CHEMICAL CHANGES
DURING FOOD PROCESSING

PT.2

VITAMINS

4.1 INTRODUCTION

Vitamins, although fairly limited in number, are a diverse group of very important and physiologically-active natural compounds. They are almost invariably destroyed to some extent during food processing, and the extent of vitamin destruction is often a critical factor in balancing the search for economies in the processing against the quality of the resulting food.

The stability of vitamins in solutions is generally influenced by the pH of the medium, the presence of air (oxygen), light, metals, and especially in foods, by a number of other factors (Table 4.1). As a rule, the water-soluble vitamins are less stable than fat-soluble vitamins and, furthermore, their losses by solution in water during operations such as washing, blanching and boiling often exceed losses due to their destruction. The most labile vitamins under conditions normally encountered in food processing are thiamine and ascorbic acid. The fat-soluble vitamins are especially unstable at high temperatures, in the presence of oxygen. Furthermore, they interact readily with the products of lipid oxidation.

Despite the huge volume of literature published on vitamin losses in foods, it is not possible to make a comprehensive review of vitamin losses in all food commodities which have undergone pre-process handling, processing operations and storage. In this chapter, therefore, the available information is given regarding the mechanisms of vitamin destruction and the products formed. Relatively less space is given to actual losses of vitamins during processing and storage of foods, especially of those foods which do not contribute significantly to the total vitamin intake in the normal diet. Additional information may be found in special monographs and review articles [1—4].

4.2 THIAMINE (VITAMIN B_1)

The heating of thiamine in water and aqueous neutral solutions of about pH 5 to 7 results in the cleavage of its methylene bridge, yielding pyrimidine and thiazole fragments, i.e. 4-amino-5-hydroxymethyl-2-methylpyrimidine and 4-methyl-5-(2-hydroxyethyl)-thiazole [5—7]. The same products arise by the action of thiaminases (p. 232). In strongly acid solutions (at pH equal to 1 or lower) substitution of the amino group gives rise to oxythiamine (Fig. 4.1) [8].

In alkaline solutions (Fig. 4.2) the breakdown is very fast and leads to a variety of reactive products, of which thiamine thiol and disulphide represent an important redox system. Thiochrome is quantitatively derived from thiamine by a one-electron oxidation [9—11]. Thiamine phosphate esters (mono-, di-

TABLE 4.1

Stability of vitamins under various conditions [1, 3]

	Conditions								
	pH								
	Acid	Neutral	Alkaline	Optimal stability	Air	Light	Reducing agents	Heat	Metal ions
Water-soluble vitamins:									
Thiamine (vitamin B_1)	+	–	–	2–3	–	+	–	–	–
Riboflavin (vitamin B_2)	+	+	–	4–6	+	–	–	–	–
Nicotinic acid (niacin) and nicotinamide* (vitamin PP, B_3)	+	+	+	6–7	+	+	+	+	–
D-pantothenic acid (vitamin B_5)	–	+	–	4–5	+	+	+	–	+
Pyridoxine (vitamin B_6)	+	+	+	2–3	+	–	+	–	–
Folacin (vitamin B_c)	–	–	+	6–7	–	–	–	–	–
Cyanocobalamin (vitamin B_{12})	–	+	–	4–5	–	–	–	+	–
Biotin (vitamin B_8 or H)	–	+	–	6–7	–	–	+	–	+
L-ascorbic and L-dehydroascorbic acid (vitamin C)	+	–	–	5–6	–	–	+	–	–
Fat-soluble vitamins:									
Retinoids (vitamin A)	–	+	–	5–7	–	–	+	–	–
Calciferols (vitamin D)	–	+	–	5–7	–	–	+	–	–
Tocopherols and tocotrienols (vitamin E)	+	+	–	5–7	–	–	+	–	–
Phylloquinone and related compounds (vitamin K)	–	+	–	5–7	+	–	–	+	+

+ = stable, – = unstable, * unstable in strong acids and alkalies

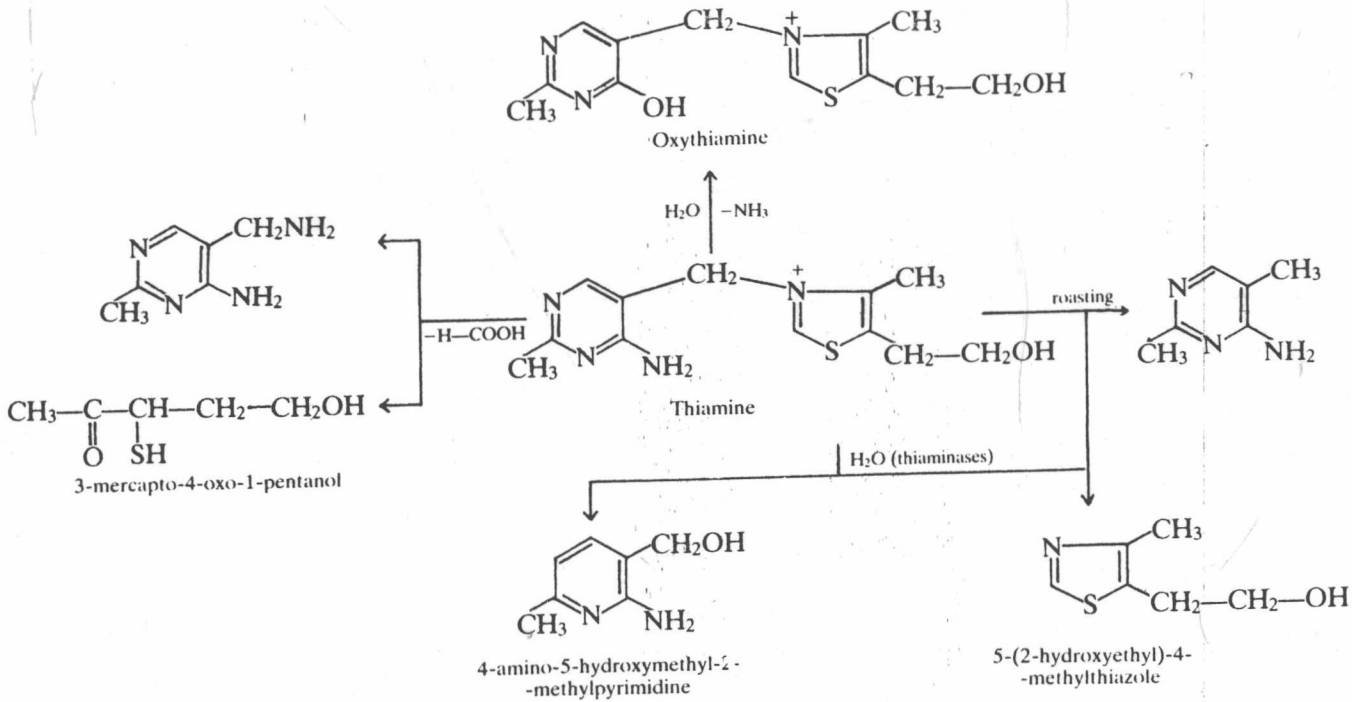


Fig. 4.1. Degradation of thiamine in neutral and acid medium and by enzymes.

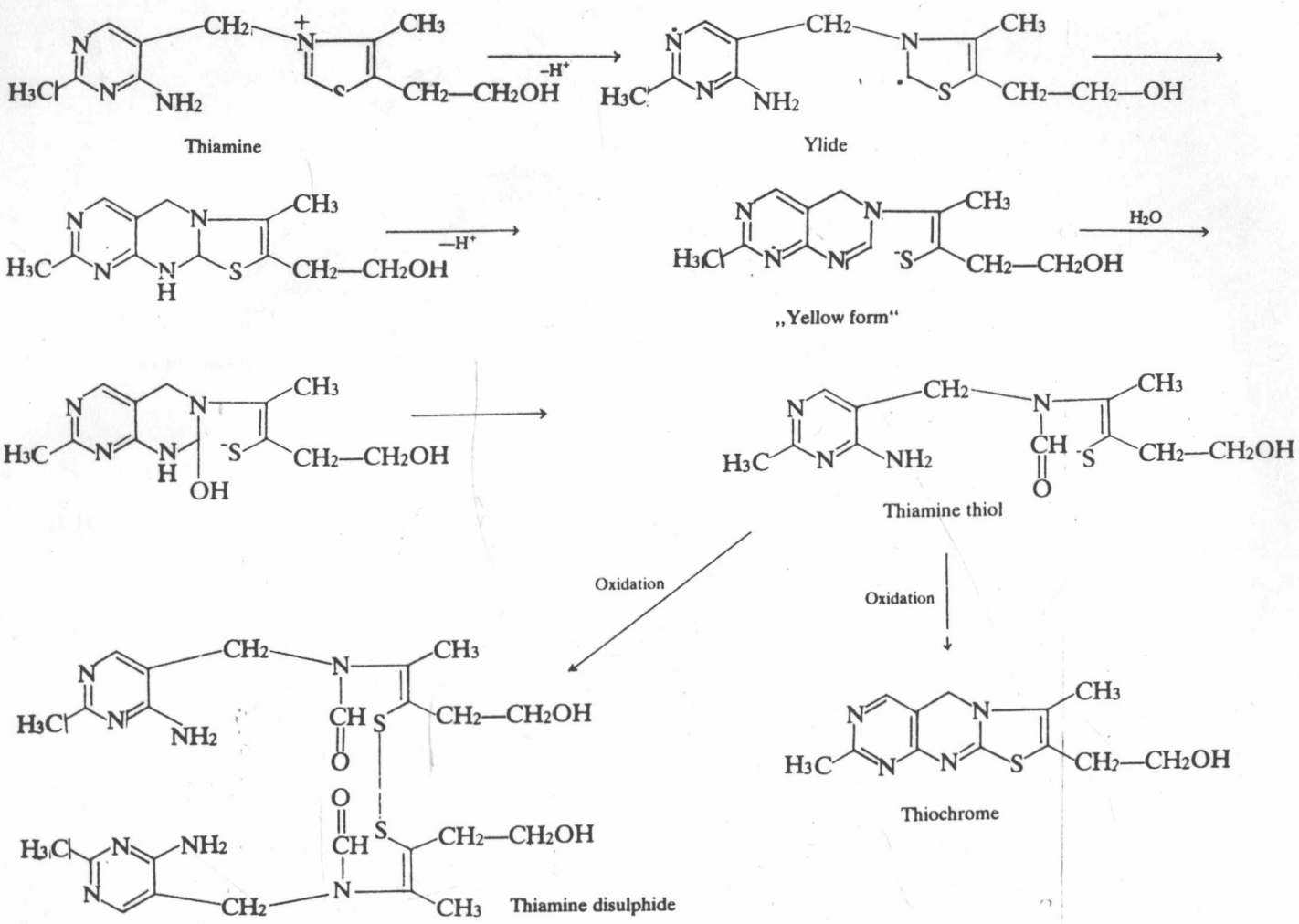


Fig. 4.2. Degradation of thiamine in alkaline medium.

and triphosphate) commonly found in foods are converted to thiochrome phosphate esters without degradation of the phosphate bond [4].

As minor degradation products 4-amino-5-aminomethyl-2-methylpyrimidine (Fig. 4.1), 3-mercapto-4-oxo-1-pentanol and formic acid [12] are formed in aqueous solutions. At neutral pH thiamine gives rise to hydrogen sulphide, 2-methyl-3-furanone, 2-methylthiophene and 2-methyl-4,5-dihydrothiophene [13], and in slightly alkaline solutions to 2,5-dimethylthiazole, 2-methyl-3-oxothiophene and 2-acetyltetrahydrothiophene [7]. Bis(2-methyl-3-furyl) disulphide, a compound with an extremely low odour threshold, was identified as the impurity of the natural thiamine isolate [14]. Sixteen new volatile degradation products of thiamine in water at 135°C (carbonyls, furans, thiophenes, thiazoles and other sulphur-containing compounds) have recently been identified [15].

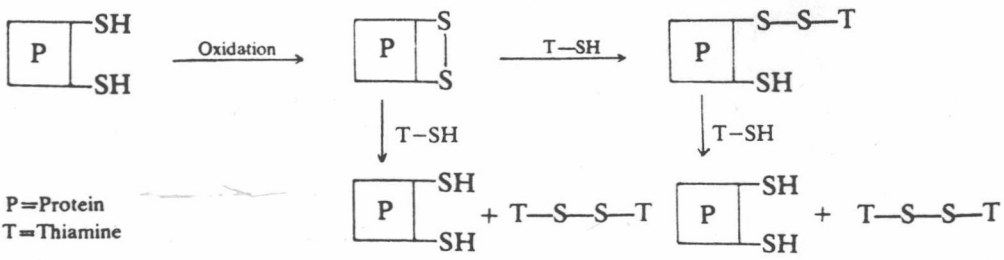
The volatile products formed by thermal degradation of thiamine again include thiophenes, thiazoles, furans and other sulphur-containing compounds. For example, during the roasting of beans thiamine yields 4-amino-2,5 dimethylpyrimidine and 4-methyl-5-(2-hydroxyethyl)-thiazole (Fig. 4.1), which in an alkaline medium (after soaking the beans in sodium bicarbonate solution), via an unstable intermediate gives hydrogen sulphide and a 5C-fragment from which sulphur-containing heterocycles are formed [16].

Compounds possessing a potent, highly characteristic odour of thiamine preparations, i.e. 1-methylbicyclo[3.3.0]-2,4-dithia-8-oxaoctane [17], 1-methylbicyclo[3.3.0.]-4-thia-2,8-dioxaoctane and 1,3-dimethylbicyclo[3.3.0]-4-thia--2,8-dioxaoctane [18], along with some other compounds have been reported to be formed upon photolysis of thiamine. The proposed pathways of the formation of some of the above mentioned volatile degradation products from thiamine via the proposed key 5C-intermediate, 3-mercapto-4-oxo-1-pentanol (Fig. 4.1) are schematically outlined in Fig. 4.3 [15, 18, 19].

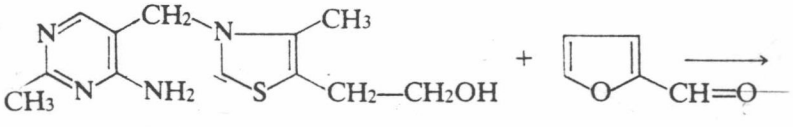
The flavour-active volatile products resulting from thiamine breakdown in solutions during thermal degradation and photolysis, may be of great importance as possible contributors to food aroma.

The presence of amino acids can influence thiamine degradation in various ways. Generally, the presence of α-and β-amino acids lowers its degradation rates. In an alkaline medium, however, glycine, alanine, valine and glutamic acid cause its desulphuration, evolution of hydrogen sulphide and the formation of desthiothiamine [20, 21] (Fig. 4.4). In the presence of glutamic acid changes of aroma during heating were demonstrated [22].

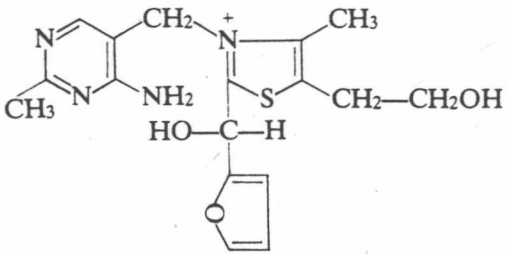
Thiamine reacts readily with cysteine and/or cystine and similarly also with proteins [23]. The reaction of cystine with thiamine thiol yields the disulphide and cysteine, which, in turn, reduces thiamine disulphide. The stabilizing effect of proteins on thiamine degradation is also based on this reaction [24, 25].



Thiamine reacts relatively easily with aldehydes [26]. In aqueous solutions at neutral pH and normal temperatures aldehydes react with ylide to form α-hydroxyalkylderivatives of thiamine. With acetaldehyde hydroxyethylthiamine [27] is formed. The degradation of thiamine in the presence of ascorbic acid (especially in an acid medium) can be thus explained by its interaction with 2-furaldehyde (p. 264):



Ylide

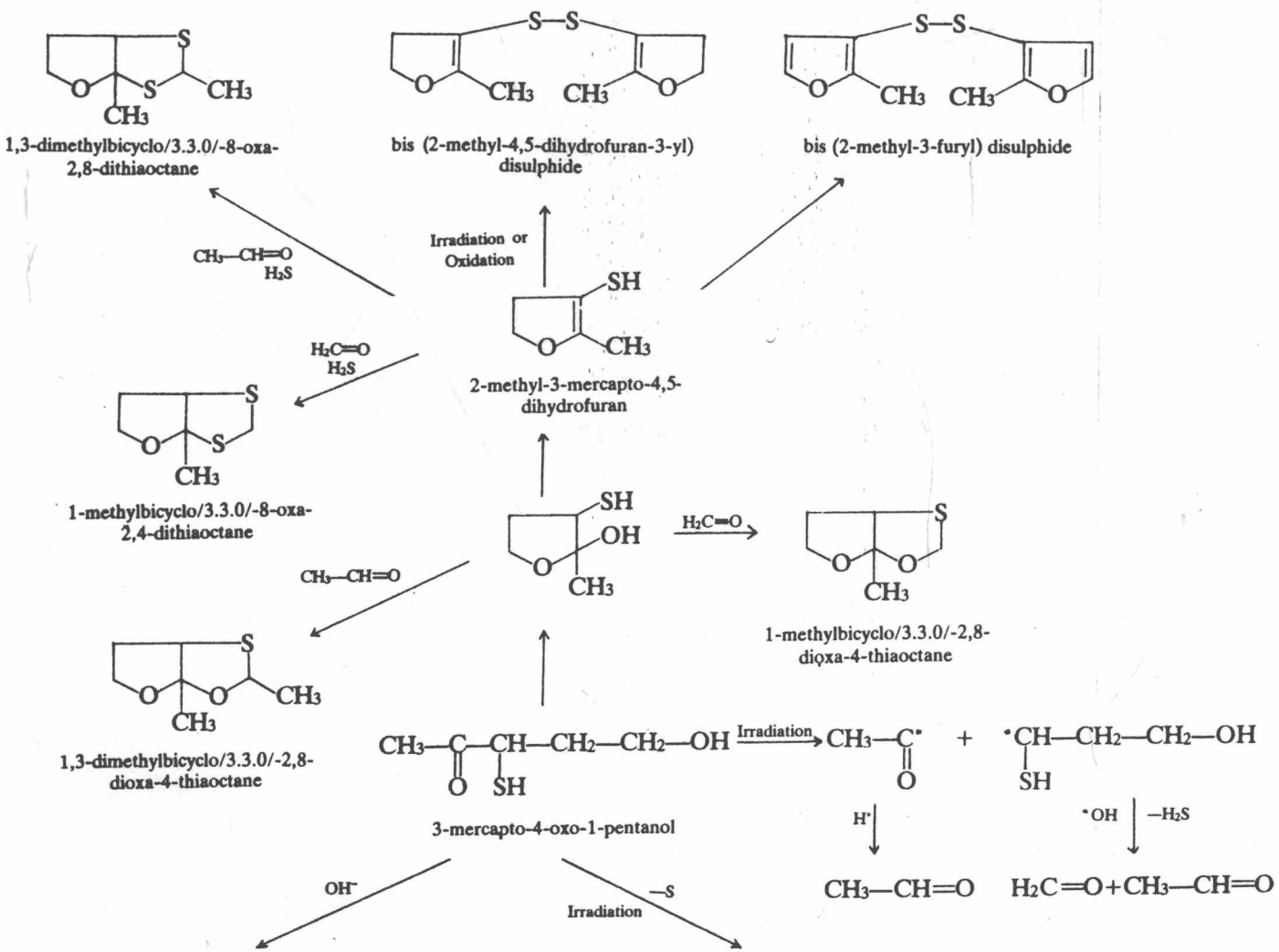


In the presence of reducing sugars, thiamine may take part in non-enzymatic browning reactions (p. 117). Reaction with glucose (in a solid state as well as in aqueous solutions at pH < 4) yields 2-glucothiamine as one of the products [28]. The rate of thiamine loss was found to depend on both the nature and the concentration of the reducing sugar present [19].

Sulphite ions added to foods as salts or formed by the reaction between sulphur dioxide and water [29]:

$$SO_2(H_2O) \rightleftharpoons HSO_3^- + H^+ \rightleftharpoons SO_3^{2-} + 2H^+,$$

rapidly react by a multi-step mechanism with thiamine [30—32] which leads to its destruction, especially in a neutral medium. The equivalent process whereby thiamine-free base reacts with bisulphite ions seems unlikely [32]. Sulphite ion first adds to the pyrimidine ring and this is followed by expulsion of the leaving group. The resultant intermediate then reacts with a second sulphite ion and expulsion of the first one leads to the final product:


1,3-dimethylbicyclo/3.3.0/-8-oxa-2,8-dithiaoctane
bis (2-methyl-4,5-dihydrofuran-3-yl) disulphide
bis (2-methyl-3-furyl) disulphide
CH3—CH=O
H2S
Irradiation or Oxidation
H2C=O
H2S
2-methyl-3-mercapto-4,5-dihydrofuran
1-methylbicyclo/3.3.0/-8-oxa-2,4-dithiaoctane
H2C=O
CH3—CH=O
1-methylbicyclo/3.3.0/-2,8-dioxa-4-thiaoctane
1,3-dimethylbicyclo/3.3.0/-2,8-dioxa-4-thiaoctane
Irradiation
3-mercapto-4-oxo-1-pentanol
H•
•OH
—H2S
CH3—CH=O
H2C=O+CH3—CH=O
OH⁻
—S
Irradiation

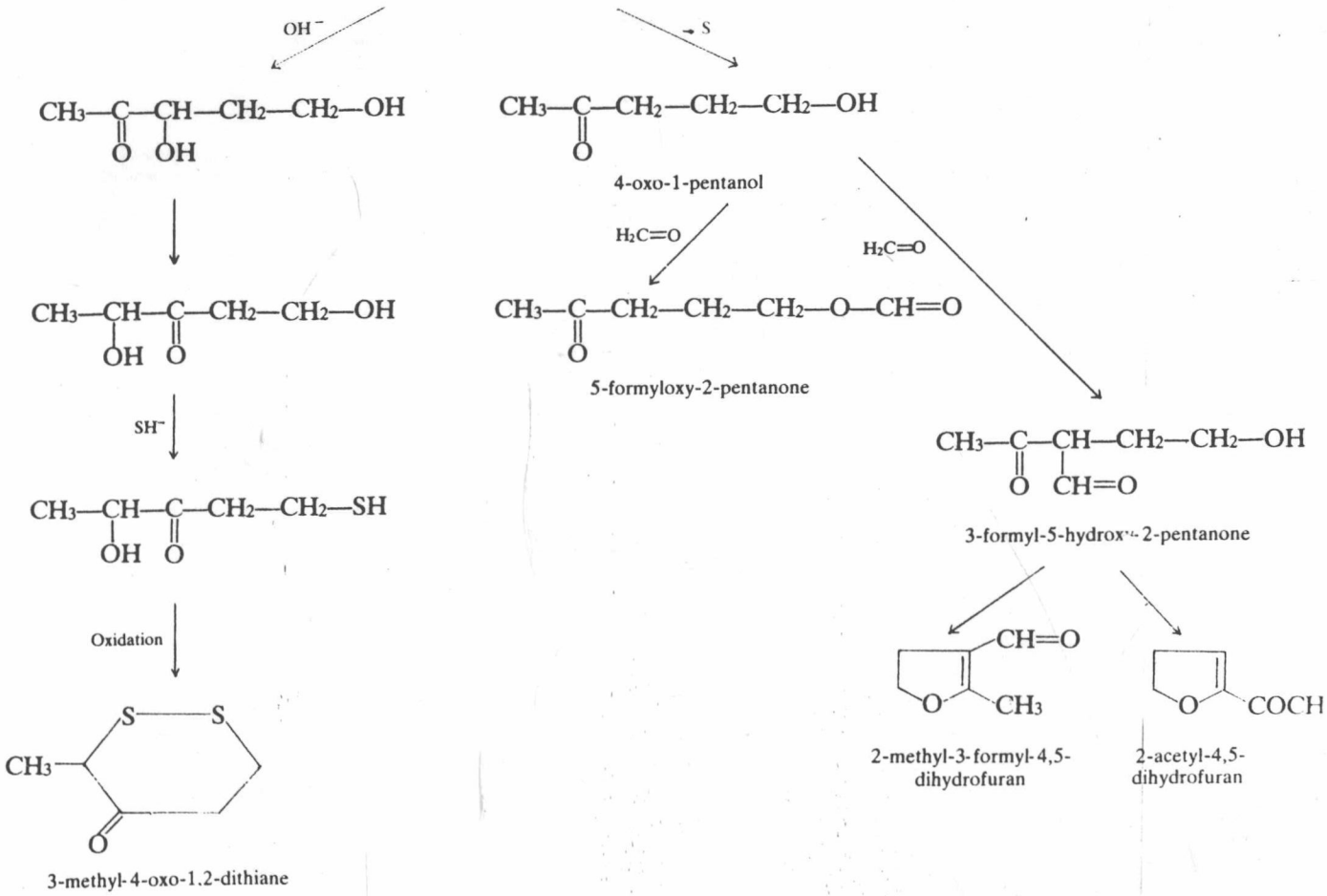


Fig. 4.3. Formation of flavour-active compounds from thiamine breakdown.

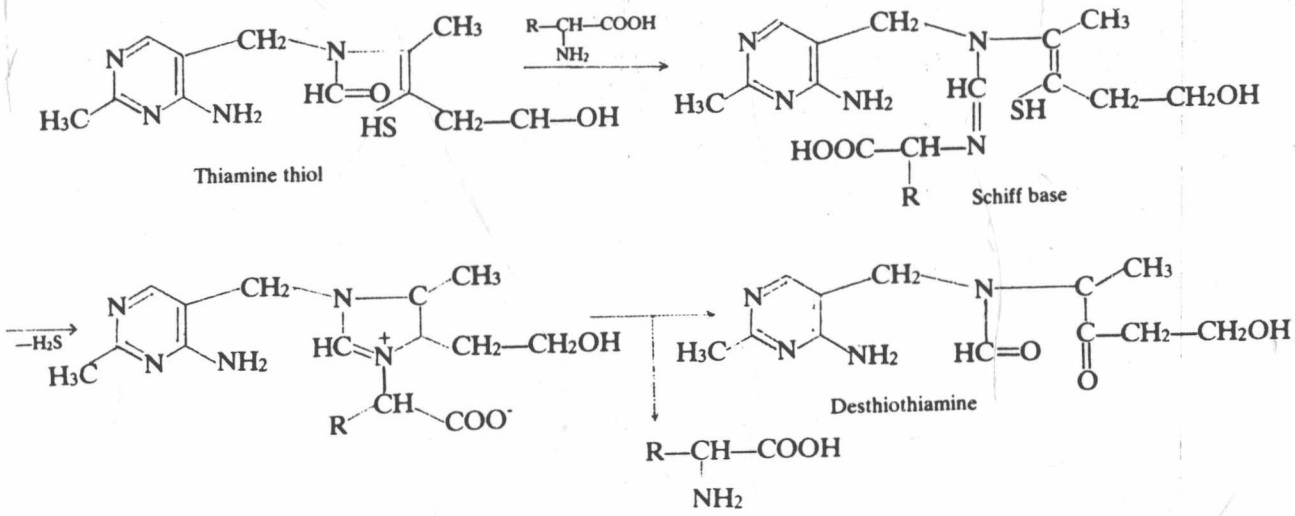
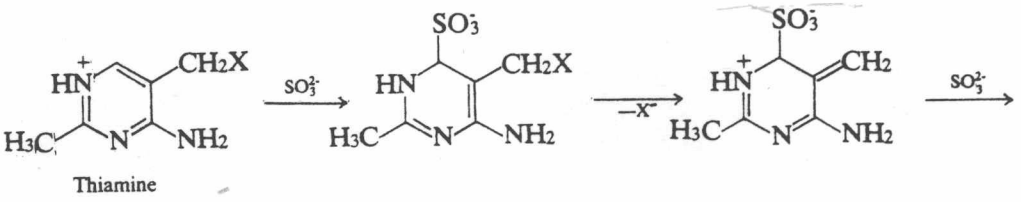
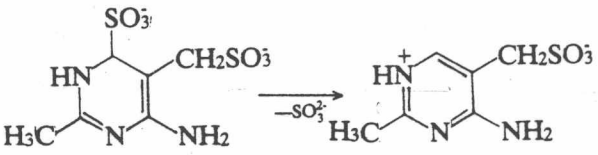


Fig. 4.4. Reaction of thiamine with amino acids.



Thiamine



(4-amino-2-methyl-5-pyrimidinyl)-methylsulphonic acid

Thiamine in natural products is generally more stable to heat than in model systems due to the stabilizing effect of proteins and other factors [4].

Thiamine present in meat, mainly in the form of diphosphate, thiol, either free or bound to protein, is partially decomposed in the course of such processes as curing, smoking, cooking, canning, dehydration, and treatment with ionizing radiation, etc. The extent of its retention ranges from about 15% in cured and smoked meat to 90% in fried meat [33]. Losses of about 30—60% on roasting, 11—50% on frying, about 60% on irradiation and 50—75% on boiling, stewing and canning appear to be related to several factors such as the size of the cut, fat and water content and the method of heat processing. During microwave heating and heating in vapour the thiamine losses were 4—14%, and 16—19% during heating by infrared radiation [34].

Pork cooked for 3 hours, for example, lost 42% of the original amount. Evidence was provided that 15% thiamine was decomposed and that substituted pyrimidine and thiazole were formed (Fig. 4.1); the remainder, i.e. 27%, was decomposed in a different manner [35, 36]. On curing, owing to the interaction with nitrites, thiamine is decomposed to elemental sulphur, thiochrome and possibly oxythiamine. Thiamine oxidation in meat is also catalyzed by the presence of heavy metals.

Refrigeration and frozen storage has only little effect on the vitamin levels in meat [34]. Vitamin retention depends, however, to a considerable extent on the temperature and other factors [37]. During one-year storage at -12 to -24° C the thiamine losses in pork loins were for instance 15—50%, in ground meat 22—23% and in salamis 13—21% [38].

Thiamine is present in milk mainly as free thiamine, diphosphate and a proportion of both these forms bound to protein. In mature cow's milk, the distribution of the vitamin between the individual forms is: 50—57% free thiamine, 18—45% phosphorylated (more labile than free thiamine) and 5—17% protein bound [39, 40]. Thiamine complexes with milk proteins containing disulphide groups have a thiamine activity of approximately 90%. During the storage of milk and its heat treatment they can decompose, so that under certain circumstances the thiamine content may increase [41].

During heat treatment of milk by pasteurization or sterilization the thiamine content of treated milk declines, depending on the temperature and period of heating [42, 43]. For instance in milk with a 4 % fat content pasteurized at 75—85°C for 16—18 seconds the thiamine content dropped to 63—68%, during pasteurization using infra-red rays the thiamine content declined only to 88% [45]. In milk pasteurized at 72°C for 16 seconds the thiamine content decreased by 18.5% [44]. Normal commercial heat treatment, including HTST and UHT treatment, result in a thiamine loss of about 10—20% [39, 46]. During the storage of full cream milk at 10°C for several days no changes in the thiamine content were found [47], and under certain circumstances the content may even increase by 3.5% [39]. Storage of UHT milk at room temperature for six weeks leads to a 10% reduction of the thiamine content (total losses are 20—25% and in some instances as much as 40%), and in sterilized milk stored at room temperature for 1—2 years there was a 20—40% thiamine loss [48, 49].

Thiamine losses occur also during evaporation and drying of milk. Losses during evaporation are in general considerable, higher than losses during heat treatment and drying [41, 50] and vary around 50%. In dried milk the thiamine content depends to a considerable extent on storage conditions, in particular on temperature and access of oxygen. As a rule thiamine retention is higher than 80% [51, 52]. Milk processing by sour fermentation generally leads to a decrease of the vitamin content due to its consumption by the growing microflora. Yoghurt, for example, contains somewhat smaller amounts of thiamine than the original milk [53]. When rod-shaped forms of lactic bacteria were used, the thiamine content decreased by 5—23%, while non-rod-shaped forms of cultures reduced the thiamine content by as much as 28—49% [54].

The application of mixed microbial cultures, mainly in the form of selected starters, decreases the loss of vitamins owing to the mutual compensation of the microflora requirements. In such cases even an increased vitamin content may be observed as a result of thiamine overproduction. Sour milk products such as yoghurt, kefir and other milk drinks containing increased levels of thiamine and other vitamins are now commercially available [55].

In cereals, thiamine, as well as the other vitamins, is not uniformly distributed throughout the kernel, but is predominantly concentrated in the outer

TABLE 4.2

Vitamin content in flours as a percentage of content in wheat [57, 58]

Vitamin	Extraction rate (%)		
	85	70	60
Thiamine	84	24	15
Riboflavin	56	35	30
Niacin	42	20	16
Pantothenic acid	67	44	37
Pyridoxine	55	26	15
Folacin	53	31	25
Biotin	44	21	11
Tocopherols	78	55	45

parts of the kernel-bran and embryo. Therefore, the content of most vitamins decreases during milling as a result of the distribution in the kernel. For example, the thiamine content of wheat is 4 $mg.kg^{-1}$, while that of flour is 0.104 $mg.kg^{-1}$ [56]. The dependence on the extraction rate of wheat flours is shown in Table 4.2. Similar losses of thiamine were found for rye flours [59]. Rice milling (i.e. dehulling) results in a loss of 76.3% of the thiamine [60]. Parboiled milled rice usually has a higher vitamin content than raw milled rice due to a lower degree of milling and to diffusion of the vitamin from the aleurone layer and germ into the endosperm during parboiling [61, 62]. Storage of milled rice results in a significant vitamin loss which is enhanced by the increasing moisture content and higher storage temperature. During 2.5-year storage at room temperature, the loss of thiamine was 29.4% [63].

TABLE 4.3

Changes of vitamin contents during conventional and continuous doughmaking ($mg.100^{-1}$ g) [65]

Production step	Thiamine	Riboflavin	Niacin
Flour	0.47	0.29	4.2
Dough, conventional: mixing	0.53	0.35	4.8
fermentation	0.59	0.30	4.3
proofing	0.48	0.29	4.2
Dough, continuous: premixing	0.60	0.37	4.9
continuous mixing	0.46	0.27	4.8
proofing	0.52	0.31	4.3

Thiamine is relatively stable during wheat bread production. Its losses average 22%, while the losses during biscuit production reached 80% [64]. Changes of thiamine levels during conventional and continuous techniques of wheat bread production are shown in Table 4.3 and Fig. 4.5. The increase of

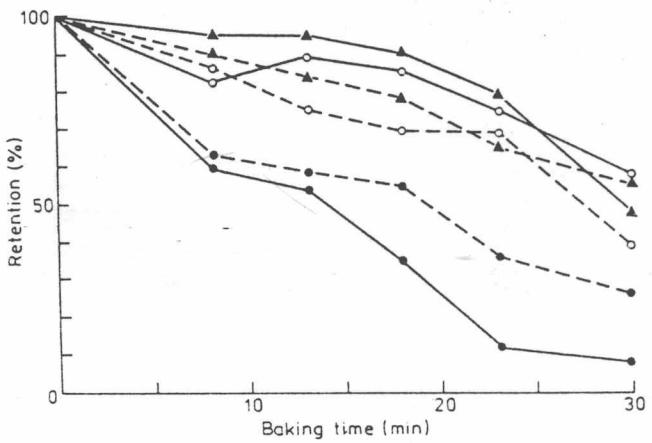


Fig. 4.5. Changes of thiamine retention with baking time of wheat bread [41]. ◗ = crust, ○ = crumb, ▲ = whole bread, ——— = conventional, — — — = continuous.

thiamine concentration in dough compared to flour results from the addition of other dough components such as yeasts, milk powder etc. A particularly low retention of thiamine is found in bread crust (7 to 26%, respectively) [65]. The losses of thiamine during bread baking depend on the extraction rate of the used flour [66]. In the baking of bread from wheat flour T 1150, 50% of thiamine was lost. In the case of flours T 700 and T 500 the losses were 35% and 17%, respectively. During baking of vitamin-enriched bread the losses are in most instances higher as compared to unfortified bread production [67]: losses of 49% of thiamine were found. A slight decrease of thiamine level in wheat bread was detected after a storage of one week and in biscuits after long-time storage [64].

Thiamine retention in cooked pasta products ranged from 54% (spaghetti) to 58% (noodles) [68]. The losses found during production and storage of spaghetti fortified with thiamine and other B-group vitamins are shown in Table 4.4. During cooking 39% thiamine were lost due to leaching and degradation [69].

TABLE 4.4

Changes of vitamin contents during production of enriched spaghetti and during storage ($mg.100^{-1}$ g) [69]

	Thiamine	Riboflavin	Niacin
Semolina	0.49	0.84	5.4
After extrusion	0.47	0.78	5.1
After drying (39°C)	0.50	0.68	5.8
After 3-month storage (darkness, 23°C)	0.52	0.64	5.8

Thiamine seems to be the most sensitive vitamin during extrusion-cooking. Increased extrusion temperature causes greater destruction of thiamine, while an increased moisture content improves its stability. Increased screw-speed has a negative effect [70—73]. Retentions from 20 to 84%, depending upon the amount of water added as well as on other variables, were found during pilot-scale extrusion of vitamin-enriched flat bread [74]. Subsequent drying of the extruded product had almost no effect.

Fruits and vegetables are primarily a source of ascorbic acid, carotenoids and phylloquinone. As a source of B-group vitamins these commodities (with some exceptions) are of only limited importance. Boiling, pressure cooking and other heat treatments of fresh vegetables may, for instance, result in thiamine loss of about 25%. Larger losses, up to 80%, may occur especially on prolonged hot-keeping and in canning [1, 4].

Levels of thiamine and some other water-soluble vitamins in barley, malt and beer are given in Table 4.5. Barley is a rich source of several vitamins, a part of which is leached out during germination and destroyed during kilning. The thiamine content of beer is low due to absorption of thiamine from

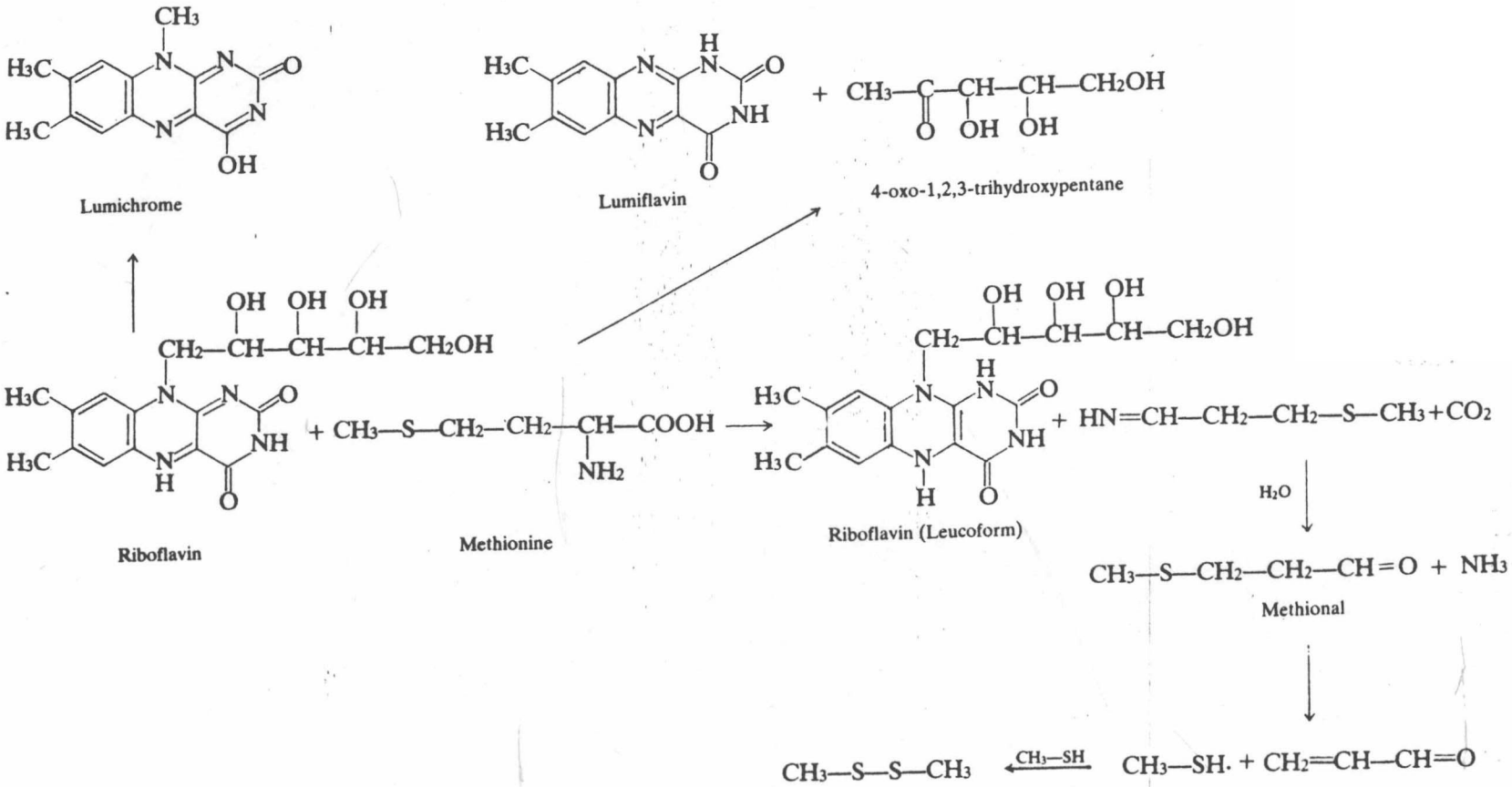


Fig. 4.6. Degradation of riboflavin.

TABLE 4.5

B-group vitamin content of barley, malt and beer [75—80]

Vitamin	Content		
	Barley ($mg.kg^{-1}$)	Malt ($mg.kg^{-1}$)	Beer ($mg.l^{-1}$)
Thiamine	1.2—7.4	1.2—8.0	0.01—0.06
Riboflavin	0.8—3.7	1.2—5.0	0.30—1.00
Niacin	80—150	90—150	4.6—14
Pantothenic acid	2.9—11.0	4.3—12.9	0.4—1.4
Pyridoxine	2.7—11.5	3.8—7.5	0.40—0.71
Cyanocobalamin	probably present	during malting	0.00009—0.00014
Biotin	0.11—0.17	0.09—0.20	0.007

fermenting wort by brewing yeasts. Because of the low content of vitamins in beer, attempts have been made to increase, at least, the content of thiamine as a preventive factor of brain damage caused by increased alcohol consumption [81—85]. Stable yeast mutants of *Saccharomyces uvarum* synthetizing and excreting thiamine from their living cells during both aerobic and anaerobic fermentation are used for the production of thiamine-rich beers [86, 87]. Mutants of *Saccharomyces cerevisiae* are suitable for the production of thiamine for pharmaceutical purposes and for the production of baker's yeasts [86].

4.3 RIBOFLAVIN (VITAMIN B_2)

Riboflavin, occurring mainly as a dinucleotide, phosphate ester (free or bound to proteins), is relatively heat stable in acid solutions. In a neutral medium it is less stable and it is rapidly destroyed in an alkaline medium. It is also stable in the presence of oxidizing agents but very sensitive to light, being converted either to lumichrome (at pH < 7) or lumiflavin (at pH > 7). The reaction of riboflavin with methionine is responsible for the so-called sunlight flavour of milk and wines [88, 89]. Riboflavin acts in this reaction as a photosensitizer and an oxidizing agent in methionine photodegradation. The primary reaction product, methional, gives rise to some other sulphur-containing compounds (Fig. 4.6). The kinetics of light-induced riboflavin degradation have been evaluated in foods such as macaroni and milk [90].

Riboflavin in meat is essentially stable to standard cooking and processing procedures, retentions higher than 90% being common [4]. Losses after heat treatment and freeze-drying of veal ranged between 14—16%, and in chicken meat 24—26% [91].

After a 1-year storage at -12 to -24°C losses of riboflavin in pork loin ranged from 10—12%, in ground meat 15—16%, and in sausages from 7 to 34% [38].

Sterilizing dosages of ionizing radiation may destroy about 25% of the vitamin [33].

Riboflavin in liquid milk is almost stable to normal technological heat treatments such as pasteurization and sterilization [27, 49, 92—94] although small losses were reported [41]. The mean retention in UHT milk, for example, was 97.6% [46], during storage for six weeks at room temperature, the losses were 10% and the total losses caused by heat treatment and storage varied between 10—15% [49]. It exhibits, however, a decrease in concentration on exposure of milk to sunlight (during subsequent refrigerated storage no further loss occurs). The sunlight induced decrease in riboflavin concentrations is followed by destruction of amino acids, especially methionine (Fig. 4.6) and oxidation of other labile compounds such as ascorbic acid and pyridoxal [94, 95]. The concentration of riboflavin in milk stored in glass or plastic containers when exposed to direct sunlight, especially light of wavelengths 490—590 nm, decreases by 20—40% in 1 hour. Light of wavelength of 400—500 nm caused a 8—14% loss of riboflavin after 1 day of exposure, the loss being 25% and 40 % after 4 and 7 days, respectively [96]. Photodegradation of riboflavin in liquid milk samples exposed to ultraviolet radiation followed first order reaction kinetics [97]. Diffuse light can result in a 10—80 % loss within a few hours [94, 98, 99].

Milk drinks produced by sour fermentation by specific microbes producing riboflavin have been described and used commercially [100].

During the drying of milk where the riboflavin losses are approximately 2 %, in some instances its content may also increase as a result of release from bound forms. Some 25- 30 % of the total amount of riboflavin in milk at refrigeration temperatures are bound to proteins [41].

The dependence of the riboflavin content on the extraction rate of wheat flours is shown in Table 4.2. Relatively little destruction occurs during baking of cereal goods. The retention of riboflavin during conventional and continuous technologies of wheat bread production (retentions for dough-making are summarized in Table 4.3) is considerable, being higher than 93% after 30 minutes of baking [65]. During the baking of riboflavin-enriched bread, losses of 29% were recorded [67]. Vitamin retention in cooked pasta products was 54% in noodles, 56% in spaghetti and 63% in macaroni [68]. Losses of about 30% due to leaching and vitamin degradation were found in spaghetti fortified with riboflavin (Table 4.4). Extrusion of flat bread fortified with the B-group vitamins had a riboflavin retention of 85%. Retention was not influenced by changes in the parameters of the process [74]. During extrusion at 154°C the retention of riboflavin in a corn/soya blend was 65—72%, while 95—100% were retained at 171°C [70, 71]. In corn grits extruded at 149 or 193°C and 13% moisture content, retention of riboflavin was complete, while at 16% moisture, retention of 53.6% was recorded [4]. A 30% vitamin retention was found in commercial soybean meal hydrolysates [101].

Losses in canned fruits and vegetables range from about 25 to 70% including leaching losses. Steaming and boiling of vegetables results in losses up to 20 and 40%, respectively. Re-heating frozen vegetables results in losses up to 20%, about two-thirds being due to leaching [4].