8 Food Additives

8.1 Foreword

A food additive is a substance (or a mixture of substances) which is added to food and is involved in its production, processing, packaging and/or storage without being a major ingredient. Additives or their degradation products generally remain in food, but in some cases they may be removed during processing. The following examples illustrate and support the use of additives to enhance the:

- *Nutritive Value of Food*
  Additives such as vitamins, minerals, amino acids and amino acid derivatives are utilized to increase the nutritive value of food. A particular diet may also require the use of thickening agents, emulsifiers, sweeteners, etc.
- *Sensory Value of Food*
  Color, odor, taste and consistency or texture, which are important for the sensory value of food, may decrease during processing and storage. Such decreases can be corrected or readjusted by additives such as pigments, aroma compounds or flavor enhancers. Development of "off-flavor", for instance, derived from fat or oil oxidation, can be suppressed by antioxidants. Food texture can be stabilized by adding minerals or polysaccharides, and by many other means.
- *Shelf Life of Food*
  The current forms of food production and distribution have increased the demand for longer shelf life. Furthermore, the world food supply situation requires preservation by avoiding deterioration as much as possible. The extension of shelf life involves protection against microbial spoilage, for example, by using antimicrobial additives and by using active agents which suppress and retard undesired chemical and physical changes in food. The latter is achieved by stabilization of pH using buffering additives or stabilization of texture with thickening or gelling agents, which are polysaccharides.
- *Practical Value*
  The common trend towards foods which are easy and quick to prepare (convenience foods) can also necessitate the increasing use of additives.

It is implicitly understood that food additives and their degradation products should be non-toxic at their recomended levels of use. This applies equally to acute and to chronic toxicity, particularly the potential carcinogenic, teratogenic (causing a malformed fetus) and mutagenic effects. It is generally recognized that additives are applied only when required for the nutritive or sensory value of food, or for its processing or handling. The use of additives is regulated by Food and Drug or Health and Welfare administrations in most countries. The regulations differ in part from country to country but there are endeavors under way to harmonize them on the basis of both current toxicological knowledge and the requirements of modern food technology. The most important groups of additives are outlined in the following sections. No reference

Table 8.1. Utilization of food additives in United States (1965 as % of total additives used)[a]

Additives, class	% of total	Additives, class	% of total
Aroma compounds	42.5	Chelating agents	2.6
Natural aroma substances	21	Colors	2.1
Nutritional fortifiers	6.9	Chemical preservatives	1.8
Surface active agents (tensides)	5	Stabilizers	1.8
Buffering substances, acids, bases	3.5	Antioxidants	1.7
		Maturing and bleaching agents	1.4
		Sweeteners	0.5
		Other additives	9.4

[a] In 1965 a total of 1696 substances (= 100%) were utilized.

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is made to legislated regulations or definitions provided therein. A compilation of the relative importance of various groups of additives is presented in Table 8.1.

8.2 Vitamins

Many food products are enriched or fortified with vitamins to adjust for processing losses or to increase the nutritive value. Such enrichment is important, particularly for fruit juices, canned vegetables, flour and bread, milk, margarine and infant food formulations. Table 8.2 provides an overview of vitamin enrichment of food.

Several vitamins have some desirable additional effects. Ascorbic acid is a dough improver, but can play a role similar to tocopherol as an antioxidant. Carotenoids and riboflavin are used as coloring pigments, while niacin improves the color stability of fresh and cured and pickled meat.

8.3 Amino Acids

The increase in the nutritive value of food by addition of essential amino acids and their derivatives is dealt with in sections 1.2.5 and 1.4.6.3.

Table 8.2. Examples of vitamin fortification of food

Vitamin	Food product
B_1	Cocoa powder and its products, beverages and concentrates, confectionary and other baked products
B_2	Baked products, beverages
B_6	Baked and pasta products
B_{12}	Beverages, etc.
Pantothenic acid	Baked products
Folic acid	Cereals (cf. 6.3.7.2)
C	Fruit drinks, desserts, dairy products, flour
A	Skim milk powder, breakfast cereals (flakes), beverage concentrates, margarine, baked products, etc.
D	Milk, milk powder, etc.
E	Various food products, e. g. margarine

8.4 Minerals

Food is usually an abundant source of minerals. Fortification is considered for iron, which is often not fully available, and for calcium, magnesium, copper and zinc. Iodization of salt is of importance in iodine deficient areas (cf. 22.2.4).

8.5 Aroma Substances

The use of aroma substances of natural or synthetic origin is of great importance (cf. Table 8.1). The aroma compounds are dealt with in detail in Chapter 5 and in individual sections covering some food commodities.

8.6 Flavor Enhancers

These are compounds that enhance the aroma of a food commodity, though they themselves have no distinct odor or taste in the concentrations used. An enhancer's effect is apparent to the senses as “feeling”, “volume”, “body” or “freshness” (particularly in thermally processed food) of the aroma, and also by the speed of aroma perception (“time factor potentiator”).

8.6.1 Monosodium Glutamate (MSG)

Glutamic acid was isolated by *Ritthausen* (cf. 1.2.2.2). In 1908 *Ikeda* found that MSG is the beneficial active component of the algae *Laminaria japonica*, used for a long time in Japan as a flavor improver of soup and similarly prepared food. The consumption of MSG in 1978 was 200,000 tonnes worldwide.

The taste of MSG cannot be explained by a combination of sweet, salty, sour and bitter tastes. It is, as the fifth quality, of an elementary nature. This assumption, which was made as early as 1908 by a Japanese researcher to explain the special taste called *umami*, was confirmed by the identification of a taste receptor for MSG. The sixth quality of taste is “fatty” (cf. 3.1). Indeed, MSG is one of the most important taste-bearing substances in meat (cf. 12.9) and cheese ripened

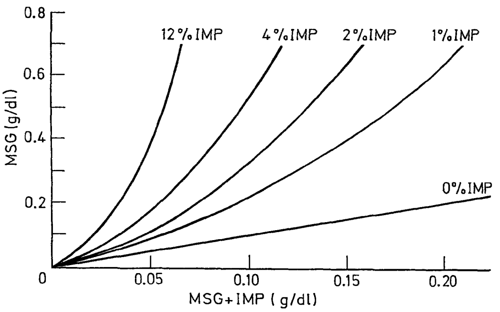


Fig. 8.1. Synergistic activities of Na-glutamate (MSG) and disodium-inosine monophosphate (IMP). The *curves* give the concentrations of MSG and MSG + IMP in water that are rated as being sensory equivalent by a taste panel

for longer periods of time (cf. 10.3.5). Reports by Japanese researchers that glutamyl peptides, e. g., Glu-Glu, also taste like MSG have not been confirmed.

The taste of MSG is intensified by certain nucleotides (Fig. 8.1). Glutamate promotes sensory perception, particularly of meat-like aroma notes, and is frequently used as an additive in frozen, dehydrated or canned fish and meat products. MSG is added in the concentration range of 0.2–0.8%.

The intake of larger amounts of MSG by some hypersensitive persons can trigger a “Chinese restaurant syndrome”, which is characterized by temporary disorders such as drowsiness, headache, stomach ache and stiffening of joints. These disappear after a short time.

8.6.2 5′-Nucleotides

5′-Inosine monophosphate (IMP, disodium salt) and 5′-guanosine monophosphate (GMP, disodium salt) have properties similar to MSG but heightened by a factor of 10–20. Their flavor enhancing ability at 75–500 ppm is good in all food (e. g. soups, sauces, canned meat or tomato juice). However, some other specific effects, besides the “MSG effect”, have been described for nucleotides. For example, they imprint a sensation of higher viscosity in liquid food. The sensation is often expressed as “freshness” or “naturalness”, the expressions “body” and “mouthfeel” being more appropriate for soups. Synergistic flavor-enhancing effects are experienced with simultaneous use of MSG and IMP or GMP (Fig. 8.1). A mixture of MSG (59 mmol) and GMP (2.75 mmol) can replace 1230 mmol of MSG.

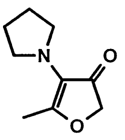
8.6.3 Maltol

Maltol (3-hydroxy-2-methyl-4-pyrone, cf. 5.3.1.2) has a caramel-like odor (melting point 162–164 °C). It enhances the perception of sweetness in carbohydrate-rich food (e. g. fruit juices, marmalades, fruit jelly). Addition of 5–75 ppm maltol allows a decrease of sugar content by about 15%, while retaining the sweetness intensity.

8.6.4 Compounds with a Cooling Effect

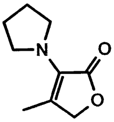
A cooling feeling in the mouth is produced by both fats (cf. 14.3.2.2.2), which melt on consumption, as well as low-molecular compounds which are capable of stimulating receptors for cold perception. Menthol is well known (cf. 5.3.2.4). Its threshold for the cooling effect is 9 µmol/kg of water. In comparison with the cooling effect, however, the retronasal threshold for the characteristic menthol odor is lower by a factor of 9.5, which is a disadvantage for the wider application of menthol.

In dark roasted malt, α-keto enamines with a cooling effect have been identified as products of the *Maillard* reaction. The odorless 5-methyl-4-(1-pyrrolidinyl)-3(2H)-furanone (Formula 8.1) was especially active. Its threshold for the cooling effect was 13.5 µmol/l, which is comparable with that of menthol. Studies on the relationship



(8.1)

between structure and effect showed that the substitution of a methylene group for the oxygen in the five-membered ring resulted in an increase in the threshold to 218 µmol/l, i. e., 16 fold. However, shifting the ring oxygen from position 4 to 5 resulted in an odourless, extremely cooling-intensive α-keto enamine (Formula 8.2) having a threshold of 0.24 µmol/l, which is 38 times lower than that of menthol.



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8.7 Sugar Substitutes

Sugar substitutes are those compounds that are used like sugars (sucrose, glucose) for sweetening, but are metabolized without the influence of insulin. Important sugar substitutes are the sugar alcohols, sorbitol, xylitol and mannitol and, to a certain extent, fructose (cf. 19.1.4.5–19.1.4.7).

8.8 Sweeteners

Sweeteners are natural or synthetic compounds which imprint a sweet sensation and possess no or negligible nutritional value ("nonnutritive sweeteners") in relation to the extent of sweetness.

There is considerable interest in new sweeteners. The rise in obesity in industrialized countries has established a trend for calorie-reduced nutrition. Also, there is an increased discussion about the safety of saccharin and cyclamate, the two sweeteners which were predominant for a long time. The search for new sweeteners is complicated by the fact that the relationship between chemical structure and sweetness perception is not yet satisfactorily resolved. In addition, the safety of suitable compounds has to be certain. Some other criteria must also be met, for example, the compound must be adequately soluble and stable over a wide pH and temperature range, have a clean sweet taste without side or post-flavor effects, and provide a sweetening effect as cost-effectively as does sucrose. At present, some new sweeteners are on the market (e. g., acesulfame and aspartame). The application of a number of other compounds will be discussed here.

The following sections describe several sweeteners, irrespective of whether they are approved, banned or are just being considered for future commercial use.

8.8.1 Sweet Taste: Structural Requirements

8.8.1.1 Structure–Activity Relationships in Sweet Compounds

A sweet taste can be derived from compounds with very different chemical structures. *Shallenberger* and *Acree* consider that for sweetness, a compound must contain a proton donor/acceptor system (AH_s/B_s-system), which has to meet some steric requirements and which can interact with a complementary receptor system (AH_r/B_r-system) by involvement of two hydrogen bridges (Fig. 8.2). The expanded model of *Kier* has an additional hydrophobic interaction with a group, X, present at a distinct position of the molecule (Fig. 8.3). The examples in Figs. 8.2 and 8.3 show that these models are applicable to many sweet compounds from highly different classes. An enlarged model substitutes a nucleophilic/electrophilic system (n_s/e_s system) for the AH_s/B_s system and an extended hydrophobic contact for the localized contact with group X.

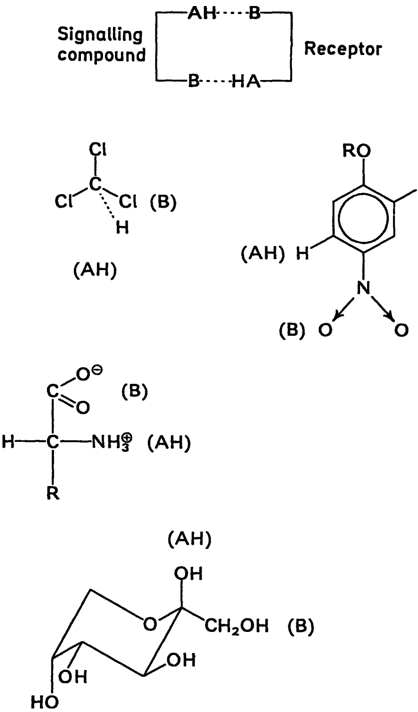


Fig. 8.2. AH/B-systems of various sweet compounds

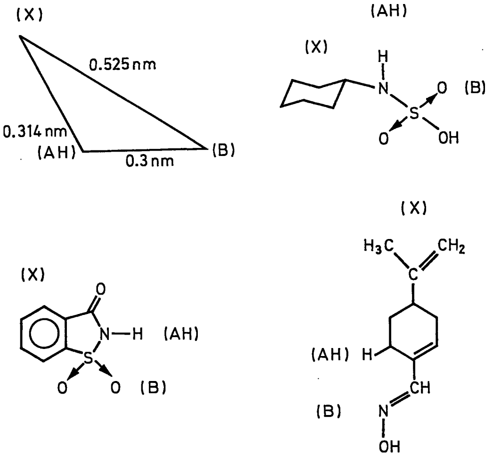


Fig. 8.3. AH/B/X-systems of various sweet compounds

Thus, a receptor for sweet compounds is to be depicted schematically as a hydrophobic pocket, containing a complementary n_r/e_r system.

It has been shown with numerous compounds that as the hydrophobicity and the space-filling properties of hydrophobic groups increase, the sweetening strength increases, passes through a maximum, and finally reaches a limit beyond which the sweet taste is either quenched or changes into a bitter taste.

According to *Nofre* and *Tinti*, even the AH/B/X system does not adequately explain the effect of hyperpotent sweeteners, e. g., guanidine (cf. 8.8.12.2). They propose a sweetness receptor which should make the large differences in the structure and sweetening strength understandable.

It is postulated that at least eight amino acid residues form the recognition sites B, AH, XH, G1, G2, G3, G4 and D in the sweetness receptor (Fig. 8.4 a). With the exception of D, two functional groups of an amino acid residue can interact with the sweet substance in each case through H-bridges, ionic relationships and *van der Waals* contacts. The last mentioned interactions involve G1–G4 (Fig. 8.4 a). The OH group of a serine or threonine residue located in the neighborhood of the phenyl ring of a phenylalanine residue is assumed for D. According to this theory, substances with weak sweetening strength, e. g., glucose (Fig. 8.4 b), make contact with only two or three amino acid residues. On the other hand, sucrose makes contact with seven, but not with D (Fig. 8.4 c). A functional group, e. g., a CN group, which accepts a H-bridge involving D and the appropriate steric orientation towards the groups G1, G2 and G4 of the receptor are characteristic of hyperpotent sweeteners, e. g., lugduname (Fig. 8.4 d), which is 230,000 times sweeter than sucrose.

The sweetening strength of a compound can be measured numerically and expressed as:

- Threshold detection value, c_{tsv} (the lowest concentration of an aqueous solution that can still be perceived as being sweet).
- Relative sweetening strength of a substance X, related to a standard substance S, which is the quotient of the concentrations c (w/w per cent or mol/l) of isosweet solutions of S and X:

$$f(c_S) = \frac{C_S}{C_X} \quad \text{for} \rightarrow c_S \text{ isosweet } c_X \qquad (8.3)$$

Saccharose in a 2.5 or 10% solution usually serves as the standard substance ($f_{sac,\,g}$). Since the sweetening strength is concentration dependent (cf. Fig. 8.5), the concentration of the reference solution must always be given ($f(c_S)$). When the sweetening strength of a substance is expressed as $f_{sac,g}(10) = 100$, this means, e. g., that the substance is 100 times sweeter than a 10% saccharose solution or a 0.1% solution of this substance is isosweet with a 10% saccharose solution.

8.8.1.2 Synergism

In mixtures of sweet tasting substances, synergistic intensification of taste occurs, i. e., the sweetness intensity is higher than the calculated value. An example is the intensification of sweetness in acesulfame–aspartame mixtures (Fig. 8.6).

8.8.2 Saccharin

Saccharin is an important sweetener ($f_{sac,\,g}(10) = 550$) and is mostly used in the form of the water-soluble Na salt, which is not so sweet ($f_{sac,\,g}(10) = 450$). At higher concentrations, this compound has a slightly metallic to bitter after-taste. The present stipulated ADI value

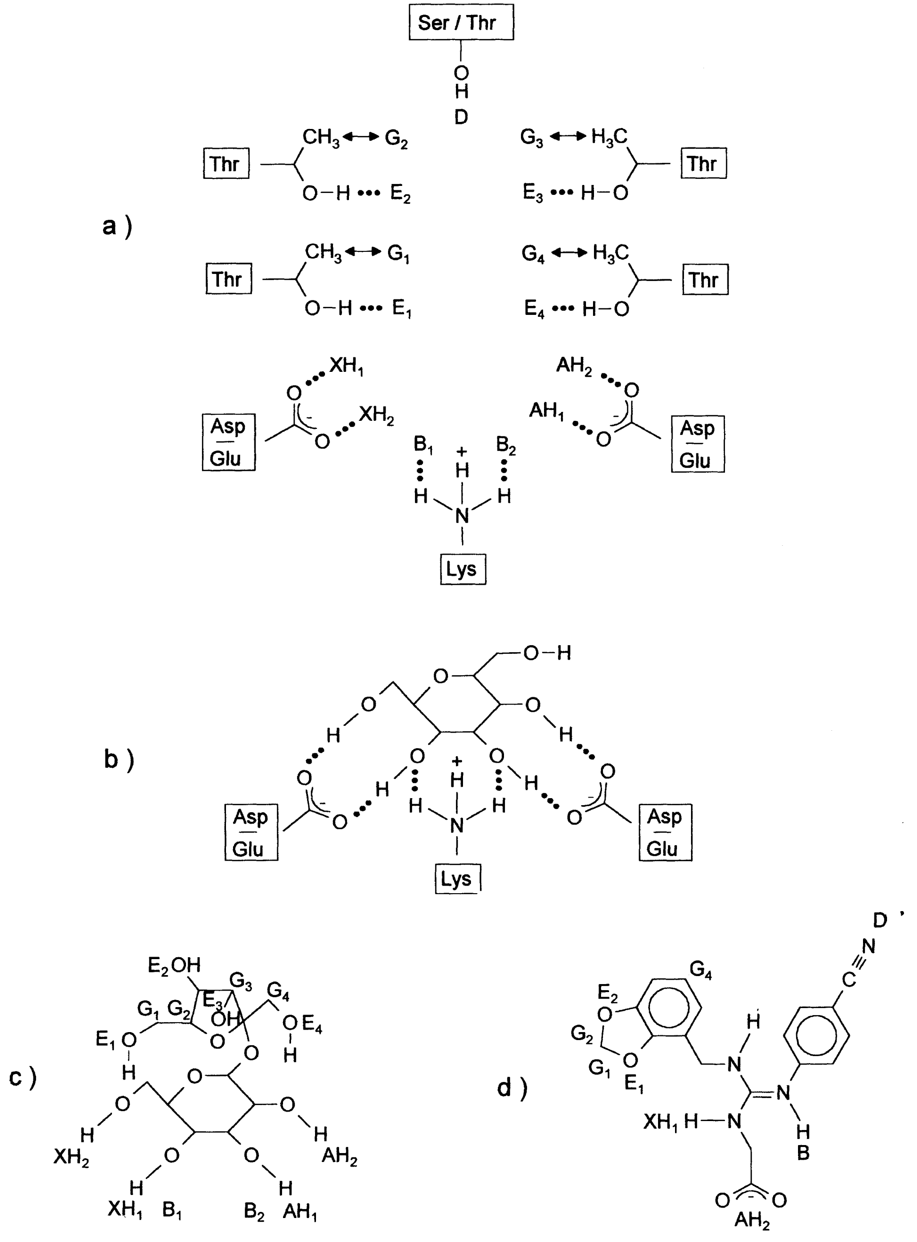


Fig. 8.4. Model of a sweetness receptor according to *Nofre* and *Tinti* (1996). **a)** Possible interactions of a sweet substance with the receptor. Interactions of the receptor with **b)** glucose, **c)** sucrose and **d)** lugduname

is 0–2.5 mg/kg of body weight. The synthesis of saccharin usually starts with toluene (*Remsen/Fahlberg* process, Formula 8.4) or sometimes with the methyl ester of anthranilic acid (*Maumee* process, Formula 8.5).

8.8.3 Cyclamate

Cyclamate is a widespread sweetener and is marketed as the Na- or Ca-salt of cyclohexane sulfamic acid. The sweetening strength is substan-

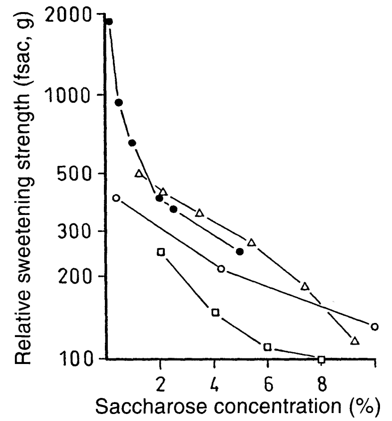
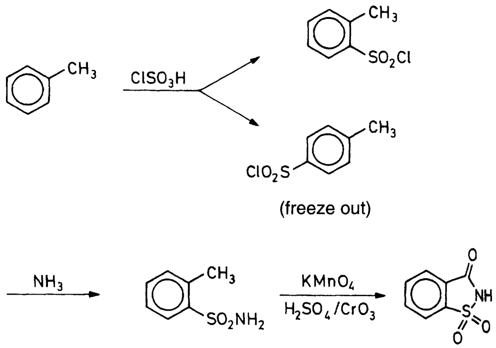


Fig. 8.5. Relative sweetening strength of some sweeteners as a function of the saccharose concentration (● neohesperidin dihydrochalcone, △ saccharin, ○ aspartame, □ acesulfame K) (according to *Bär* et al. 1990)



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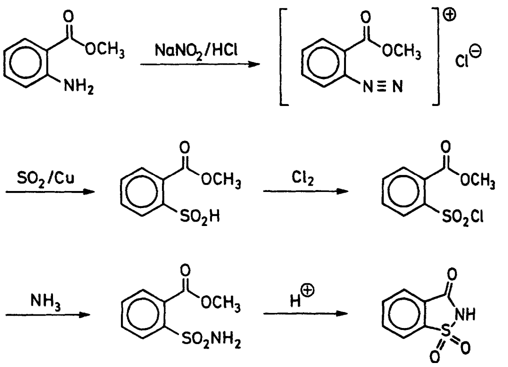

(8.5)

tially lower than that of saccharin and is $f_{\mathrm{sac,\,g}}(10) = 35$. It has no bitter after-taste. Overall, the sweet taste of cyclamate is not as pleasant as that of saccharin. The present stipulated ADI value of the acid is 0–11 mg/kg of body weight. The synthesis of the compound is based on sulfonation of cyclohexylamine:

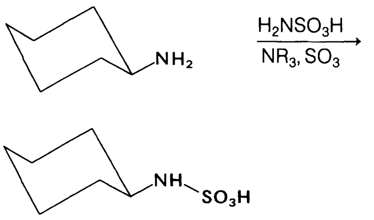

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Table 8.3 shows several homologous compounds, illustrating the dependence of sweetness intensity on cycloalkyl ring size. The larger the ring size, the higher the sweetness, i. e. the lower the sweetness threshold value.

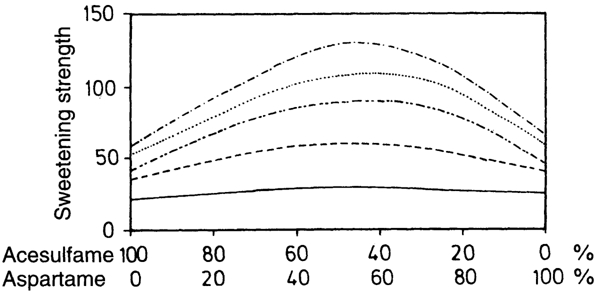


Fig. 8.6. Synergistic intensification of sweetness in acesulfame–aspartame mixtures (according to *v. Rymon Lipinski*, 1994) Ordinate: sweetening strength by comparison with a sucrose solution (g/l). Sweetener mixture: (—) 100 mg/l, (- - - - -) 200 mg/l, (— — — —) 300 mg/l, (· · · · · ·) 400 mg/l, (–·–·) 500 mg/l

Table 8.3. Taste threshold values of some alicyclic sulfamic acids (Na-salts)

R	c_{tsw} (mmol/l)	R	c_{tsw} (mmol/l)
Cyclobutyl	100	Cycloheptyl	0.5–0.7
Cyclopentyl	2–4	Cyclooctyl	0.5–0.8
Cyclohexyl	1–3		

8.8.4 Monellin

The pulp of *Dioscoreophyllum cumminsii* fruit contains monellin, a sweet protein with a molecular weight of 11.5 kdal. It consists of two peptide chains, A and B, which are not covalently bound. Their amino acid sequences are shown in Table 8.4.

The conformation is known (Fig. 8.7 and 8.8). As a result of cross reactions with an antiserum against thaumatin (cf. 8.8.5), sequence Y(13)ASD in a β-turn is regarded as the site of contact with the sweetness receptor. It corresponds to sequence Y(57)FD of thaumatin. The separated individual chains are not sweet. When the chains are recombined, a sweet taste is restored slowly, but the sweetness intensity of the original native protein is not reached. This strongly suggests that peptide chain separation results in irreversible conformational changes. However, combination of synthesized A and B chains gave a product with the same sweetening strength as natural monellin. The thermal stability of the protein was increased by covalently bonding the two peptide chains via the amino acid residues A2 and B50 (cf. Fig. 8.9). For this purpose, a synthetic gene

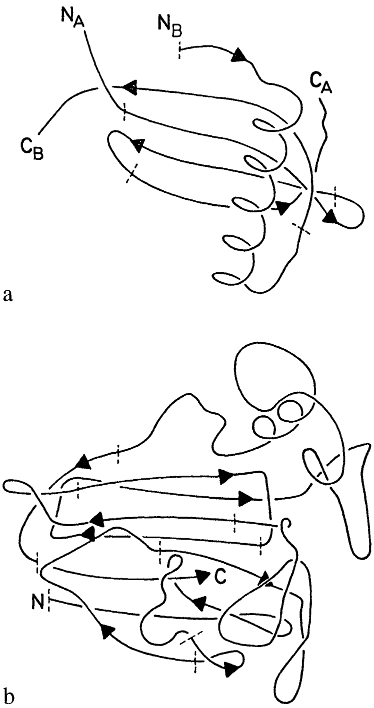


Fig. 8.7. A two dimensional representation of the conformation of the peptide chains of monellin (**a**) and thaumatin (**b**). (β-structure: →| ; α-helix: ∂; β-turn: ⊃; N, C, or N_A, N_B, C_A, C_B: N and C termini of the chains) (according to *Kim* et al., 1991)

Table 8.4. Amino acid sequences of the A and B chains of monellin. The sequence YASD shown in **bold type**, which is localized in a β-turn, is regarded as a part of the structure responsible for the cross reaction of monellin with antibodies against thaumatin as well as for making contact with the sweetness receptor (cf. Table 8.5 and Fig. 8.7 and 8.8)

						5					10					15					20
A-chain:	F[a]	R	E	I	K	G	Y	E	Y	Q	L	Y	V	**Y**	**A**	**S**	**D**	K	L	F	R
		A	D	I	S	E	D	Y	K	T	R	G	R	K	L	L	R	F	N	G	P
		V	P	P	P																
						5					10					15					20
B-chain	T[b]	G	E	W	E	I	I	D	I	G	P	F	T	Q	N	L	G	K	F	A	V
		D	E	E	N	K	I	G	Q	Y	G	R	L	T	F	N	K	V	I	R	P
		C	M	K	K	T	I	Y	E	N	E										

[a] Ca. 10% of the A chains also contain phenylalanine at the N-terminal (Phe-A-chain).

[b] Ca. 19% of the B chains also contain threonine at the N-terminal (Thr-B-chain) and N-terminal glycine is absent in ca. 24% (de-gly^1-B-chain).

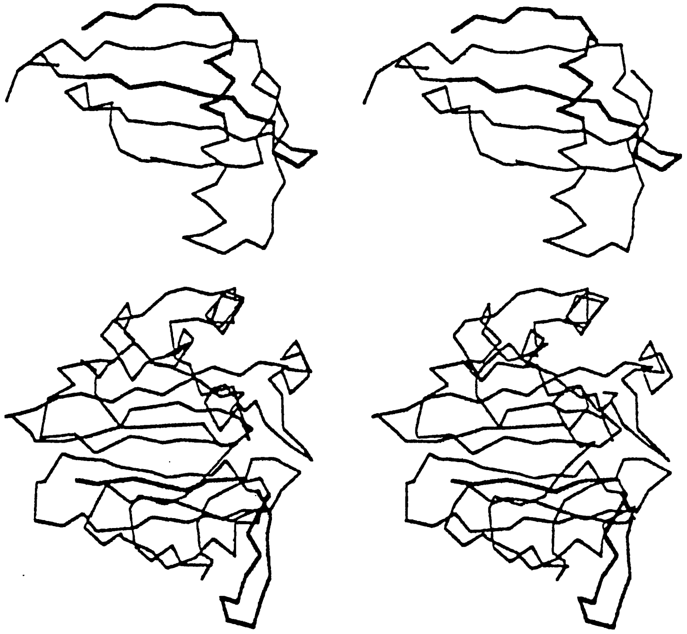


Fig. 8.8. A stereoscopic representation of the conformation of the peptide chains of monellin (*top*) and thaumatin (*bottom*) (the location of tryptic peptides that cross react with heterologous antibodies is indicated by *thicker lines*) (according to *Kim* et al., 1991)

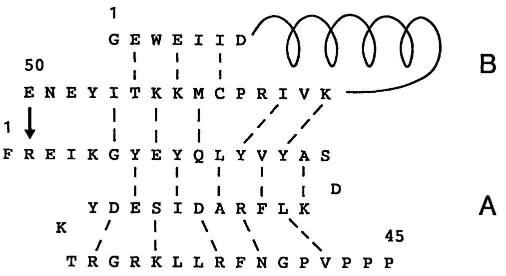


Fig. 8.9. Monellin: schematic representation of the A and B chains, showing the intra- and intermolecular hydrogen bonds (— —). Using genetic engineering techniques, the two chains were linked via a peptide bond (→) between the amino acids residues E (B50) and R (A2) (according to *Kim* et al., 1991)

was cloned and expressed in *E. coli* and yeast. The protein (I) thus obtained was as sweet as natural monellin (II). While the sweet taste of II was completely quenched at pH 2 by heating to 50 °C, I exhibited its full sweetness at room temperature even after being heated to 100 °C.

The threshold value is $f_{\text{sac, g}} = 3000$. Based on its low stability, slow triggering and slow fading away of taste perception, monellin probably will not succeed as a commercial sweetener.

8.8.5 Thaumatins

The fruit of *Thaumatococcus daniellii* contains two sweet proteins: thaumatin I and II, with $f_{\text{sac, g}} \sim 2000$. There are also low amounts of three other sweet proteins (thaumatin a, b and c). The complete amino acid sequence and the conformation (Fig. 8.7 and 8.8) of thaumatin I, a peptide chain with 207 amino acid residues, has been established (Table 8.5). As a result of cross reactions with an anti-serum against monellin (cf. 8.8.4), sequence Y(57)FD in a β-turn is regarded as the site of contact with the sweetness receptor. It corresponds to sequence Y(A13)ASD of monellin.

The sweet taste gets weaker with the increasing acetylation of the 11 ε-amino groups of the protein, being lost already with four acetyl groups. In contrast, up to 7 ε-amino groups could be modified by reductive methylation with $HCHO/NaBH_4$ without reduction in the taste intensity. The isoelectric point of the protein is obviously of importance for its activity. Thaumatin which is regarded as toxicologically safe is used, e. g., in chewing gum and milk products. Synergistic effects have been observed when thaumatin is used in combination with saccharin and acesulfame.

Table 8.5. Amino acid sequence of thaumatin I. (Disulfide bonds: 9–204, 55–66, 71–77, 121–193, 126–177, 134–145, 149–158, 159–164; the sequence YFD shown in *bold type*, which is localized in a β-turn, is regarded as a part of the structure responsible for the cross reaction of thaumatin with antibodies against monellin as well as for making contact with the sweetness receptor (cf. Table 8.4 and Fig. 8.7 and 8.8)

	5	10	15	20	25	30	35	40
	A T F E I	V N R C S	Y T V W A	A A S K G	D A A L D	A G G R Q	L N S G E	S W T I N
41	V E P G T	N G G K I	W A R T D	C **Y F D D**	S G S G I	C K T G D	C G G L L	R C K R F
81	G R P P T	T L A E F	S L N Q Y	G K D Y I	D I S N I	K G F N V	P M N F S	P T T R G
121	C R G V R	C A A D I	V G Q C P	A K L K A	P G G G C	N D A C T	V F Q T S	E Y C C T
161	T G K C G	P T E Y S	R F F K R	L C P D A	F S Y V L	D K P T T	V T C P G	S S N Y R
201	V T F C P	T A						

8.8.6 Curculin and Miraculin

Curculin is a sweet protein ($f_{\mathrm{sac,\,g}}(6.8) = 550$) of known sequence (Table 8.6). It occurs in the fruit of *Curculigo latifolia*. The sweet taste induced by this protein disappears after a few minutes, only to reappear with the same intensity on rinsing with water. It is assumed that $Ca^{2\oplus}$ and/or $Mg^{2\oplus}$ ions in the saliva suppress the sweet taste. Rinsing with citric acid (0.02 mol/l) considerably enhances the impression of sweetness ($f_{\mathrm{sac,\,g}}(12) = 970$). Thus, like miraculin, curculin acts as a taste modifier.

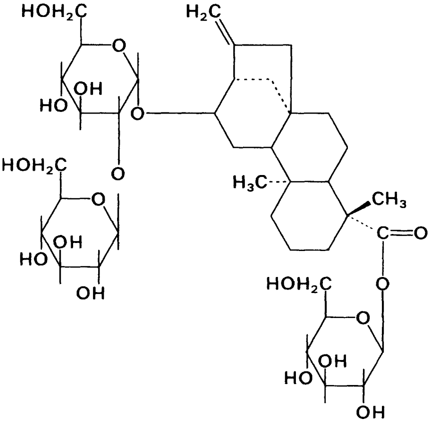
Miraculin is a glycoprotein present in the fruit of *Synsepalum dulcificum* (a tropical fruit known as miracle berry). Although it is tasteless, it has the property of giving sour solutions a sweet taste and therefore it is called a taste modifier. Thus, lemon juice seems sweet when the mouth is first rinsed with a solution of miraculin. The molecular weight of this taste modifier is 42–44 kdal.

8.8.7 *Gymnema silvestre* Extract

The extract from *Gymnema silvestre* is related to the taste modifier miraculin. It has the property of eliminating the ability to perceive sweetness for a few hours, without interfering with the perception of other taste qualities. The active substance has not yet been identified.

8.8.8 Stevioside

Leaves of *Stevia rebaudiana* contain approx. 6% stevioside ($f_{\mathrm{sac,\,g}}(4) \sim 300$). Its structure is shown in Formula 8.7. This compound is of interest as a sweetener, however its toxic properties are unclear.



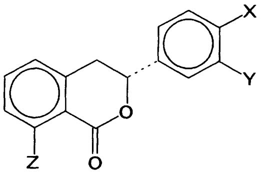
(8.7)

Table 8.6. Amino acid sequence of curculin

	5	10	15	20	25	30	35	40
	D N V L L	S G Q T L	H A D H S	L Q A G A	Y T L T I	Q N N C N	L V K Y Q	N G R Q I
41	W A S N T	D R R G S	G C R L T	L L S D G	N L V I Y	D H N N N	D V N G S	A C C G D
81	A G K Y A	L V L Q K	D G R F V	I Y G P V	L W S L G	P N G C R	R V N G	

8.8.9 Phyllodulcin

The leaves of *Hydrangea macrophylla* contain a 3,4-dihydroisocoumarin derivative, phyllodulcin (Formula 8.8). Its sweetness matches that of dihydrochalcones and of licorice root.



Z = OH, X = OMe, Y = OH (8.8)

The taste perception builds relatively slowly and also fades away slowly. The sweetening strength is $f_{\mathrm{sac}}(5) = 250$. A study of a number of related isocoumarin derivatives shows that taste quality and strength are very much dependent on the substitution pattern of the molecule (cf. Table 8.7).

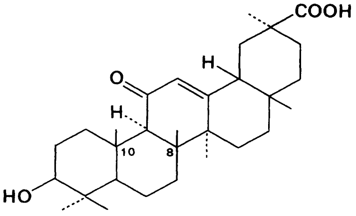
Table 8.7. Sensory properties of some 2,3-dihydroisocoumarins

Compound[a]			
X	Y	Z	Taste
OMe	OH	OH	very sweet
OMe	OMe	OH	bitter
OMe	OMe	OMe	no taste
OMe	OAc	OAc	slightly sweet
OH	OH	OH	no taste
OH	H	OH	no taste
OH	OH	H	no taste
OMe	OH	H	very sweet
OH	OMe	H	no taste

[a] Formula 8.8.

8.8.10 Glycyrrhizin

The active substance from licorice root (*Glycyrrhiza glabra*) is a β,β'-glucuronido-glucuronide of glycyrrhetic acid:

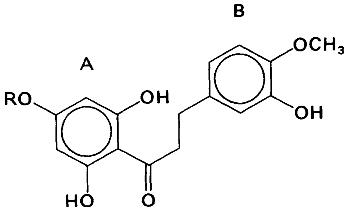


(8.9)

The sweetening strength is $f_{\mathrm{sac,\,g}}(4) = 50$. The compound is utilized for production of licorice (also spelled as liquorice). Its cortisone-like side effect limits its wide application.

8.8.11 Dihydrochalcones

Some dihydrochalcones are derived from flavanones (cf. 18.1.2.5.4) and have a relatively clean sweet taste that is slowly perceived but persists for some time. The sweetening strength of β-neohesperidin dihydrochalcone is $f_{\mathrm{sac,\,g}} = 1100$ (threshold value) or $f_{\mathrm{sac,\,g}}(10) = 667$ (R = β-neohesperidosyl in Formula 8.10).



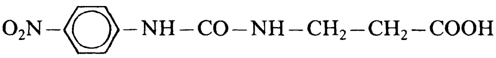
(8.10)

In different countries, this compound is used in chewing gum, mouthwashes, beverages, and various types of candy. The quality and strength of the sweet taste of dihydrochalcone are related particularly to the substitution pattern in ring B. The prerequsite for a sweet taste is the presence in ring B of at least one hydroxy group, but not three adjacent hydroxy and alkoxy substituents.

8.8.12 Ureas and Guanidines

8.8.12.1 Suosan

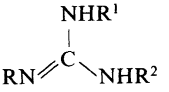
Suosan, N-[(p-nitrophenyl)carbamoyl]-β-alanine (Formula 8.11), $f_{\mathrm{sac,\,g}}(2) = 700$, is clearly sweeter than saccharin. The e/n system could be the NH/COO$^-$ system of β-alanine, which corresponds to the e/n system of aspartame (cf. 1.3.3 and 8.8.15). The p-cyanophenyl compound ($f_{\mathrm{sac,\,g}}(2) = 450$), the N-glycine homolog and the thiocarbamoyl compound are also sweet.



(8.11)

8.8.12.2 Guanidines

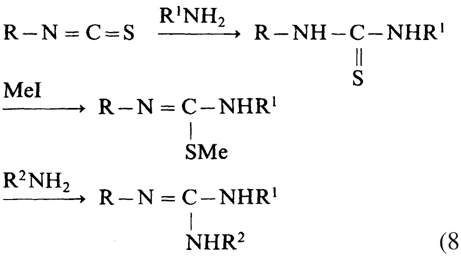
Derivatives of guanidinoacetic acid (Formula 8.12) are among the sweetest compounds known until now (Table 8.8).



$$RN{=}C(NHR^1)\text{–}NHR^2$$

(8.12)

Replacement of the carboxyl group by a tetrazole residue results in loss of sweetening strength (Table 8.8).
The guanidines can be synthesized, e. g., via the isothiocyanates:



$$R\text{–}N{=}C{=}S \xrightarrow{R^1NH_2} R\text{–}NH\text{–}C({=}S)\text{–}NHR^1 \xrightarrow{MeI} R\text{–}N{=}C(SMe)\text{–}NHR^1 \xrightarrow{R^2NH_2} R\text{–}N{=}C(NHR^2)\text{–}NHR^1$$

(8.13)

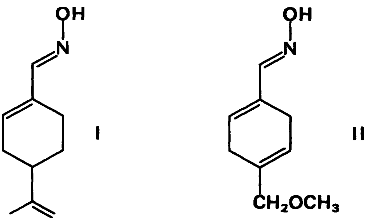
Table 8.8. Taste of some guanidines (Formula 8.12)

R	R^1	R^2	$f_{\text{sac, g}}(2)$
p-Cyanophenyl	H	Carboxy-methyl	2700
	Benzyl		30,000
	Phenylsulfonyl		45,000
	1-Naphthyl		60,000
	Cyclohexyl		12,000
	Cyclooctyl		170,000
	Cyclononyl		200,000
3,5-Dichloro-phenyl	Benzyl		80,000
	Cyclooctyl		60,000
p-Cyanophenyl	Cyclohexyl	Tetrazolyl-methyl	400[a]
	Cyclooctyl		5000[b]

[a] $f_{\text{sac, g}}(4)$.
[b] $f_{\text{sac, g}}(5)$.

8.8.13 Oximes

It has long been known that the anti-aldoxime of perillaldehyde (discovered in the essential oil of *Perilla nankinensis*) has an intensive sweet taste ($f_{\text{sac, g}} \sim 2000$). For its structure see Formula 8.14 (I).

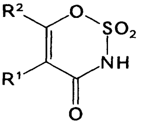


(8.14)

In the meantime, a related compound, (II), with improved solubility, has been reported, but its sweetness is just moderately high ($f_{\text{sac, g}} \sim 450$).

8.8.14 Oxathiazinone Dioxides

These compounds (Formula 8.15) belong to a class of sweeteners with an AH/B-system corresponding to that of saccharin. Based on their properties and present toxicological data, they are suitable for use. The sweetening strength depends on substituents R^1 and R^2 and is $f_{\text{ac, g}}(10) = 200$ for acesulfame K (Table 8.9).
The ADI value stipulated for the potassium salt of acesulfame is 0–9 mg/kg of body weight.



(8.15)

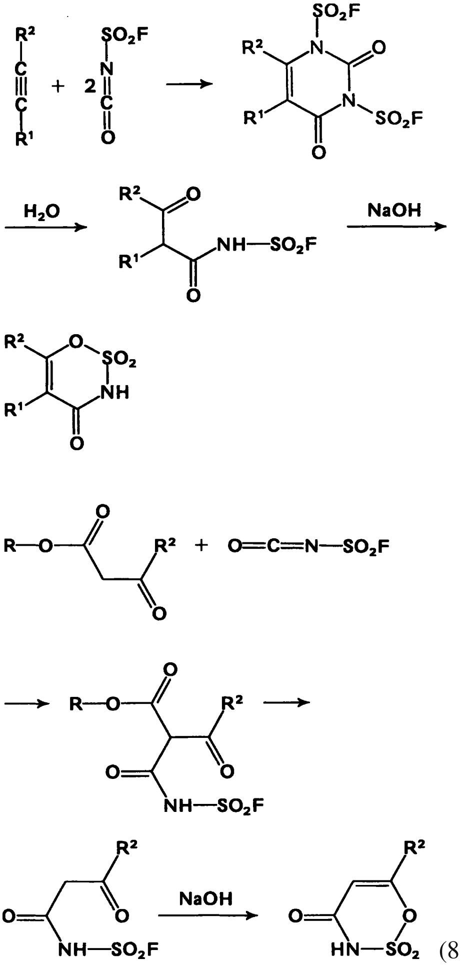
Oxathiazinone dioxides are obtained from fluorosulfonyl isocyanate and alkynes, or from com-

Table 8.9. Sweetness of some oxathiazinone dioxides (Na-salts) (Formula 8.15)

R^1	R^2	$f_{\text{sac, g}}$	R^1	R^2	$f_{\text{sac, g}}$
H	H	10	Et	H	20
H	Me	130[a]	Et	Me	250
Me	H	20	Pr	Me	30
Me	Me	130	i-Pr	Me	50
H	Et	150			

[a] Acesulfame.

pounds with active methylene groups, as exemplified by 1,3-diketones, 3-oxocarboxylic acids and 3-oxocarboxylic acid esters:



(8.16)

The sweetness of acesulfame is perceived quickly and this substance is practically stable in foods under the common processing and storage conditions. It is used in a large number of different products.

8.8.15 Dipeptide Esters and Amides

8.8.15.1 Aspartame

A dipeptide, L-aspartyl-L-phenylalanine methyl ester (L-Asp-L-Phe-OMe), has recently been approved for use as a sweetener in North America (aspartame, "NutraSweet"). It is as sweet as a number of other dipeptide esters of L-aspartic acid and D,L-aminomalonic acid. The relationship between chemical structure and taste among these compounds was outlined in more detail in 1.3.3. The sweetening strength relative to saccharose is concentration dependent (Table 8.10). Aspartame is used worldwide, though its stability is not always satisfactory. Unlike sweetening of drinks (coffee or tea) which are drunk immediately, problems arise in the use of aspartame in food which has to be heated or in sweetened drinks which have to be stored for longer periods of time. Possible degradation reactions involve α/β-rearrangement, hydrolysis

Table 8.10. Comparison of sweetening strengths of aspartame and saccharose (concentrations of isosweet aqueous solutions in %)

Saccharose	Aspartame	$f_{\text{sac, g}}$
0.34[a]	0.001[a]	340
4.3	0.02	215
10.0	0.075	133
15.0	0.15	100

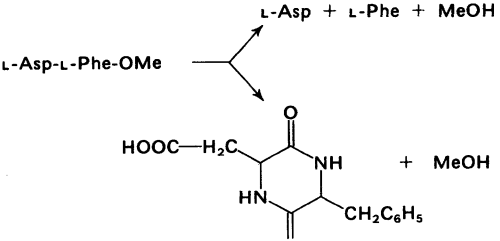
[a] Threshold value.

Table 8.11. Taste of some dipeptide amides of the type L-Asp-D-Ala-NHR

R	$f_{\text{sac, g}}(10)$
Cyclopentyl	50
Cyclohexyl	90
(2,2,5,5-tetramethyl)-cyclopentyl	800
(2,2,6,6-tetramethyl)-cyclohexyl	300
(Diethyl)-methyl	100
(Diisopropyl)-methyl	250
(Di-tert-butyl)-methyl	450
(Di-cyclopropyl)-methyl	1200
(Cyclopropyl)-(tert-butyl)-methyl	1200
(Cyclopropyl)-(methyl)-methyl	100
(2,2,4,4-Tetramethyl)-cyclobutyl	300
(2,2,4,4-Tetramethyl)-cyclobutan-3-onyl	240
(3-Hydroxy-2-2,2,4,4-tetramethyl)-cyclobutyl	125
3-(2,2,4,4-Tetramethyl)-thietanyl	2000[a]
3-(1-cis-Oxo-2,2,4,4-tetramethyl)-thietanyl	300
3-(1-trans-Oxo-2,2,4,4-tetramethyl)-thietanyl	350
3-(1,1-Dioxo-2,2,4,4-tetramethyl)-thietanyl	805

[a] Alitame.

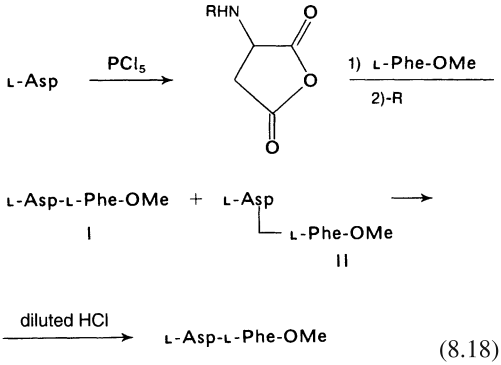
into amino acid constituents and cyclization to the 2,5-dioxopiperazine derivative:



(8.17)

The ADI values stipulated for aspartame and diketopiperazine are 0–40 mg/kg of body weight and 0–7.5 mg/kg of body weight.

Aspartame synthesis on a large scale is achieved by the following reactions:



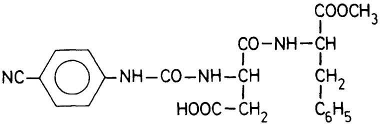
(8.18)

Separation of the two dipeptide isomers (I, II) is possible since there are solubility differences between the two isomers as a consequence of their differing isoelectric points ($IP_I > IP_{II}$).

Other possible syntheses are based on a plastein reaction (cf. 1.4.6.3.2) with an N-derivatized aspartic acid and phenylalanine methylester or on bacterial synthesis of an Asp–Phe polymer, achieved by genetic engineering techniques, enzymatic cleavage of the polymer to Asp–Phe, followed by acid or enzyme catalyzed esterification of the dipeptide with methanol.

8.8.15.2 Superaspartame

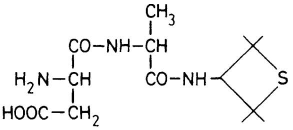
Substitution of a (p-cyanophenyl)carbamoyl residue for the free amino group of aspartame produces a compound called superaspartame (Formula 8.19), $f_{\text{sac,g}}(2) = 14{,}000$, which is sweeter than aspartame by about two powers of ten. This molecule contains structural elements of aspartame and of cyanosuosan.



(8.19)

8.8.15.3 Alitame

Amides of dipeptides consisting of L-aspartic acid and D-alanine are sweet (Table 8.11). The compound alitame is the N-3-(2,2,4,4-tetramethyl)-thietanylamide of L-Asp-D-Ala (Formula 8.20) and with $f_{\text{sac,g}}(10) = 2000$, it is a potential sweetener.



(8.20)

Since the second amino acid has a D configuration, its side chain must be small, corresponding to the structure activity relationships discussed for dipeptide esters of the aspartame type (cf. 1.3.3). On the other hand, the carbonyl group should carry the largest possible hydrophobic residue.

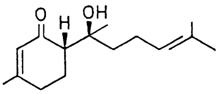
The stability of dipeptide amides of the alitame type is substantially higher than that of dipeptide esters of the aspartame type. Therefore, alitame can also be used in bread and confectionery.

Like aspartame, alitame also undergoes α/β-rearrangement. Both isomers hydrolyze slowly to give L-aspartic acid and D-alanine amide, which is excreted either directly or as the glucuronide. A small part is oxidized to sulfoxides and sulfone. Cyclization to diketopiperazine which is typical of dipeptide methylesters does not occur.

8.8.16 Hernandulcin

(+)-Hernandulcin is a sweet sesquiterpene from *Lippia dulcis Trev.* (*Verbenaceae*), with the structure 6-(1,5-dimethyl-1-hydroxy-hex-4-enyl)

-3-methyl-cyclohex-2-enone: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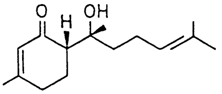
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(8.21)

In comparison with sucrose, the sweetening strength of this compound is $f_{\mathrm{sac,mol}}(0.25) = 1250$. Hernandulcin is somewhat less pleasant in taste than sucrose and exhibits some bitterness.

The racemic compound was synthesized via a directed aldol-condensation reaction by adding 6-methyl-5-hepten-2-one to a mixture of 3-methyl-2-cyclohexen-1-one and lithium diisopropylamide in tetrahydrofuran, followed by chromatographic separation of (±)-hernandulcin (I, 95%) from the diastereomeric counterpart (±)-epihernandulcin (II, 5%). Whereas I is sweet, II exhibits no sweet taste.

The carbonyl and hydroxyl groups, which are located about 0.26 nm apart in the preferred conformation, are considered as an AH/B-system. The sweet taste is lost when these groups are modified (reduction of the carbonyl group to an alcohol, or acetylation of the hydroxy group).

8.9 Food Colors

A number of natural colors are available and used to adjust or correct food discoloration or color change during processing or storage. Carotenoids (cf. 3.8.4.5) are used the most, followed by red beet pigment and brown colored caramels. The number of approved synthetic dyes is low. Table 8.12 lists the pigments of importance in food coloring. Yellow and red colors are used the most. Food products which are often colored are confections, beverages, dessert powders, cereals, ice creams and dairy products.

8.10 Acids

The acid taste is caused only by the $H^{\oplus}$ ion. The intensity depends on the potential and not on the actual $H^{\oplus}$-ion concentration, which indicates the pH. Consequently, the solution of a weak acid, which is not completely dissociated, tastes as sour as the solution of a strong acid of the same concentration. Therefore, the first step in the detection of an acid is comparable with an acid–base titration, the receptor for the sour taste functioning as the base.

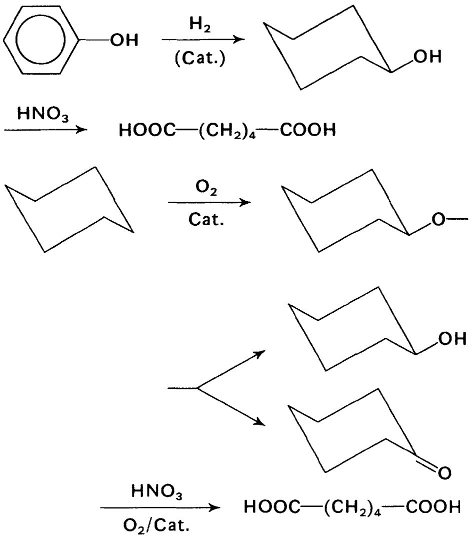
Apart from the taste effect and antimicrobial activities, acids have a number of other functions in foods. The most important acids used in food processing and storage are outlined in this section.

8.10.1 Acetic Acid and Other Fatty Acids

Acetic, propionic and sorbic acids are dealt with under antimicrobial agents (8.10). Other short chain fatty acids, such as butyric and higher homologues, are used in aroma formulations.

8.10.2 Succinic Acid

The acid ($pK_1 = 4.19$; $pK_2 = 5.63$) is applied as a plasticizer in dough making. Succinic acid monoesters with glycerol are used as emulsifiers in the baking industry. The acid is synthesized by catalytic hydrogenation of fumaric or maleic acids.



(8.22)

Table 8.12. Examples of food colorants (natural and synthetic)

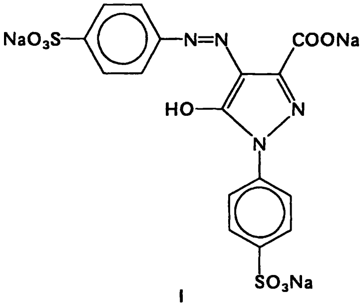
Number	Name	FD & C (USA)	EU No.	Color	λ max (nm) (solvent[b])	Formula[a]	Examples for utilization in food processing
1	Tartrazine	Yellow No 5	E 102	lemon-yellow (W)	426 (W)	I	Pudding powders, confectionary and candies, ice creams, pop (effervescent) drinks
2	Riboflavin		E 101	yellow (W)	445 (W)		Mayonnaise, soups, puddings, desserts, confectionary and candy products
3	Curcumin		E 100	yellow-red (E)	426 (E)	II	Mustard
4	Zeaxanthin			yellow (oil)	455–460 (CH)		Fat, hot and cold drinks, puddings, water
5	Sunset Yellow FCF	Yellow No 6	E 110	orange (W)	485 (W)	III	Beverages, fruit preserves, confectionary and candy products, honey-like products, sea salmon, crabs
6	β-Carotene		E 160a	orange (oil)	453–456 (CH)		Fat, beverages, soups, pudding, water, confectionary and candy products, yoghurt
7	Bixin		E 160b	orange (oil)	471/503 ($CHCl_3$)		Fat, mayonnaise
8	Lycopene		E 160d	orange (oil)	478 (H)		Mayonnaise, ketchup, sauces
9	Canthaxanthin	Food Orange 8	E 161g	orange (oil)	485 ($CHCl_3$)		Sea salmon, beverages, tomato products
10	Astaxanthin			orange (oil)	488 ($CHCl_3$)		Beverages, tomato products, confectionary and candy products
11	β-Apo-8′- carotenal		E 160e	orange (oil)	460–462 (CH)		Sauces, beverages, confectionary and candy products
12	Carmoisine		E 122	red with bluish tint (W)	516 (W)	IV	Beverages, confectionary and candy products, ice cream, pudding, fruit preserves
13	Amaranth	Red No 2	E 123	red with bluish tint (W)	520 (W)	V	Beverages, fruit preserves, confectionary and candy products, jams
14	Ponceau 4R		E 124	scarlet-red (W)	505 (W)	VI	Beverages, candy products (bonbons), sea salmon, cheese coatings

Table 8.12. (*Continued*)

Number	Name	FD & C (USA)	EU No.	Color	λ max (nm) (solvent[b])	Formula[a]	Examples for utilization in food processing
15	Carmine		E 120	bright-red	518 (W ammonia solution)	VII	Alcoholic beverages
16	Anthocyanidin (from red grape pomace)		E 163a–f	red-violet[c] (W) (M + 0.01% HCl)	520–546		Jams, pop (effervescent) drinks
17	Erythrosine	Red No 3	E 127	cherry-red (W)	527 (W)	VIII	Fruits, jams, confectionary and candy products
18	Red 2G			red with bluish tint	532 (W)	IX	Confectionary and candy products
19	Indigo Carmine (Indigotine)	Blue No 2	E 132	purple blue (W)	610 (W)	X	Also in combination with yellow colorant for confectionary and candy products and liqueurs
20	Patent Blue V		E 131	blue with a greenish tint (W)	638 (W)	XI	Mostly in combination with yellow colorants for confectionary and candy products, beverages
21	Brilliant blue FCF	Blue No 1		blue with a greenish tint (W)	630 (W)	XII	Mostly in combination with yellow colorants for confectionary and candy products, beverages
22	Chlorophyll		E 140	green	412 ($CHCl_3$)		Edible oils
23	Chlorophyllin copper complex		E 141	green (W)	405 (W)		Confectionary and candy products, liqueurs, jellies, cream food products
24	Green S (Brillant Green BS)		E 142	green (W)	632 (W)	XIII	
25	Black BN		E 151	violet with bluish tint	570 (W)	XIV	Fish roe coloring, confectionary and candy products

[a] Formulas in Table 8.13;
[b] Solvent W: water, CH: cyclohexane, M: methanol, H: hexane, and E: ethanol;
[c] Color is pH dependent.

Table 8.13. Structures of the synthetic food colorants listed in Table 8.12



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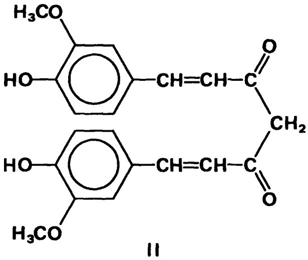

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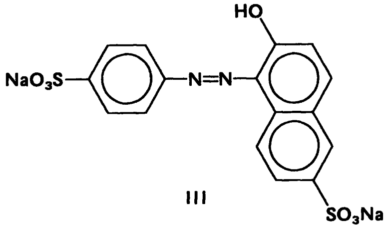

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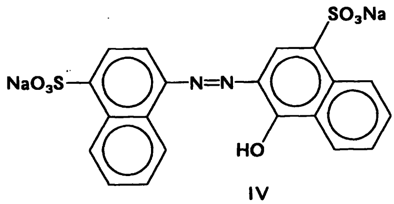

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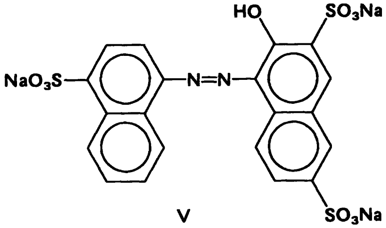

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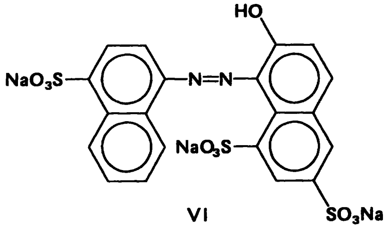

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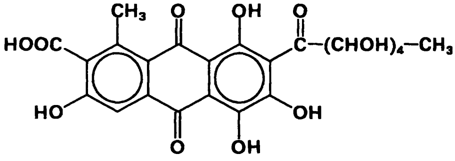
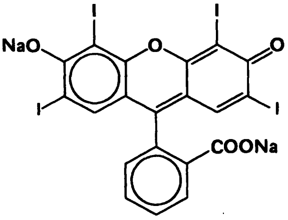


VII



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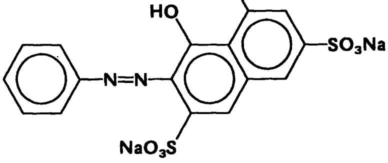
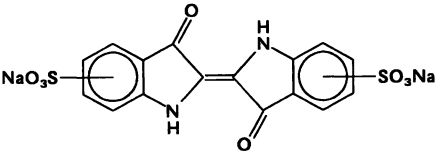


IX

Table 8.13. (Continued)



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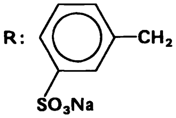

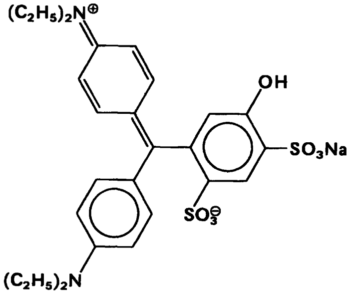

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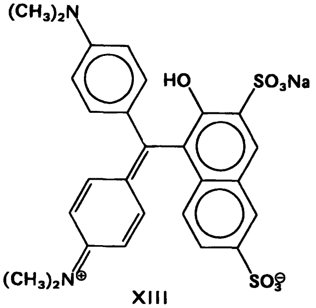

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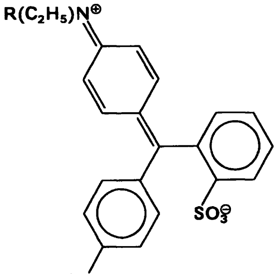

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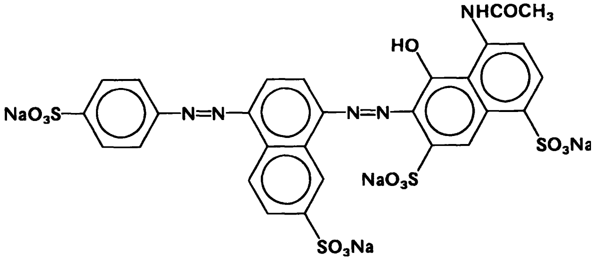


XIV

8.10.3 Succinic Acid Anhydride

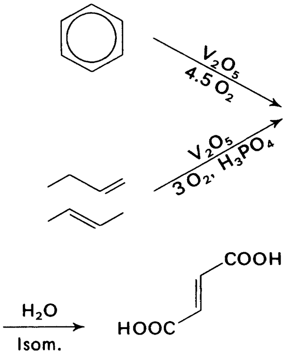
This is the only acid anhydride used as a food additive. The hydrolysis proceeds slowly, hence the compound is suitable as an agent in baking powders and for binding of water in some dehydrated food products.

8.10.4 Adipic Acid

Adipic acid ($pK_1 = 4.43$; $pK_2 = 5.62$) is used in powdered fruit juice drinks, for improving the gelling properties of marmalades and fruit jellies, and for improving cheese texture. It is synthesized from phenol or cyclohexane (cf. Reactions 8.22).

8.10.5 Fumaric Acid

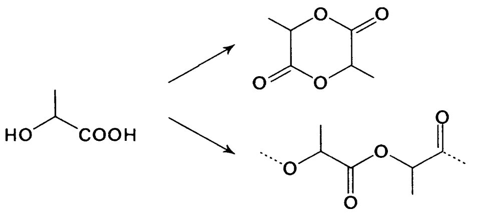
Fumaric acid ($pK_1 = 3.00$; $pK_2 = 4.52$) increases the shelf life of some dehydrated food products (e. g. pudding and jelly powders). It is also used to lower the pH, usually together with food preservatives (e. g. benzoic acid), and as an additive promoting gel setting. Fumaric acid is synthesized via maleic acid anhydride which is obtained by catalytic oxidation of butene or benzene (cf. Reaction 8.23) or is produced from molasses by fermentation using *Rhizopus spp.*



(8.23)

8.10.6 Lactic Acid

D,L- or L-lactic acid (pK = 3.86) is utilized as an 80% solution. A specific property of the acid is its formation of intermolecular esters, providing oligomers or a dimer lactide:



(8.24)

Such intermolecular esters are present in all lactic acid solutions with an acid concentration higher than 18%. More dilute solutions favor complete hydrolysis to lactic acid. The lactide can be utilized as an acid generator. Lactic acid is used for improving egg white whippability (pH adjustment to 4.8–5.1), flavor improvement of beverages and vinegarpickled vegetables, prevention of discoloration of fruits and vegetables and, in the form of calcium lactate, as an additive in milk powders.

Lactic acid production is based on synthesis starting from ethanal, leading to racemic D,L-lactic acid (Formula 8.25) or on homofermentation (*Lactobacillus delbruckii, L. bulgaricus, L. leichmannii*) of carbohydrate-containing raw material, which generally provides L-but also D,L-lactic acids under the conditions of fermentation.

$$CH_3CHO \xrightarrow{HCN} CH_3CHOHCN \longrightarrow \text{Lactate} \qquad (8.25)$$

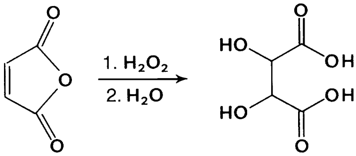
8.10.7 Malic Acid

Malic acid ($pK_1 = 3.40$; $pK_2 = 5.05$) is widely utilized in the manufacturing of marmalades, jellies and beverages and canning of fruits and vegetables (e. g. tomato). The monoesters with fatty alcohols are effective antispattering agents in cooking and frying fats and oils.

Malic acid synthesis, which provides the racemic D,L-form, is achieved by addition of water to maleic/fumaric acid. L-Malic acid can be synthesized enzymatically from fumaric acid with fumarase (*Lactobacillis brevis, Paracolobactrum spp.*) and from other C sources (paraffins) by fermentation with *Candida spp.*

8.10.8 Tartaric Acid

Tartaric acid ($pK_1 = 2.98$; $pK_2 = 4.34$) has a "rough", "hard" sour taste. It is used for the acidification of wine, in fruit juice drinks, sour candies, ice cream, and because of its formation of metal complexes, as a synergist for antioxidants. The production of (2R,3R)-tartaric acid is achieved from wine yeast, pomace, and cask tartar, which contain a mixture of potassium hydrogentartrate and calcium tartrate. This mixture is first converted entirely to calcium tartrate, from which tartaric acid is liberated by using sulfuric acid. Racemic tartaric acid is obtained by cis-epoxidation of maleic acid, followed by hydrolysis:



(8.26)

8.10.9 Citric Acid

Citric acid ($pK_1 = 3.09$; $pK_2 = 4.74$; $pK_3 = 5.41$) is utilized in candy production, fruit juice, ice cream, marmalade and jelly manufacturing, in vegetable canning and in dairy products such as processed cheese and buttermilk (aroma improver). It is also used to suppress browning in fruits and vegetables and as a synergetic compound for antioxidants. Its production is based on microbial fermentation of molasses by *Aspergillus niger*. The yield of citric acid is 50–70% of the fermentable sugar content.

8.10.10 Phosphoric Acid

Phosphoric acid ($pK_1 = 2.15$; $pK_2 = 7.1$; $pK_3 \sim 12.4$) and its salts account for 25% of all the acids used in food industries. The bulk of the acids (salts) used in the industry is citric acid (about 60%), while the use of other acids accounts for only 15%. The main field of use of phosphoric acid is the soft drink industry (cola drinks). It is also used in fruit jellies, processed cheese and baking powder and as an active buffering agent or pH-adjusting ingredient in fermentation processes. Acid salts, e. g., $Ca(H_2PO_4)_2 \cdot H_2O$ (fast activity), NaH_{14}-$Al_3(PO_4)_8 \cdot 4\ H_2O$ (slow activity) and Na_2H_2-P_2O_7 (slow activity) are used in baking powders as components of the reaction to slowly or rapidly release the CO_2 from $NaHCO_3$.

8.10.11 Hydrochloric and Sulfuric Acids

Both acids are used in starch and sucrose hydrolyses. Hydrochloric acid is also used in protein hydrolysis in industrial production of seasonings.

8.10.12 Gluconic Acid and Glucono-δ-lactone

Gluconic acid is obtained by the oxidation of glucose, which proceeds either by metal catalysis or enzymatically (*Aspergillus niger, Guconobacter suboxidans*).

Gluconic acid is used, e. g., in the production of invert sugar, beverages, and candies. The δ-lactone is produced by evaporating a gluconic acid solution at 35–60 °C. Glucono-δ-lactone slowly hydrolyzes, releasing protons. Hence, it is applied as an additive when slow acidification is needed, as with baking powder, raw sausage ripening and several sour milk products.

8.11 Bases

NaOH and a number of alkaline salts, such as $NaHCO_3$, Na_{2CO_3}, $MgCO_3$, MgO, $Ca(OH)_2$, Na_{2HPO_4} and Na-citrate, are used in food processing for various purposes, for example:

Ripe olives are treated with 0.25–2% NaOH to eliminate the bitter flavor and to develop the desired dark fruit color.

In alkali-baked goods (bread and cakes that keep) molded dough pieces are dipped into 1.25% NaOH at 85–88 °C or, in the case of larger fresh alkali-baked goods, into 3.5% NaOH at room temperature in the baking process in order to form the typical smooth, light to dark brown surface.

In chocolate manufacturing, $NaHCO_3$ enhances the *Maillard* reaction, providing dark bitter chocolates.

In production of molten processed cheese, the pH rise needed to improve the swelling of casein gels is achieved by addition of alkali salts.

8.12 Antimicrobial Agents

Elimination of microflora by physical methods is not always possible, therefore, antimicrobial agents are needed. The spectrum of compounds used for this purpose has hardly changed for a long time. It is not easy to find new compounds with wider biological activity, negligible toxicity for mammals and acceptable cost.

In the use of weak acids as preservatives, their pK value and the pH value of the food are very important for the application because only the undissociated molecule can penetrate into the inside of the microbial cell. Accordingly, weak acids are suitable preferably for acidic foods.

8.12.1 Benzoic Acid

Benzoic acid activity is directed both to cell walls and to inhibition of citrate cycle enzymes (α-ketoglutaric acid dehydrogenase, succinic acid dehydrogenase) and of enzymes involved in oxidative phosphorylation.

The acid is used in its alkali salt form as an additive, since solubility of the free acid is unsatisfactorily low. However, the undissociated acid

($pK_a = 4.19$) is predominantly active. As in the case of sorbic acid and propionic acid, a certain activity is also attributed to the anion.

Benzoic acid usually occurs in nature as a glycoside (in cranberry, bilberry, plum and cinnamon trees and cloves). Its activity is primarily against yeasts and molds, less so against bacteria. Figures 8.10 and 8.12 show the pH-dependent activity of the acid against *Escherichia coli, Staphylococcus aureus* and *Aspergillus niger*.

The LD_{50} (rats; orally) is 1.7–3.7 g/kg body weight; the LD_{100} (guinea pig, cat, dog, rabbit; orally) is 1.4–2 g/kg. A daily intake of <0.5 g Na-benzoate is tolerable for humans. No dangerous accumulation of the acid occurs in the body even at a dosage of as much as 4 g/day. It is eliminated by excretion in the urine as hippuric acid while, at higher levels of intake, the glucuronic acid derivative is also excreted.

Benzoic acid (0.05–0.1%) is often used in combination with other preservatives and, on the basis of its higher activity at acidic pH's, it is used for preservation of sour food (pH 4–4.5 or lower), beverages with carbon dioxide, fruit salads, marmalades, jellies, fish preserves, margarine, paste (pâté) fillings and pickled sour vegetables. A change in aroma, occurring mostly in fruit products, may result as a consequence of benzoic acid esterification.

8.12.2 PHB-Esters

The alkyl esters of p-hydroxybenzoic acid (PHB; parabens) are quite stable. Their solubility in water decreases with increasing alkyl chain length (methyl → butyl). The esters are mostly soluble in 5% NaOH.

The esters are primarily antifungal agents and are also active against yeasts but less so against bacteria, especially those which are gram-negative. The activity rises with increasing alkyl chain length (Fig. 8.11). Nevertheless, lower members of the homologous series are preferred because of better solubility.

The LD_{50} (mice; orally) is >8 g/kg body weight. In a feeding experiment over 96 weeks using 2% PHB-ester, no weight decrease was observed, while a slight decrease was found at the 8% level. In humans, the compounds are excreted in urine as p-hydroxybenzoic acid or its glycine or glucuronic acid conjugates.

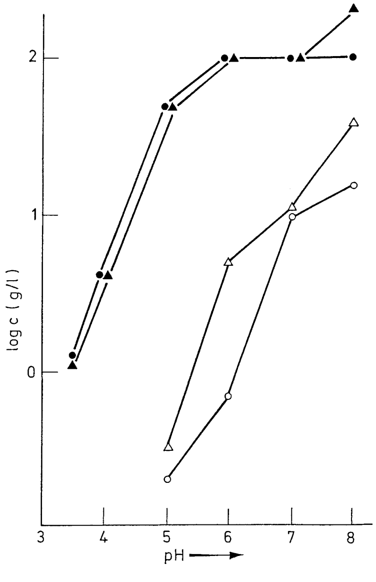


Fig. 8.10. The effect of benzoic acid on *Escherichia coli* (○ bacteriostatic. ● bactericidal activity) and *Staphylococcus aureus* (△ bacteriostatic and ▲ bactericidal activity)

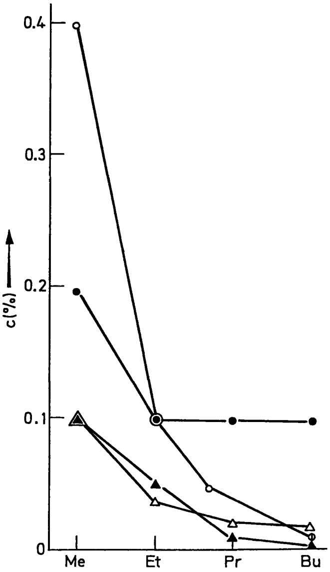


Fig. 8.11. Inhibition of *Salmonella typhosa* (●), *Aspergillus niger* (△), *Staphylococcus aureus* (○), and *Saccharomyces cerevisiae* (▲) by PHB-esters

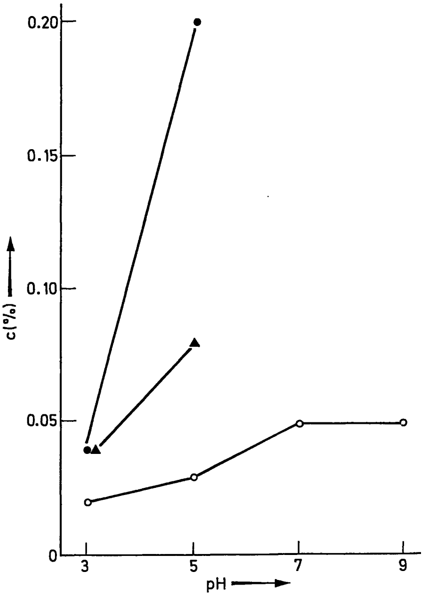


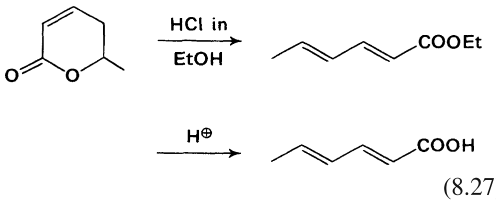
Fig. 8.12. Growth inhibition of *Aspergillus niger* by benzoic acid (●), p-hydroxybenzoic acid propyl ester (○) and sorbic acid (▲)

Unlike benzoic acid, the esters can be used over a wide pH range since their activity is almost independent of pH (cf. Fig. 8.12). As additives, they are applied at 0.3–0.06% as aqueous alkali solutions or as ethanol or propylene glycol solutions in fillings for baked goods, fruit juices, marmalades, syrups, preserves, olives and pickled sour vegetables.

8.12.3 Sorbic Acid

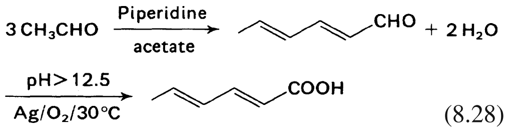
The antimycotic effect of straight chain carboxylic acids has long been known. In particular the unsaturated acids, for example crotonic acid and its homologues, are very active. Sorbic acid (2-trans, 4-trans-hexadienoic acid; pK = 4.76) has the advantage that it is odorless and tasteless at the levels of use (0.3% or less). The acid is obtained by several syntheses:

- From parasorbic acid [(S)-2-hexen-5-olide); cf. Reaction 8.27]. The acid is present in berries of the mountain ash tree (*Sorbus aucuparia*).



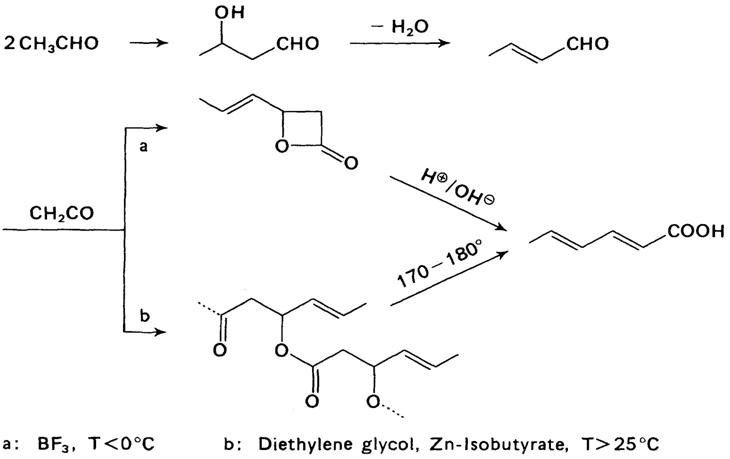
(8.27)

- From ethanal:



(8.28)

- From crotonaldehyde obtained from ethanal (cf. Reaction 8.29).



(8.29)

The third synthesis is the most important.

The microbial activity of sorbic acid is primarily against fungi and yeasts, less so against bacteria. The activity is pH dependent (Fig. 8.12). Its utilization is possible up to pH 6.5, the proportion of undissociated acid being still 1.8%.

The LD_{50} (rats) of sorbic acid is ca. 10 g/kg body weight. Feeding experiments with rats for more than 90 days, with 1–8% sorbic acid in the diet, had no effect, while only 60% of the animals survive an 8% level of benzoic acid.

Sorbic acid is degraded biochemically like a fatty acid, i. e. by a β-oxidation mechanism. A small portion of the acid is degraded by ω-oxidation, yielding trans, trans-muconic acid (cf. Reaction 8.30).

(8.30)

Some microorganisms, such as *Penicillium roqueforti*, have the ability to decarboxylate sorbic acid and thus convert it into 1,3-pentadiene, which has no antimicrobial activity and in addition may contribute to an off-flavor in cheeses:

(8.31)

Sorbic acid or its salts are effective antifungal agents in baked products, cheeses, beverages (fruit juices, wines), marmalades, jellies, dried fruits and in margarine.

8.12.4 Propionic Acid

Propionic acid is found in nature where propionic acid fermetnation occurs, e. g., in Emmental cheese, in which it is present up to 1%.

Its antimicrobial activity is mostly against molds, less so against bacteria. Propionic acid has practically no effect against yeast. Its activity is pH dependent. It is recommended and used up to pH 5 and only occasionally up to pH 6.

Propionic acid is practically nontoxic. It is used as an additive in baked products for inhibition of molds, and to prevent ropiness caused by the action of *Bacillus mesentericus*. It is added to flour at 0.1–0.2% as its Ca-salt and is used in cheese manufacturing by dipping the cheese into an 8% solution of the acid.

8.12.5 Acetic Acid

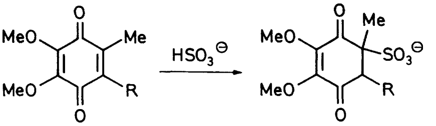
The preserving activity of vinegar (cf. 22.3) has been known from ancient times. The acid has a two-fold importance: as a preservative and as a seasoning agent. It is more active against yeasts and bacteria than against molds. It is used as the free acid, Na- and Ca-salts, or as Na-diacetate ($CH_3COOH \cdot CH_3COONa \cdot 1/2H_2O$), in ketchup, mayonnaise, acid-pickled vegetables, bread and other baked products.

8.12.6 SO_2 and Sulfite

The activity of these preserving agents covers yeasts, molds and bacteria. The activity increases with decreasing pH and is mostly derived from undissociated sulfurous acid, which predominates at a pH < 3.

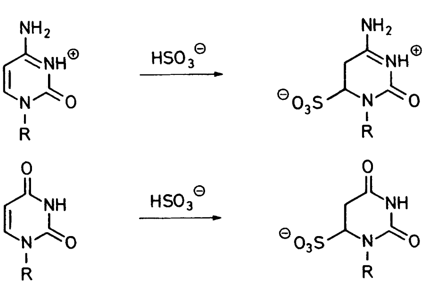
Toxicity is negligible at the levels usually applied. Possible mutagenic activity is under investigation. Excretion in the urine occurs as sulfate.

Sulfite reacts with a series of food constituents, e. g., proteins with cleavage of disulfide bonds (cf. 1.4.4.4), with various cofactors like $NAD^{\oplus}$, folic acid, pyridoxal, and thiamine (cf. 6.3.1.3) and with ubiquinone:



(8.32)

The pyrimidines in nucleic acids can also react, e. g., cytosine and uracil (cf. Formula 8.33). Anthocyanins are bleached (cf 18.1.2.5.3).



(8.33)

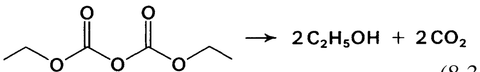
SO_2 is used in the production of dehydrated fruits and vegetables, fruit juices, syrups, concentrates or purée. The form of application is SO_2, Na_2SO_3, K_2SO_3, $NaHSO_3$, $Na_2S_2O_5$ and $K_2S_2O_5$ at levels of 200 ppm or less.

SO_2 is added in the course of wine making prior to must fermentation to eliminate interfering microorganisms. During wine fermentation with selected pure yeast cultures, SO_2 is used at a level of 50–100 ppm, while 50–75 ppm are used for wine storage.

SO_2 is not only antimicrobially active, but inhibits discoloration by blocking compounds with a reactive carbonyl group (*Maillard* reaction; nonenzymatic browning) or by inhibiting oxidation of phenols by phenol oxidase enzymes (enzymatic browning).

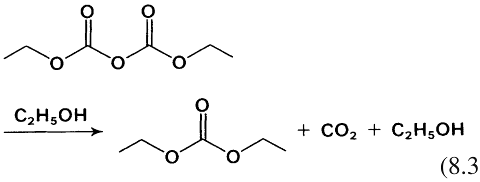
8.12.7 Diethyl (Dimethyl) Pyrocarbonate

Diethyl pyrocarbonate (DEPC or diethyl dicarbonate) is a colorless liquid of fruit-like or ester odor. Its antimicrobial activity covers yeasts (10–100 ppm), bacteria (*Lactobacilli*: 100–170 ppm) and molds (300–800 ppm). The levels of the compound required for a clear inhibition are given in brackets. Diethyl pyrocarbonate readily hydrolyzes to yield carbon dioxide and ethanol:



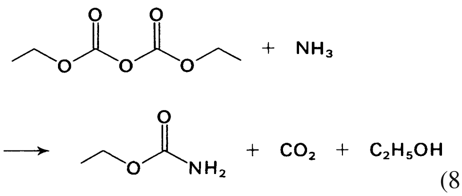
(8.34)

or it reacts with food ingredients. In alcoholic beverages it yields a small amount of diethyl carbonate:



(8.35)

In the presence of ammonium salts, DEPC can form ethyl urethane in a pH-dependent reaction:



(8.36)

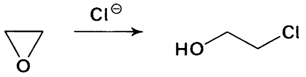
Since diethyl carbonate may be a teratogenic agent and ethyl urethane is a carcinogen, the use of diethyl pyrocarbonate is discussed under toxicological aspects. The compound should be replaced by dimethyl pyrocarbonate, since methyl urethane, unlike ethyl urethane, is not carcinogenic.

Both compounds are used in cold pasteurization of fruit juices, wine and beer at a concentration of 120–300 ppm.

8.12.8 Ethylene Oxide, Propylene Oxide

These compounds are active against all microorganisms, particularly vegetative cells and spores, and also against viruses. Propylene oxide is somewhat less reactive than ethylene oxide.

Since they are efficient alkylating agents, the pure compounds are very toxic. After application, all the residual amounts must be completely removed. The glycols resulting from their hydrolysis are not as toxic (ethylene glycol: LD_{50} for rats is 8.3 g/kg body weight). Toxic reaction products can be formed, as exemplified by chlorohydrin obtained in the presence of chloride:



(8.37)

In addition, some essential food constituents react with formation of biologically inactive derivatives. Examples are riboflavin, pyridoxine, niacin, folic acid, histidine or methionine. However, these reactions are not of importance under the conditions of the normal application of ethylene oxide or propylene oxide.

Both compounds are used as gaseous sterilants (ethylene oxide, boiling point 10.7 °C; propylene oxide, 35 °C) against insects and for gaseous sterilization of some dehydrated foods for which other methods, e. g. heat sterilization, are not suitable. Examples are the gaseous sterilization of walnuts, starches, dehydrated foods (fruits and vegetables) and, above all, spices, in which a high spore count (and plate count in general) is often a sanitary problem. The sterilization is carried out in pressure chambers in a mixture with an inert gas (e. g. 80–90% CO_2). The need to remove the residual unreacted gas (vacuum, "gaseous rinsing") has already been emphasized. An alternative method of sterilization for the above-mentioned food products is high energy irradiation (UV-light, X-ray, or gamma irradiation).

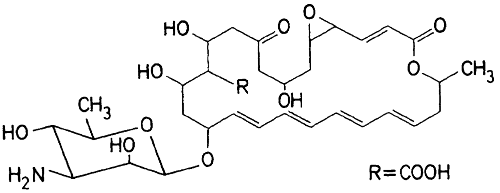
8.12.9 Nitrite, Nitrate

These additives are used primarily to preserve the red color of meat (cf. 12.3.2.2.2). However, they also have antimicrobial activity, particularly in a mixture with common salt. Of importance is their inhibitive action, in nonsterilized meat products, against infections by *Clostridium botulinum* and, consequently, against accumulation of its toxin. The activity is dependent on the pH and is proportional to the level of free HNO_2. Indeed, 5–20 mg of nitrite per kg are considered sufficient to redden meat, 50 mg/kg for the production of the characteristic taste, and 100 mg/kg for the desired antimicrobial effects. Acute toxicity has been found only at high levels of use (formation of methemoglobin). A problem is the possibility of the formation of nitrosamines, compounds with powerful carcinogenic activity. Numerous animal feeding tests have demonstrated tumor occurrence when the diet contained amines (sensitive to nitroso substitution) and nitrite. Consequently, the trend is to exclude or further reduce the levels of nitrate and nitrite in food. No suitable replacement has been found for nitrite in meat processing.

8.12.10 Antibiotics

The use of antibiotics in food preservation raises a problem since it might trigger development of more resistant microorganisms and thus create medical/therapeutic difficulties.

Of some importance is nisin, a polypeptide antibiotic, produced by some *Lactococcus lactis* strains. It is active against Gram-positive microorganisms and all spores, but is not used in human medicine. This heat-resistant peptide is applied as an additive for sterilization of dairy products, such as cheeses or condensed or evaporated milk (cf. 1.3.4.3). Natamycin (pimaricin, Formula 8.38), which is produced by *Streptomyces natalensis* and *S. chattanogensis*, is active at 5–100 ppm against yeasts and molds and is used as an additive in surface treatment of cheeses. It also finds application for suppressing the growth of molds on ripening raw sausages.



(8.38)

The possibility of incorporating the wide spectrum antibiotics chlortetracycline and oxytetracycline into fresh meat, fish and poultry, in order to delay spoilage, is still under investigation.

8.12.11 Diphenyl

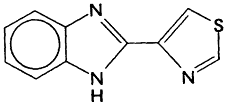
Diphenyl, due to its ability to inhibit growth of molds, is used to prevent their growth on peels of citrus fruits (lemon, orange, lime, grapefruit). It is applied by impregnating the wrapping paper and/or cardboard packaging material (1–5 g diphenyl/m^2).

8.12.12 o-Phenylphenol

This compound, at a level of 10–50 ppm and a pH range of 5–8, inhibits the growth of molds. The inhibition effect, which increases with increasing pH, is utilized in the preservation of citrus fruits. It is applied by dipping the fruits into a 0.5–2% solution at pH 11.7.

8.12.13 Thiabendazole, 2-(4-Thiazolyl)benzimidazole

This compound (Formula 8.39) is particularly powerful against molds which cause the socalled blue mold rots, e. g., *Penicillium italicum* (blue-green-spored "contact mold") and *Penicillium digitatum* (green-spored mold). It is used for preserving the peels of citrus fruits and bananas. The application mode is by dipping or spraying the fruit with a wax emulsion containing 0.1–0.45% thiabendazole.



(8.39)

8.13 Antioxidants

Since lipids are widely distributed in food and since their oxidation yields degradation products of great aroma impact, their degradation is an important cause of food deterioration by generation of undesirable aroma. Lipid oxidation can be retarded by oxygen removal or by using antioxidants as additives. The latter are mostly phenolic compounds, which provide the best results often as a mixture and in combination with a chelating agent. The most important antioxidants, natural or synthetic, are tocopherols, ascorbic acid esters, gallic acid esters, tert-butylhydroxyanisole (BHA) and di-tert-butylhydroxytoluene (BHT). They are covered in 3.7.3.2.2.

8.14 Chelating Agents (Sequestrants)

Chelating agents have acquired greater importance in food processing. Their ability to bind metal ions has contributed significantly to stabilization of food color, aroma and texture. Many natural constituents of food can act as chelating agents, e. g., carboxylic acids (oxalic, succinic), hydroxy acids (lactic, malic, tartaric, citric), polyphosphoric acids (ATP, pyrophosphates), amino acids, peptides, proteins and porphyrins.

Table 8.14 lists the chelating agents utilized by the food industry, while Table 8.15 gives the stability constants for some of their metal complexes.

Traces of heavy metal ions can act as catalysts for fat or oil oxidation. Their binding by chelating agents increases antioxidant efficiency and inhibits oxidation of ascorbic acid and fat-soluble vitamins. The stability of the aroma and color of canned vegetables is substantially improved.

Table 8.14. Chelating agents used as additives in food processing. (Compounds given in brackets are utilized only as salts or derivatives)

(Acetic acid)	Na-, K-, Ca-salts
Citric acid	Na-, K-, Ca-salts, monoisopropyl ester, monoglyceride ester, triethyl ester, monostearyl ester,
EDTA	Na-, Ca-salts
(Gluconic acid)	Na-, Ca-salts
Oxystearin	
Orthophosphoric acid	Na-, K-, Ca-salts
(Pyrophosphoric acid)	Na-salt
(Triphosphoric acid)	Na-salt
(Hexametaphosphoric acid, 10–15 residues)	Na-, Ca-salts
(Phytic acid)	Ca-salt
Sorbitol	
Tartaric acid	Na-, K-salts
(Thiosulfuric acid)	Na-salt

Table 8.15. Stability constants (pK-values) of some metal complexes

Chelating agent	Ca^{2+}	Co^{2+}	Cu^{2+}	Fe^{2+}	Fe^{3+}	Mg^{2+}	Zn^{2+}
Acetate	0.5	2.2				0.5	1.0
Glycine	1.4	5.2	8.2	4.3	10.0	3.5	5.2
Citrate	3.5	4.4	6.1	3.2	11.9	2.8	4.5
Tartrate	1.8		3.2		7.5	1.4	2.7
Gluconate	1.2		18.3			0.7	1.7
Pyrophosphate	5.0		6.7		22.2	5.7	8.7
ATP	3.6	4.6	6.1			4.0	4.3
EDTA	10.7	16.2	18.8	14.3	25.7	8.7	16.5

In the production of herb and spice extracts, the combination of an antioxidant and a chelating agent provides an improved extract quality. Chelating agents are also used in dairy products, wherein their deaggregating activity for the casein complexes is often utilized; in blood recovery processes to prevent clotting; and in the sugar industry to facilitate sucrose crystallization, a process which is otherwise retarded by sucrose-metal complexes.

8.15 Surface-Active Agents

Naturally occurring and synthetic surface-active agents (tensides), some of which are listed in Table 8.16, are used in food processing when a decrease in surface tension is required e. g., in production and stabilization of all kinds of dispersions (Table 8.17).

Dispersions include emulsions, foams, aerosols and suspensions (Table 8.18). In all cases an *outer, continuous* phase is distinct from an *inner*, discontinuous, *dispersed* phase. Emulsions are of particular importance and they will be outlined in more detail.

8.15.1 Emulsions

Emulsions are dispersed systems, usually of two immiscible liquids. When the outer phase consists of water and the inner of oil, it is considered as an "oil in water" (o/w) type of emulsion. When this is reversed, i. e., water is dispersed in oil, a w/o emulsion exists. Examples of food emulsions are: milk (o/w), butter (w/o) and mayonnaise (o/w).

The visual appearance of an emulsion depends on the droplet diameter. If the diameter is in the range of 0.15–100 μm, the emulsion appears milky-turbid. In comparison, micro-emulsions (diameter: 0.0015–0.15 μm) are transparent and considerably more stable because the sedimentation rate depends on the droplet diameter (Table 8.19).

Table 8.16. Surfactants (surface active agents) in food

I. Naturally occurring:
A. Ions: proteins (cf. 1.4.3.6), hydrocolloids (gum arabic, cf. 4.4.4.5.2), phospholipids (lecithin, cf. 3.4.1.1), bile acids
B. Neutral substances: glycolipids (cf. 3.4.1.2), saponins
II. Synthetic:
A. Ions: stearyl-2-lactylate, Datem, Citrem (cf. Table 8.24)
B. Neutral substances: mono-, diacylglycerols and their acetic- and lactic acid esters, saccharose fatty acid esters, sorbitan fatty acid esters, polyoxyethylene sorbitan fatty acid esters
Polyglycerol-polyricinoleate (PGPR)

Table 8.17. Examples of surfactant utilization in the food industry

Utilization in production of	Effect
Margarine	Stabilization of a w/o emulsion
Mayonnaise	Stabilization of an o/w emulsion
Ice cream	Stabilization of an o/w emulsion, achievement of a "dry" consistency
Sausages	Prevention of fat separation
Bread and other baked products	Improvement of crumb structure, baked product volume, inhibition of starch retrogradation (bread staling)
Chocolate	Improvement of rheological properties, inhibition of "fat blooming"
Instant powders	Solubilization
Spice extracts	Solubilization

Table 8.18. Dispersion systems

Type	Inner phase	Outer phase
Emulsion	liquid	liquid
Foam	gaseous	liquid
Aerosol	liquid or solid	gaseous
Suspension	solid	liquid

Table 8.19. Sedimentation rate (v) as a function of droplet diameter (d)

d (μm)	v (cm/24 h)
0.02	3.75×10^{-4}
0.2	3.76×10^{-2}
2	3.76
20	3.76×10^{2}
200	3.76×10^{4}

Each emulsifier can disperse a limited amount of an inner phase, i. e. it has a fixed *capacity*. When the limit is reached, further addition of outer phase breaks down the emulsion. The capacity and other related parameters differ among emulsifiers and can be measured accurately under standardized conditions.

8.15.2 Emulsifier Action

8.15.2.1 Structure and Activity

Emulsions are made and stabilized with the aid of a suitable tenside, usually called an emulsifier. Its activity is based on its molecular structure. There is a lipophilic or hydrophobic part with good solubility in a nonaqueous phase, such as an oil or fat, and a polar or hydrophilic part, soluble in water. The hydrophobic part of the molecule is generally a long-chain alkyl residue, while the hydrophilic part consists of a dissociable group or of a number of hydroxyl or polyglycolether groups.

In an immiscible system such as oil/water, the emulsifier is located on the interface, where it decreases interfacial tension. Thus, even in a very low concentration, it facilitates a fine distribution of one phase within the other. The emulsifier also prevents droplets, once formed, from aggregating and coalescing, i. e. merging into a single, large drop (Fig. 8.13).

Ionic tensides stabilize o/w emulsions in the following way (Fig. 8.14a): at the interface, their alkyl residues are solubilized in oil droplets, while the charged end groups project into the aqueous phase. The involvement of counter ions forms an electrostatic double layer, which prevents oil droplet aggregation.

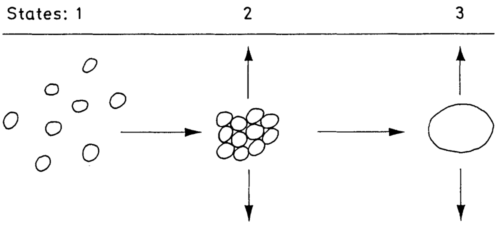


Fig. 8.13. Changes in an emulsion. *1* The droplets are dispersed in a continuous phase. *2* The droplets form aggregates. An increase in particle diameter results in acceleration of their flotation or sedimentation. *3* Coalescence: the aggregated droplets merge into larger droplets. Finally, two continuous phases are formed; the emulsion is destroyed

Nonionic, neutral tensides are oriented on the oil droplet's surface with the polar end of the tenside projecting into the aqueous phase. The coalescence of the droplets of an o/w emulsion is prevented by an anchored "hydrate shell" built around the polar groups.

The coalescence of water droplets in a w/o emulsion first requires that water molecules break through the double-layered hydrophobic region of emulsifier molecules (Fig. 8.14 b). This escape is only possible when sufficient energy is supplied to rupture the emulsifier's hydrophobic interaction.

The stability of an emulsion is increased when additives are added which curtail droplet mobility. This is the basis of the stabilization effect of hydrocolloids (cf. 4.4.3) on o/w emulsions since they increase the viscosity of the outer, aqueous phase.

A rise in temperature negatively affects emulsion stability, and can be applied whenever an emulsion has to be destroyed. Elevated temperatures are used along with shaking, agitation or pressure

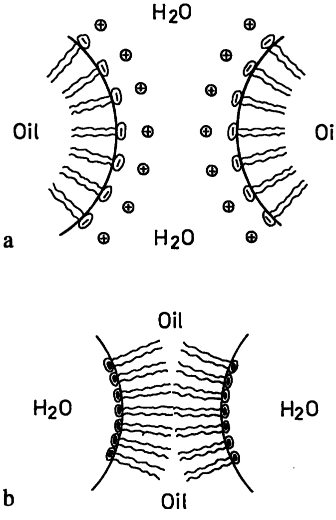


Fig. 8.14. Stabilization of an emulsion. **a** Activity of an ionic emulsifier in an o/w emulsion. **b** Activity of a nonpolar emulsifier in w/o emulsion. ○ Polar groups, $\sim$ apolar tails of the emulsifier

(mechanical destruction of interfacial films as, for example, in butter manufacturing, cf. 10.2.3.3). Other ways of decreasing the stability of an emulsion are addition of ions which collapse the electrostatic double layer, or hydrolysis to destroy the emulsifier.

Table 8.20. Effect of fatty acid residues on the critical micelle temperature T_c of lecithins

Fatty acid	T_c (°C)
12:0	0
14:0	23
16:0	41
18:0	58
18:1	−20

8.15.2.2 Critical Micelle Concentration (CMC), Lyotropic Mesomorphism

The surface tension of an aqueous solution of an o/w emulsifier decreases down to the critical micelle concentration (CMC) as a function of the emulsifier concentration. Above this limiting value, the emulsifier aggregates reversibly to give spherical micelles, the surface tension changing only slightly. The CMC is a characteristic value of the emulsifier, which decreases as the hydrophobic part of the molecule increases. It is also influenced by the temperature, pH value, and electrolyte concentration.

The temperature at which the solubility of an emulsifier reaches the CMC is called the critical micelle temperature (Tc, Krafft point). Crystals, micelles, and the dissolved emulsifier are in equilibrium at the Tc (Fig. 8.15). An emulsifier cannot form micelles below the Tc which, e. g., depends on the structure of the fatty acid residues in lecithin (Table 8.20).

Emulsifiers are lyotropic mesomorphous, i. e., they form one of the following liquid crystalline mesophases depending on the water content and the temperature (shown schematically in Fig. 8.16):

Hexagonal I: Cylindrical aggregates of emulsifier molecules; the polar groups are oriented towards the outer water phase.

Lamellar: Emulsifier bilayers which are separated by thin water zones.

Hexagonal II (Inverse Hexagonal): Cylindrical aggregates of emulsifier molecules; the polar groups are oriented towards the inner water phase.

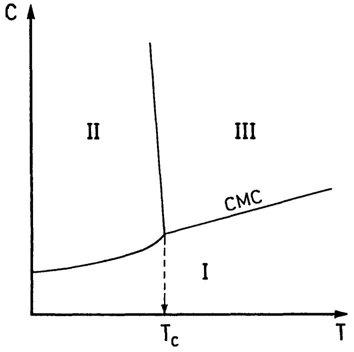


Fig. 8.15. Solubility of an emulsifier in water. *Ordinate*: concentration, *abscissa*: temperature. I: Solution, II: Crystals, III: Micelles, T_C: Critical micelle temperature

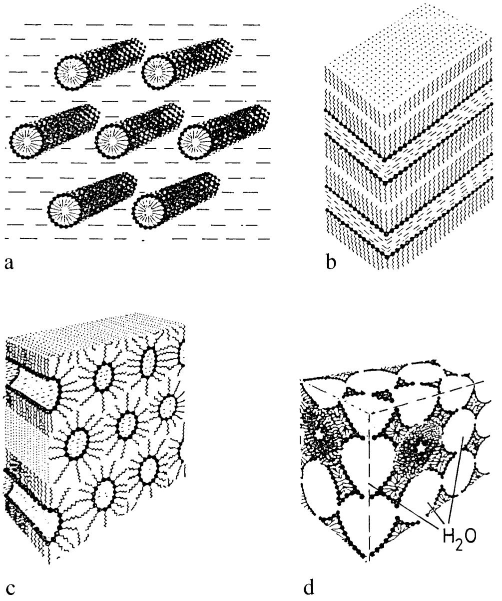


Fig. 8.16. Lyotropic mesophases of emulsifiers (according to *Schuster*, 1985). **(a)** Hexagonal I, **(b)** Lamellar, **(c)** Hexagonal II, **(d)** Cubic • •∿∿∿ Emulsifier -=-=- Water

Cubic: Cubic space- and face-centered water aggregates in a matrix of emulsifier molecules; the polar groups are oriented towards the water.

Phase diagrams show the mesophase present as a function of water content and temperature. In the phase diagram of the o/w emulsifier lysolecithin (Fig. 8.17, a), micelles, a lamellar, and a hexagonal phase appear. The w/o emulsifier 1-monoelaidin (Fig. 8.17, b) crystallizes at temperatures below 30 °C. The β-modification formed first is converted to the more stable β-form, which unlike the α-form, has no emulsifying properties. The melted 1-monoelaidin forms a microemulsion with little water and lamellar and cubic mesophases with much water. 1-Monoolein (Fig. 8.17, c) melts at lower temperatures and an inverse hexagonal mesophase appears.

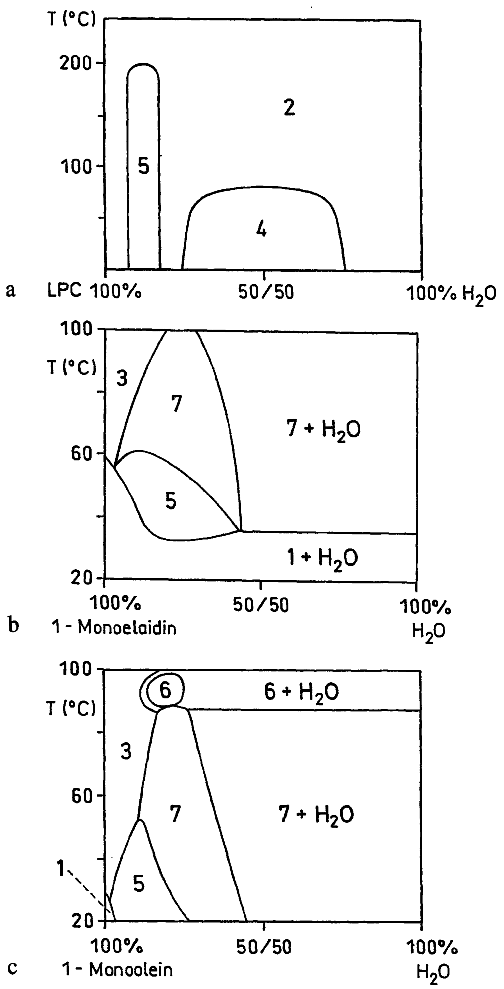


Fig. 8.17. Binary phase diagrams for the system emulsifier/water (according to *Krog*, 1990) (**a**) Lysolecithin, (**b**) 1-Monoelaidin, (**c**) 1-Monoolein
1 Crystals, *2* Micelles, *3* Microemulsion, *4* Hexagonal I, *5* Lamellar, *6* Hexagonal II, *7* Cubic

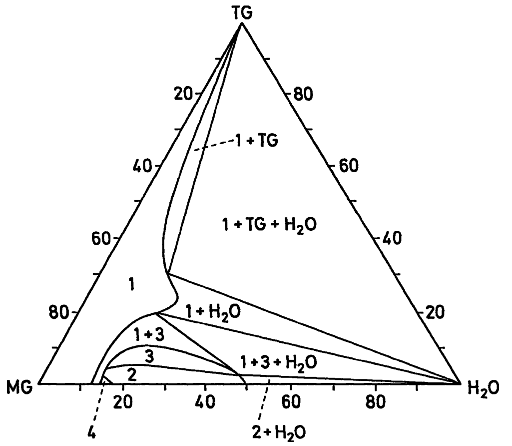


Fig. 8.18. Ternary phase diagram for the system monoglycerides (from sunflower oil)/water/soybean oil at 40 °C (according to *Larsson* and *Dejmek*, 1990)
1 Microemulsion, *2* Cubic, *3* Hexagonal II, *4* Lamellar

The phases of simply constituted food emulsions, which are present at a certain temperature depending on the composition, show a ternary phase diagram, e. g., in Fig. 8.18.

8.15.2.3 HLB-Value

A tenside with a relatively strong lipophilic group and weak hydrophilic group is mainly soluble in oil and preferentially stabilizes a w/o emulsion, and vice versa. This fact led to the development of a standard with which the relative strength or “activity” of the hydrophilic and lipophilic groups of emulsifiers can be evaluated. It is called the *HLB value* (hydrophilic–lipophilic balance). It can be determined, e. g., from dielectric constants or from the chromatographic behavior of the surface-active substance. The HLB value of the fatty acid esters of polyhydroxy alcohols can also be calculated as follows (SV =

saponification number of the emulsifier, AV = acid value of the separated acid):

$$\mathrm{HLB} = 20\left(1 - \frac{\mathrm{SV}}{\mathrm{AV}}\right) \tag{8.40}$$

On the basis of experimental group numbers (Table 8.21), the HLB value can be calculated using the formula:

$$\begin{aligned}\mathrm{HLB} = {} & \sum(\text{hydrophilic group number}) - \\ & \sum(\text{hydrophobic group number}) + 7\end{aligned} \tag{8.41}$$

Some examples listed in Table 8.22 show very good correspondence between calculated and experimentally found HLB values.

Table 8.21. Group number N_H and N_L for HLB calculation

Hydrophilic group	N_H	Lipophilic group	N_L
$-OSO_3^-, Na^+$	38.7	$-\overset{\vert}{C}H-$	0.475
$-SO_{3^-}, Na^+$	37.4	$-CH_2-$	0.475
$-COO^-, Na^+$	21.1	$-CH_3$	0.475
$-COO^-, K^+$	19.1	$=CH-$	0.475
Sorbitan ring	6.8	$-\underset{CH_3}{\underset{\vert}{C}}H-CH_2-O-$	0.15
Ester	2.4		
$-COOH$	2.1		
$-OH$ (free)	1.9	Benzene ring	1.662
$-O-$	1.3		
$-(CH_2-CH_2\text{-}O)-$	0.33		

Table 8.22. Hydrophilic lipophilic balance (HLB) values of some surfactants

	HLB-value	
Compound	Found	Calculated
Oleic acid	1.0	
Sorbitol tristearate	2.1	2.1
Stearyl monoglyceride	3.4	3.8
Sorbitol monostearate	4.7	4.7
Sorbitol monolaurate	8.6	
Gelatin	9.8	
Polyoxyethylene sorbitol tristearate	10.5	10
Methylcellulose	10.5	
Polyoxyethylene sorbitol monostearate	14.9	
Polyoxyethylene sorbitol monooleate	15.0	15
Sodium oleate	18.0	
Potassium oleate	20.0	

Table 8.23. HLB-values related to their industrial application

HLB-range	Application
3–6	w/o-Emulsifiers
7–9	Humectants
8–18	o/w-Emulsifiers
15–18	Turbidity stabilization

The HLB values indicated the first industrial applications (Table 8.23). For a detailed characterization, however, comprehensive knowledge of possible interactions of the emulsifier with the many components of a food emulsion is still lacking. Hence, emulsifiers are mainly used in accordance with empirical considerations.

It has been observed with neutral emulsifiers that the degree of hydration of the polar groups decreases with a rise in temperature and the influence of the lipophilic groups increases. Phase inversion occurs o/w → w/o. The temperature at which inversion occurs is called the phase conversion temperature.

8.15.3 Synthetic Emulsifiers

Today, 150,000–200,000 t of emulsifiers are produced worldwide. Of this amount, mono-and diacylglycerides and their derivatives account for the largest part, i. e. about 75%. Synthetic emulsifiers include a series of nonionic compounds. Unlike the ionic compounds, the nonionic emulsifiers are not in danger of decreasing in interfacial activity through salt formation with food constituents. The utilization of emulsifiers is legislated and often differently regulated in some countries. The synthetic emulsifiers described below are used worldwide.

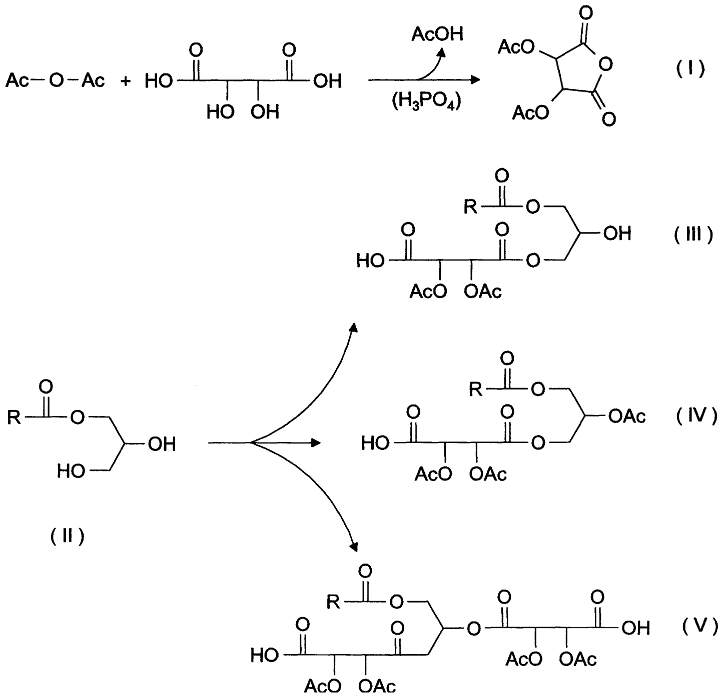
8.15.3.1 Mono-, Diacylglycerides and Derivatives

Mono- and diacylglycerides, which are mostly used as mixtures, are produced as described in 3.3.2. Other emulsifiers with special activities are obtained by derivatization (cf. Table 8.24). As a result of the diverse reaction possibilities

Table 8.24. Emulsifiers from mono- and diacylglyceride mixtures

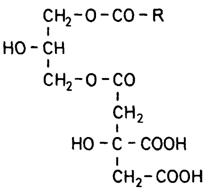
Name			Production by conversion of mixtures of mono- and diacylglycerides with
Mono- and diglycerides esterified with		EU-number	
Acetic acid (acetylated mono- and diglycerides)	*Acetem*	E472a	Acetic anhydride
Lactic acid	*Lactem*	E472b	Lactic acid
Citric acid	*Citrem*	E472c	Citric acid
Monoacetyl- and diacetyltartaric acid	*Datem*	E472e anhydride	Tartaric acid and acetic

of the starting compounds, complex products are obtained in this process. An example is represented by the diacetyltartaric acid ester of monoglycerides (DATEM). At concentrations of ca. 0.3% (based on the amount of flour), this ester increases the volume of wheat biscuits. For the production of this emulsifier, acetic anhydride and tartaric acid are heated, diacetyltartaric acid anhydride (I in Formula 8.43) being formed on removal of acetic acid by distillation. Compound I is converted to DATEM with monoacylglycerides (II). In the series 6:0 to 22:0 as well as 18:1 (9) and 18:2 (9,12), the baking activity of DATEM is the highest with stearic acid as the acyl residue. DATEMs on the basis of diacylglycerides containing the acyl residues 10:0 or 18:0 exhibit only slight activity. Of the 10 components of a DATEM preparation which quantitatively appeared, the main product (III in Formula 8.43) gave the largest increase in volume of white bread, closely followed by compounds IV and V.



(8.43)

Unlike *acetem* and *lactem, citrem* is an acid (cf. Formula 8.43).



$$\begin{array}{l} CH_2{-}O{-}CO{-}R \\ HO{-}CH \\ CH_2{-}O{-}CO \\ \quad\quad\quad CH_2 \\ \quad HO{-}C{-}COOH \\ \quad\quad\quad CH_2{-}COOH \end{array}$$

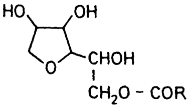
(8.43)

8.15.3.2 Sugar Esters

They are obtained, among other methods, by transesterification of fatty acid methyl esters (14:0, 16:0, 18:0 and/or 18:1, double bond position 9) with sucrose and lactose. The resultant mono- and diesters are odorless and tasteless. Depending on their structure, they cover an HLBrange of 7–13, and are used in stabilization of o/w emulsions, or in stabilization of some instant dehydrated and powdered foods.

8.15.3.3 Sorbitan Fatty Acid Esters

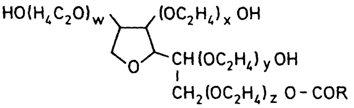
Esters of sorbitan (cf. 19.1.4.6) with fatty acids (*Span's*) serve the stabilization of w/o emulsions. Sorbitan tristearate is used in the production of chocolate to delay the fat bloom formation.



(8.44)

8.15.3.4 Polyoxyethylene Sorbitan Esters

Polyoxyethylene groups are introduced into the molecules to increase the hydrophilic property of sorbitan esters:



(8.45)

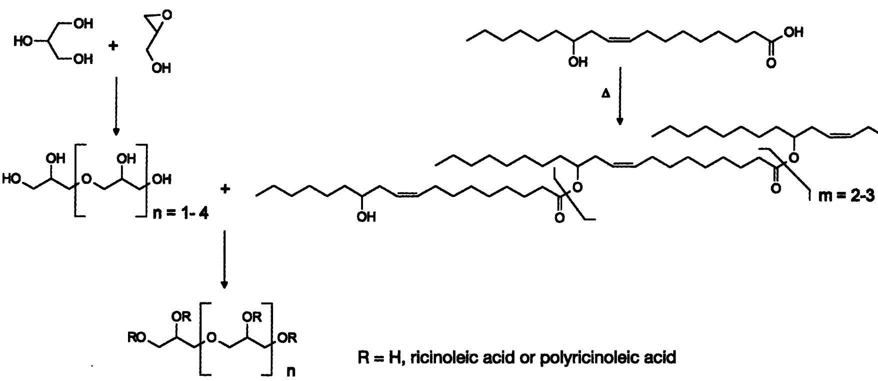
Polyoxyethylene sorbitan monoesters (examples in Table 8.22) are used to stabilize o/w emulsions.

8.15.3.5 Polyglycerol – Polyricinoleate (PGPR)

In the production of the emulsifier PGPR (cf. Formula 8.46), oligomeric glycerol is made by attachment of 2,3-epoxy-l-propanol (glycid) to glycerol and at the same time ricinoleic acids are esterified with each other under controlled heating conditions. In a third step, the oligomeric glycerol is esterified with polyesterricinoleic acid.

The emulsifier has a very complicated composition: apart from different types of esters, oligomeric glycerol and free ricinoleic acid are present.

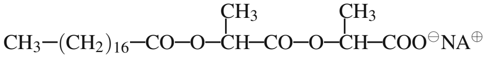
Together with lecithin, PGPR is used in the production of chocolate. It completely eliminates the flow point of a molten chocolate mass, but hardly lowers the viscosity.



(8.46)

8.15.3.6 Stearyl-2-Lactylate

In the presence of sodium or calcium hydroxide, esterification of stearic acid with lactic acid gives a mixture of stearyl lactylates (Na or Ca salt), the main component being stearyl-2-lactylate:



$$CH_3-(CH_2)_{16}-CO-O-\overset{CH_3}{\overset{|}{CH}}-CO-O-\overset{CH_3}{\overset{|}{CH}}-COO^{\ominus}NA^{\oplus}$$

(8.47)

The free acid acts as a w/o emulsifier and the salts as o/w emulsifiers. The HLB-value of the sodium salt is 8–9, and that of the calcium salt, 6–7. The sodium salt is used to stabilize an o/w emulsion which is subjected to repeated cycles of freezing and thawing.

8.16 Substitutes for Fat

In the industrial, highly developed countries, the intake of energy with food is higher than the physiological requirements. To avoid the consequences manifested by, e. g., overweight and adipositas, attempts are made to substitute the fat, the main source of energy. However, fat has many functions in food which cannot be completely taken on by a substitute. For this reason, various substances are offered which make partial solutions possible. They are divided into two groups depending on their origin:

- natural (fat mimetics)
- synthetic (fat substitutes, fat replacers)

8.16.1 Fat Mimetics

8.16.1.1 Microparticulated Proteins

The mouth feeling of substances depends on their chemical composition and on the particle size. Protein particles with a diameter of more than 8 μm are experienced as sandy, those in the range of 3–8 μm as powdery, 0.1–3 μm as creamy, and less than 0.1 μm as watery. Therefore, by means of microparticulation of protein concentrates to particles of 0.1–3 μm, it is possible to achieve the melt-in-the-mouth feeling produced by fat globules. In this process, concentrates of ovalbumin, casein and whey protein are exposed to varying pressures and temperatures, the proteins being ground by high shear forces. Rapid cooling to 4−1 °C yields a thick cream.

These substitutes are suitable for milk products (ice cream, desserts etc.) which are not strongly heated. In fact, 3 g of fat can be replaced by 3 g of swollen substitute (1 g of protein + 2 g of water) or 27 kcal by 4 kcal.

8.16.1.2 Carbohydrates

Polymeric carbohydrates are used as fat substitutes. They are nondigestible in the small intestine and classed as fiber. However, a number of these substances are degraded by the bacteria in the large intestine with the formation of short-chain acids (2:0, 3:0, 4:0). These acids are absorbed, the gain in energy at 2 kcal/g being half as much as with digestible carbohydrates. The energy (kcal/g) provided by fiber substances which can be used to replace fat are: wheat bran (1.5), barley brans (0.9), oat bran (0.1), apple fibers (1.6), soybean bran (0.7) and pea fibers (0.2). In the production of foods, attention must be paid to the taste of the preparations. The carbohydrate-based fat substitutes include the resistant starches (cf. 4.4.4.14.6), which can be formed during starch retrogradation, but also occur in some fruits, e. g., bananas. Fructose polymers (cf. 4.4.4.22.1), pectin (cf. 4.4.4.13), modified starch and cellulose, e. g., carboxymethyl cellulose (4.4.4.17.2), also play a role.

From corn starch, e. g., non-sweet oligosaccharides (maltodextrins, DE5) which dissolve completely in hot water are obtained. When this solution is cooled, a gel is formed which has the texture of edible oil. It can partially replace fat, e. g., in margarine, allowing a 35% reduction of the energy content.

8.16.2 Synthetic Fat Substitutes

Energetically inefficient fat substitutes can basically be made as follows:

- replacement of glycerol with other alcohols,
- replacement of the usual fatty acids with branched, polybasic or especially long-chain carboxylic acids,

- introduction of inverse ester bonds (retrofats),
- use of ether instead of ester bonds.

8.16.2.1 Carbohydrate Polyester

Mono-, oligo- and polysaccharides yield fatlike products when esterified with fatty acids. In general, the starting material is sucrose as the acetate, which is melted with fatty acid alkylesters in the presence of alkali metals. The degree of esterification of sucrose should be high because otherwise the ester bonds are hydrolyzed in the gastrointestinal tract. In the best known product, *Olestra*®, 6–8 OH groups are esterified with fatty acids 8:0–12:0. This product is tasteless and thermally stable so that it can be as strongly heated as an edible fat during baking and frying.

8.16.2.2 Retrofats

These are esters of polybasic acids (e. g., malonic acid, citric acid, propane 1,2,3-tricar-boxylic acid, butane 1,2,3,4-tetracarboxylic acid) with long-chain alcohols.

8.17 Thickening Agents, Gel Builders, Stabilizers

A number of polysaccharides and their modified forms, even at low concentrations, are able to increase a system's viscosity, to form gels and to stabilize emulsions, suspensions or foams. These compounds are also active as crystallization inhibitors (e. g. in confections, ice creams) and are suitable for aroma encapsulation, as is often needed for dehydrated food. These properties make polysaccharides important additives in food processing and storage. The compounds of importance, together with their properties and utilization, were described in detail in the chapter on carbohydrates. Among proteins, gelatin is an important gel-forming agent used widely in food products (cf. 12.3.2.3.1).

8.18 Humectants

Some polyols (1,2-propanediol, glycerol, mannitol, sorbitol) have distinct hygroscopic properties and act as humectants, i. e. additives for retaining food moisture and softness and inhibiting crystallization. They are often required in a confectionery product. When glycerol or sorbitol is added to mashed vegetables or fruits or in the production of other powdered foods before the final drying stage, the dehydrated products have improved rehydration characteristics.

8.19 Anticaking Agents

Some food products, such as common salt, seasoning salt (e. g. a mixture of onion or garlic powder with common salt), dehydrated vegetable and fruit powders, soup and sauce powders and baking powder, tend to cake into a hard lump. Lumping can be avoided by using any of a number of compounds that either absorb water or provide protective hydrophobic films. Anticaking compounds include sodium, potassium and calcium hexacyanoferrate (II), calcium and magnesium silicate, tricalcium phosphate and magnesium carbonate.

8.20 Bleaching Agents

Bleaching is used primarily in flour production. The removal of yellow carotenoids by oxidation can be achieved by a number of compounds that, in addition to bleaching, improve the baking quality of flour. Examples of some approved common bleaching agents are Cl_2, ClO_2, NOCl, NO_2 and N_2O_4. Lipoxygenase enzyme also has an efficient bleaching activity.

8.21 Clarifying Agents

In some beverages, such as fruit juices, beer or wine, turbidity and sediment formation can occur with the involvement of phenolic compounds, pectins and proteins. These defects can be corrected by: (a) partial enzymatic degradation of pectins and proteins; (b) removal of phenolic compounds with the aid of gelatin, polyamide or polyvinyl pyrrolidone powders; and (c) by protein removal with bentonite or tannin. Bentonite consists of hydrous aluminium silicate,

$Al_2SiO_9(OH)_x$, and changing amounts of iron, calcium and magnesium salts.

8.22 Propellants, Protective Gases

Food sensitive to oxidation and/or microbial spoilage can be stored in an atmosphere of protective gas or a gas mixture (N_2, CO_2, CO, etc.; modified or controlled atmosphere storage). This is often a suitable method for lengthening the shelf life of food.

Liquid food can be filled into pressurized containers and, when needed, using a propellant, discharged in the form of a cream or paste (e. g. cream cheese, ketchup), a foam (whipping cream) or a mist (herb or spice extracts in oil; liquid barbecue smoke). Propellants used are N_2, N_2O, and CO_2.

Due to its low solubility in water, fat and oil, N_2 is used preferentially as a propellant when foam formation is not desired (ketchup). On the other hand, gases such as N_2O and CO_2 are preferred for foam formation (whipped cream) due to their good solubility in water.

8.23 References

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