramatically decreased the n value for the SC system.

Highest k value data were obtained for the WPC/PF blend (at 40% peanut protein). The consistency of this blend was also most affected by increased carrageenan probably reflecting the reactivity of whey

proteins with carrageenan. k value data for the SC/PF blend follow a trend similar to PF alone rather than SC alone with respect to level of carrageenan used. Considerably higher k values were obtained for the SC/PF blend formulated with carrageenan than were observed for similarly formulated SC.

Data presented indicates that fractionation or modification of peanut flour may be necessary for optimal utilization as blended dairy ingredients or in fortification of milk. The calcium-protein-carrageenan complex using peanut protein or peanut/milk protein blends may find application in food systems where higher viscosity is desired. However, more definitive investigations are needed to provide more understanding of the interactions involved and for optimization of formulations to a desired food application.

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EFFECT OF SINE WAVE TEMPERATURE CYCLING ON THIAMIN LOSS IN FORTIFIED PASTA

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ABSTRACT

The loss of thiamin in fortified pasta was studied under steady state conditions of 30°, 37° and 45°C. Using this data and the Arrhenius relationship, the reaction kinetics for each temperature were determined and then used to predict the loss of thiamin in pasta stored under a sine wave fluctuating temperature condition (25°/45°C). The predicted values were compared to actual losses found in pasta stored in the sine wave condition. Three statistical methods were utilized to specify the confidence in the predicted values. The Hicks-Schwimmer-Labuza model was found to be adequate for the prediction of the amount of loss from steady state data (20-25% error).

INTRODUCTION

The American National Nutrition program has recognized pasta as one of the cheapest means for improving the diet in developed countries and for minimizing the problem of hunger in the underdeveloped countries (Antognelli 1980). Pasta is relatively easy to prepare, palatable, versatile, nutritious and has a long shelf life; these properties should ensure that pasta and pasta products will continue to be of primary importance as world demand for cereal derivatives increases (Antognelli 1980).

The property of a long shelf life is generally accepted for pasta. Though pasta may be organoleptically acceptable for long periods of time, nutrient losses can occur, thereby reducing the nutritional quality of the pasta.

In enriched flour, thiamin and riboflavin are the most unstable of the water soluble vitamins. These nutrients can undergo deterioration during processing and distribution because of the chemical activity generated by the variable temperature and humidity conditions en-

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countered. Kamman *et al.* (1981) have demonstrated the effect of temperature and a_w variations on the loss of riboflavin and thiamin in pasta. Further knowledge of stability of these nutrients is important because of the long term storage of most pasta products.

The prediction of nutrient losses in foods during storage requires adequate knowledge of the reaction kinetics. Labuza (1972) has demonstrated the application of chemical kinetics to the study of nutrient losses in dehydrated foods. Most recently, Kamman *et al.* (1981) and prior to this others (Farrer 1955; Feliciotti and Esselen 1956) have shown the loss of thiamin in pasta due to temperature effects could be predicted by the first order reaction which mathematically follows:

$$ln \frac{A}{A_o} = -k\theta \quad (1)$$

where: A = concentration at time θ; A_o = initial concentration; k = rate constant which depends upon temperature and water activity; and θ = time. The activation energies (E_A) derived from the Arrhenius relationship for loss of thiamin were approximately 20-30 kcal/mole.

In using steady state equations, it is assumed that the Arrhenius relationship is followed at all storage temperatures. Unfortunately, deviations in the Arrhenius approach can occur. Pope (1980) has reviewed some of the reasons for observed deviations from the Arrhenius relationship in shelf life testing of pharmaceuticals. This deviation is due in part to factors which affect the rate of reaction other than temperature itself when the temperature is raised in accelerated shelf life testing. Several of these factors can also be observed in food systems such as mechanistic effects, phase changes, solubility and a_w changes. These factors have been reviewed by Labuza and Riboh (1982) and can contribute to deviations in the Arrhenius relationship by introducing error in the reaction rates at higher temperatures. This in turn will lead to error in predicting the stability of products stored at lower temperatures, either over- or underpredicting.

Traditionally, the storage stability of products has been tested by selected steady state conditions. This research has not been applied to normal processing and distribution where unknown fluctuating conditions are usually encountered. Using a controlled fluctuating storage condition such as a sine wave or square wave cycle, the environmental conditions encountered during processing and distribution can be more closely simulated.

The original analysis of the effect of periodic temperature fluctuation on a zero order reaction rate was proposed by Hicks (1944). Schwimmer *et al.* (1955) extended Hicks' theory to zero order systems which had undergone sine, square or spike wave fluctuation. Labuza (1979) presented a corrected solution for a first order reaction based on the original Hicks-Schwimmer theory. These equations show that the amount of loss in foods subjected to regular temperature fluctuations would have a greater extent of loss than would be predicted from the mean temperature rate constant. Kamman *et al.* (1981) and others (Labuza and Saltmarch 1982; Labuza *et al.* 1982) have used dehydrated food systems to demonstrate the ability of these equations to predict actual nutrient losses during a square wave fluctuating temperature condition.

The purpose of this study was to test the predictive ability of the equations describing losses during a fluctuating temperature sine wave sequence by comparing calculated loss to actual loss. A fortified pasta system was utilized in this study with loss of thiamin being the mode of deterioration. Also, three methods of statistical analysis were utilized to analyze the kinetic data, and results of these three analyses were compared.

MATERIALS AND METHODS

Test System

The fortified pasta utilized in this study was prepared from unenriched semolina (International Multifoods Corp., Minneapolis, MN) with thiamin mononitrate (Hoffmann-LaRoche, Inc., Nutley, NJ) added at a level of 20 mg/100 g pasta. The noodles were made on a custom built extruder, cut to approximately 25 mm and dried in an Environet drier to 10% moisture content (wet basis). The noodles were then equilibrated over a saturated $MgNO_3$ slurry until an a_w of 0.49 was reached. Approximately 10 g of the humidified noodles were vacuum packaged into retort pouches to prevent moisture loss.

Thiamin Measurement

Thiamin was measured using an Auto Analyzer II system (Technicon, Tarrytown, NY) which automated the approved AOAC method (Pelletier and Madere 1975). The extraction procedure of Kamman *et al.* (1980) was used. Duplicate analyses of eight samples were analyzed to determine the initial thiamin values of the pasta and the

variability in the measurements. Triplicate samples were analyzed at each test time.

Storage Conditions—Steady/Unsteady State

The samples were stored at isothermal conditions of 30°, 37° and 45°C. Triplicate samples were removed from the 30° and 37° conditions every 4 weeks whereas triplicate samples from the 45°C condition were removed every 3 weeks for a total of 300 days. Samples were also stored in a fluctuating sine wave condition which was programmed to cycle between 25° and 45°C over a 24 h period for a total of 300 days.

To determine the confidence interval of the rate constants as well as the Arrhenius plot, three statistical approaches were utilized. First, the rate constant at each temperature was determined by applying the standard linear regression method on the data. Linear regression was then used on *ln* k versus 1/T to get E_A, the activation energy. The second method was to use the 95% confidence limits of each k (3 temperatures) and run a linear regression of the 6 points to get a measure of confidence of E_A from the *ln* k versus 1/T plot. This method was only used to estimate E_A. Thirdly, each data point was considered an independent experiment; extrapolating back to zero time gave a k_θ value. The population of k_θ's was used to get an average and the 95% confidence limits. This is called the point-by-point method. All the k_θ's were then used to also get an estimate of the 95% confidence limits of the *ln* $k_\theta$versus 1/T plot. A detailed comparison of these statistical methods had been presented by Labuza and Kamman (1982).

RESULTS AND DISCUSSION

Results of this study show, as demonstrated by Kamman *et al.* (1981), that the stability of thiamin in pasta is affected by temperature. Figure 1 presents the semilog plot of the thiamin retention at the constant temperature conditions showing a first order reaction. The rate constants and half-lives for loss of thiamin are shown in Table 1. As can be seen, at the higher temperatures, the rate constants determined by the two methods are similar, though the point-by-point analysis method gave larger 95% confidence limits for the rate constant; this suggests that the point-by-point method should not be used if there is considerable deviation of values. Poor fits of data were obtained at the lower temperatures; this could be due to two factors.

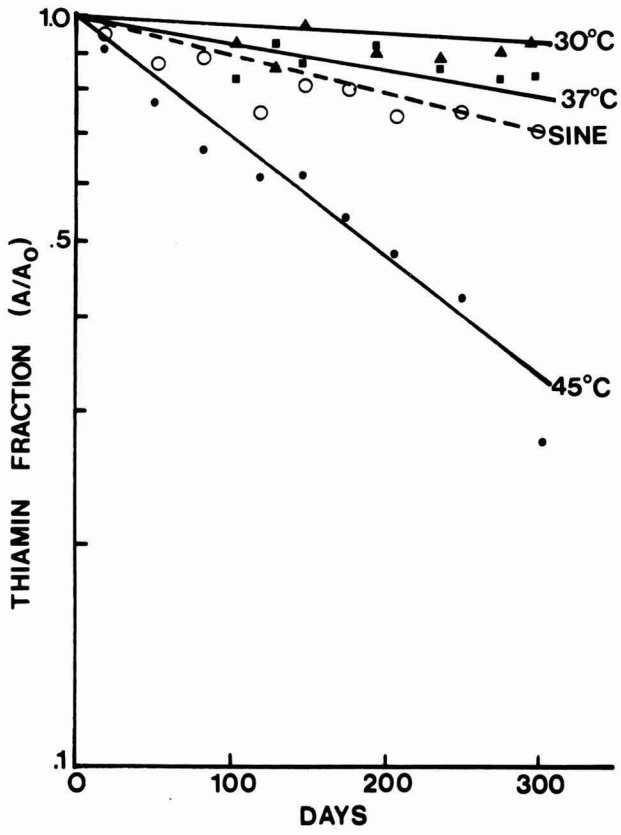

FIG. 1. COMPARISON OF LOSS OF THIAMIN UNDER CONSTANT TEMPERATURE CONDITIONS TO THE LOSS IN A SINE WAVE (25/45°C 24 H PERIOD) TEMPERATURE FLUCTUATION

Table 1. Effect of temperature on rate constant and half-life ($\theta_{1/2}$) for thiamin loss in pasta

°C	k by Linear Regression ($day^{-1} \times 10^4$)	Half Life (days)	k_θ from Point-by-Point Analysis ($day^{-1} \times 10^4$)	Half Life (days)
30	3.90 ± 2.93[a] (r^2 = .312)	1,180	6.92 ± 9.72[a]	1,000
37	8.24 ± 3.50 (r^2 = .555)	840	9.26 ± 11.03	750
45	38.22 ± 5.11 (r^2 = .911)	180	40.27 ± 19.28	170

(a) ± 95% confidence limit

First, the amount of loss measured at 30°C was less than 10% which leads to large inherent errors (Benson 1960). A second factor may be poor distribution of the thiamin in the noodle dough. In any case, these rate constants were similar to those found by Kamman *et al.* (1981). The data show that if stored at $\leq$ 37°C thiamin loss is slow, taking over two years to decrease by 50%. However, if stored at $>$ 45°C, as may occur in tropical warehouses without air-conditioning, 50% loss occurs within six months.

Table 2 lists the activation energies (E_A) calculated from the slopes of the Arrhenius plots (*ln* k versus 1/T) using the rate constants from all the statistical methods. The average values in Table 2 range from 26.4 to 34.4 kcal/mole which are fairly close to the values reported by Farrer (1955) and Feliciotti and Esselen (1956). In reviewing the three statistical methods used for calculating the activated energy, the point-by-point method gives the smallest 95% confidence interval and a reasonably low standard error. This is expected since the degrees of freedom are significantly increased in the point-by-point method which reduces the $t_{\alpha/2}$ value used in calculating the confidence interval. As reviewed by Labuza and Kamman (1982), if statistical analysis of the activation energy is to be done, the only logical method, if reasonable confidence limits are desired, is to use the point-by-point method. Most researchers use E_A values taken by regression of the three constant temperature values which is meaningless as shown since one will always get a high r^2 with a 95% confidence limit greater than the value of E_A.

The original Hicks-Schwimmer theory states the amount of loss in foods subjected to regular temperature fluctuations would show a greater extent of loss than would be predicted from steady state data.

Table 2. Arrhenius kinetic values for thiamin loss

Method	E_A (kcal/mole) ± 95% Confidence Limits	*ln* k_o ± 95% Confidence Limits	r^2	S_e[1]
1. Linear regression of average k values	29.2 ± 63.9	40.6 ± 103.8	.97	0.28
2. From end points of 95% confidence limits of k values	34.4 ± 42.2	48.8 ± 68.6	.75	0.78
3. Regression of all k_θ values from point-by-point analysis	26.4 ± 11.4	36.1 ± 18.5	.65	0.63

By taking into account the temperature dependence of reactions and correcting for the effect of a temperature on Q_{10}, Labuza (1979) has shown the ratio of loss for a first order reaction between sine wave fluctuation and constant conditions could be described by:

$$\Gamma_{sine} = 1 + \frac{(a_o b)^2}{2^2} + \frac{(a_o b)^4}{2^2 4^2} + \frac{(a_o b)^6}{2^2 4^2 6^2} + \ldots . \quad (2)$$

where: a_o = the amplitude of the sine wave fluctuation in °C; b = ln $Q_{10}/10$ as determined from the E_A value. Calculation of the Γ_{sine} value enables estimation of the effective temperature of fluctuation (T_{eff}); the constant temperature to which the fluctuating sequence was equal in rate. The effective temperature increase can be calculated by:

$$T_{eff} - T_{mean} = \Delta T_{effective} = \frac{1}{b} \; ln \; \Gamma_{sine} \quad (3)$$

For a first order reaction undergoing a sine wave temperature fluctuation, the extent of change is:

$$A = A_o e^{-k_{T_m} \Gamma_{sine} \theta} \quad (4)$$

where: k_{T_m} = rate constant at mean temperature T_m. The effective rate constant at a given sine wave fluctuation condition can be related to the rate constant at the conditions of T_m by:

$$k_{effective} = k_{T_m} \Gamma_{sine} \quad (5)$$

Kamman *et al.* (1981) examined the rates of loss of thiamin in pasta under square wave temperature fluctuations and compared them to the rates of loss predicted by square wave equations, using data generated in steady state conditions. Results of the study demonstrated that the Hicks-Schwimmer-Labuza theoretical equations gave relatively good predictions for the rates of thiamin degradation over a wide range of temperature and a_w (error of 25-35%). The data from the sine wave condition were also plotted in relationship to the steady state data in Fig. 1, and the best fit line by linear regression was drawn. These data verify what Labuza and Saltmarch (1981), Kamman *et al.* (1981) and Labuza *et al.* (1982) have shown for a square wave, that the fluctuation condition proceeds at a faster rate than the mean temperature of the fluctuation. The 25°/45° sine wave shows a greater rate of loss than the rate of loss at 37°C, which is

Table 3. Comparison of effective rate constants and shelf-life for 25% thiamin loss (θ_s) in fortified pasta subjected to a sine wave temperature fluctuation of 25/45°C

Method	$k_{Predicted\ (day^{-1} \times 4)}$	$k_{Actual \pm 95\%\ Confidence\ Interval\ (day^{-1} \times 10^4)}$	% Error[2] for k	θ_s Actual	θ_s Predicted	% Error for θ_s
1. Average value of rate constant by linear regression	12.64 range[1]—4.99 to 32.88	10.33 ± 2.85 (r^2 = .668)	22.6	279	228	18.3%
2. Rate constants from end points of 95% confidence limits	11.82 range—6.29 to 22.43	(not applicable)	—	—	—	—
3. Point-by-point analysis	13.20 range—10.89 to 16.46	16.43 ± 18.85	19.7	175	218	24.6

[1]maximum upper value—highest k_{T_m}, highest Γ_{sine}
maximum lower value—lowest k_{T_m}, lowest Γ_{sine}
[2]% error = (Δ/actual) × 100

higher than the fluctuating mean temperature of 35°C. Table 3 lists the calculated kinetic constants for the fluctuating storage conditions in comparison to the predicted $k_{effective}$ for the sine wave fluctuation. The predicted rate constants were fairly close to those actually observed for the sine wave condition if only the averages are compared. The error in the predicted rate constants was approximately 25%; this is similar to that found by Kamman *et al.* (1981). As can be seen, using the linear regression method the actual rate is smaller as compared to the predicted rate. Using the point-by-point method for analyzing the k values would result in the opposite conclusion, i.e., the equations underpredicted the actual loss by 25%. Table 3 also shows the predicted and actual shelf life for the sine wave condition which is defined as the time when 25% of the thiamin was degraded. As seen, the error (<25%) is similar to what Kamman *et al.* (1981) found for a square wave temperature fluctuation.

A factor which may be contributing to the differences between the actual and predicted values for the sine wave may be history effect on the degradation reaction between the two temperatures of fluctuation. As previously discussed, at the higher temperature of fluctuation reactions could occur producing by-products which could inhibit or catalyze the loss of thiamin at the lower temperature. For example, nonenzymatic browning could occur at the higher temperature producing compounds that would cause a faster rate of thiamin degradation at the lower temperature, thereby underpredicting the shelf life. Reactive free radicals could be formed at the higher temperature, therefore, causing a faster rate of thiamin degradation than predicted at the low temperature. Also, there could be an increase in a_w in the sealed pouches with increased temperature, thereby causing a faster rate of degradation than was predicted from the steady state data. Based on the linear regression of the actual data, it can be concluded that there is no positive history effect since the actual rate was 20% slower than was predicted. Thus, there is a negative history effect for which there is no explanation at this time. A more detailed study of the chemistry of the reaction would be needed.

Table 4 compared the predicted effective temperature of fluctuation to the measured effective fluctuation temperature as determined by the rate constants from the Arrhenius plot. The average actual and predicted values are within 1-2°C of each other and are probably not significantly different when taking into consideration that the 95% confidence limits overlap. The ranges are large, however, as previously noted with the $k_{effective}$ value. This seems to demonstrate that there is a history effect which has caused a slower rate of thiamin loss

Table 4. Comparison of effective temperatures for thiamin loss in pasta during sine wave storage

Method of Analysis		Actual[1] (°C)	Predicted (°C)
1. Average values of rate constants from regression	$T_{average}$	37.0	38.4
	T_{lower}[2]	28.8	37.8
	T_{upper}[2]	38.7	38.9
2. Point-by-point analysis	$T_{average}$	38.7	38.1
	T_{lower}	23.2	38.0
	T_{upper}	45.3	38.2

[1]mean = 35.0°C
[2]95% confidence limits

than predicted by using the Arrhenius approach. These results support the findings of Kamman *et al.* (1981) that the mean temperature is inaccurate in predicting the rate of the deteriorative reaction. In the sine wave condition the effective temperature was higher than the fluctuation mean of 35°C.

CONCLUSIONS

This study has demonstrated that the average values found from the predictive equations presented for sine wave fluctuations were reasonably good when compared to actual rates of loss. The error was about 25%. It was shown that the mean temperature of the fluctuation is an inadequate measure of expected loss. The Hicks-Schwimmer-Labuza model enables reasonable prediction of these losses utilizing steady state data generated in the laboratory without the need to conduct actual fluctuating temperature studies. As shown in this study, to obtain data suitable for kinetic analysis, it is important to have sufficient degradative loss or change to determine the steady state rate constants with some accuracy. Results of this study also seem to indicate that there is a history effect which has caused a different rate of thiamin loss than predicted by using the Arrhenius approach.

As concluded by Kamman *et al.* (1981), the loss of thiamin in pasta would be expected to be minimal during distribution and storage for periods up to one year under moderate conditions. If the product encounters fluctuating and/or high temperatures during distribution and storage for even short periods of time, reactions may occur that lead to the formation of by-products which may accelerate or decelerate the loss of thiamin at normal storage conditions.

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