16 Legumes

16.1 Foreword

Ripe seeds of the plant family *Fabaceae*, known commonly as "legumes" or "pulses", are an important source of proteins for much of the world's population*. The extent of the production of major legumes is illustrated in Table 16.1. Legumes contain relatively high amounts of protein (Table 16.2). Hence, they are an indispensable supply of protein for the "third world". Soybeans and peanuts are oil seeds (cf. 14.3.2.2.5) and, even in industrialized countries, are used as an important source of raw proteins.

With regard to the biological value, legume proteins are somewhat deficient in the S-containing amino acids (Table 16.3 and 1.8).

Antinutritive substances, e. g., allergenic proteins, proteinase inhibitors, lectins and cyanogenic glycosides, are found in food raw materials. These substances will be described in this chapter since a large variety have been identified in legumes.

16.2 Individual Constituents

16.2.1 Proteins

About 80% of the proteins from soybean can be extracted at pH 6.8. A large number of these proteins can be precipitated by acidification at pH 4.5 (cf. Figure 1.54). This pH-dependent solubility is used in large-scale preparations of soy proteins.

Fractionation of legume proteins using solubility procedures, as applied to cereals by *Osborne* (cf. 15.2.1.2), yields three fractions: albumins, globulins, and glutelins, with globulins being predominant (Table 16.4).

16.2.1.1 Glubulines

The high content of globulins in seeds indicates that they function mostly as storage proteins, which are mobilized during the course of germination.

The globulin fraction can be separated by ultracentrifugation or chromatography into two major components present in all the legumes: *vicilin* (∼7S) and *legumin* (∼11S). Legumin from soybeans is called glycinin and from peanuts is called arachin. Molecular weights and sedimentation coefficients for the 7 S and 11 S globulins isolated from various legumes are presented in Table 16.5.

The 11 S globulins originate from a protein precursor ($M_r \sim 60,000$) which is split into an acidic α-polypeptide (pI ∼ 5) and a basic β-polypeptide (pI ∼ 8.2) by cleaving the peptide bond between Asn (417) and Gly (418) (cf. Table 16.6). These two polypeptides are connected by a disulfide bridge between Cys (92) and Cys (424) and are regarded as one subunit. Six such subunits join to give 11 S globulin, the hydrophobic β-polypeptides evidently forming the core of the subunits and of the entire structure. Little is known about the tertiary and quaternary structure. On the other hand, the amino acid sequences of the subunits of the 11 S globulins of a number of legumes are known. They were mainly derived from the nucleotide sequences of the coding nucleic acids. As an example, Table 16.6 shows the sequences of legumin J from the pea (*Pisum sativum*) and glycinin A_2B_{1a} from the soybean (*Glycine max*). Homology exists between the 11 S globulins of different legumes. Variable regions are primarily found in the acidic α-polypeptide, while the basic β-polypeptide is conservative with slight variability in the region of the C-terminal. Conserved residues are uniformly distributed throughout the sequence. The α/β cleavage site is conserved in all the proteins studied until now (cf. Table 16.7). Thus,

* Semi-ripe peas and beans are considered as vegetables (cf. Chapter 17).

Table 16.1. World production of seed legumes, 2006 (1,000 t)

Continent	Pulses *Legumes total*[a]	Beans[b]	Broad beans	Peas
World	60,194	19,559	4577	10,563
Africa	11,111	2856	1321	382
America, Central	2185	1853	37	8
America, North	6025	1430	18	3405
America, South and Caribbean	6865	6153	158	94
Asia	28,505	8701	2256	2392
Europe	6841	404	719	3898
Oceania	846	15	104	392

Continent	Chick peas	Lentils	Soybeans	Groundnuts *Peanuts*[c]
World	8241	3455	221,501	47,768
Africa	324	105	1417	8967
America, Central	163	7	124	175
America, North	231	928	91,203	1479
America, South and Caribbean	170	16,759	98,885	1026
Asia	7365	2316	26,334	36,258
Europe	43	48	3607	9
Oceania	108	41	55	29

Country	*Pulses* Legumes, grand total
India	14,264
China	5557
Canada	4072
Brazil	3448
Nigeria	3091
Myanmar	2571
USA	1953
Russian Fed.	1764
Mexico	1663
Turkey	1550
France	1347
Ethiopia	1265
Iran	1007
UK	830
Australia	803
Pakistan	803
∑ (%)[d]	76

Country	Beans
Brazil	3437
India	3174
China	2007
Myanmar	1700
Mexico	1375
USA	1057
Kenya	532
Uganda	424
Canada	373
Indonesia	327
Argentina	323
∑ (%)[d]	75

Country	Broad beans
China	2100
Ethiopia	599
Egypt	315
France	290
Morocco	181
Sudan	138
United Kingdom	130
Australia	104
Italy	83
Spain	76
∑ (%)[d]	90

[a] Without soybeans and peanuts.
[b] Without broad beans.
[c] With hull included.
[d] World production = 100%.

Table 16.1. (Continued)

Country	Peas	Country	Chick peas	Country	Lentils
Canada	2806	India	5600	India	950
Russian Fed.	1158	Turkey	552	Canada	693
China	1140	Pakistan	480	Turkey	623
France	1010	Iran	293	USA	235
India	800	Canada	182	Syria	165
USA	599	Myanmar	172	Nepal	158
Ukraine	485	Mexico	163	China	150
Australia	360	Ethiopia	125	Bangladesh	120
Germany	288	Australia	108	Iran	113
Iran	265	Morocco	66	Ethiopia	65
∑ (%)[d]	84	∑ (%)[d]	94	∑ (%)[d]	95

Country	Soybeans	Country	Groundnuts *Peanuts*
USA	87,670	China	14,722
Brazil	52,356	Indonesia	14,700
Argentina	40,467	India	4980
China	15,500	Nigeria	3825
India	8270	USA	1479
Paraguay	3800	Myanmar	910
Canada	3533	Sudan	540
Bolivia	1350	Ghana	520
Ukraine	889	Argentina	496
Russian Fed.	807	Viet Nam	465
∑ (%)[d]	97	∑ (%)[d]	89

Table 16.2. Chemical composition of legumes[a]

Name	Systematic name	Crude protein[b] (%)	Lipid (%)	Available carbohydrates (%)	Dietary fiber (%)	Minerals (%)
Soybeans	*Glycine hyspida max*	41.0	19.6	7.6	24.0	5.5
Peanuts	*Arachis hypogaea*	31.4	50.7	7.9	12.3	2.7
Peas	*Pisum sativum*	25.7	1.4	53.7	18.7	3.0
Garden beans	*Phaseolus vulgaris*	24.1	1.8	54.1	19.2	4.4
Runner beans	*Phaseolus coccineus*	23.1	2.1	n.a.	n.a.	3.9
Black gram	*Phaseolus mungo*	26.9	1.6	46.3	n.a.	3.6
Green gram (mungo beans)	*Phaseolus aureus*	26.7	1.3	51.7	21.7	3.8
Lima beans	*Phaseolus lunatus*	25.0	1.6	n.a.	n.a.	3.9
Chick peas	*Cicer arietinum*	22.7	5.0	54.6	10.7	3.0
Broad beans	*Vicia faba*	26.7	2.3	n.a.	n.a.	3.6
Lentils	*Lens culinaris*	28.6	1.6	57.6	11.9	3.6

[a] The result are average values given as weight-%/dry matter.
[b] N × 6.25.
n.a.: not analyzed.

cleavage of the protein precursor evidently occurs through the same, very specific, but not yet characterized proteinase. With a few exceptions, the 11 S globulins are not glycosylated.

Table 16.3. Essential amino acids in legumes (g/16 g N)

Amino acid	Soybean	Broad bean
Cystine	1.3	0.8
Methionine	1.3	0.7
Lysine	6.4	6.5
Isoleucine	4.5	4.0
Leucine	7.8	7.1
Phenylalanine	4.9	4.3
Tyrosine	3.1	3.2
Threonine	3.9	3.4
Tryptophan	1.3	n.a.
Valine	4.8	4.4

n.a.: not analyzed.

Table 16.4. Legumes: protein distribution (%) by *Osborne* fractions

Fraction	Soybeans	Peanuts	Peas	Mungo beans	Broad beans
Albumin	10	15	21	4	20
Globulin	90	70	66	67	60
Glutelin	0	10	12	29	15

Table 16.5. Molecular weight and sedimentation coefficient of the 7 S and 11 S globulins from legumes

Legume	7 S globulin		11 S globulin	
	Sedimentation coefficient	Mol. weight (kdal)	Sedimentation coefficient	Mol. weight (kdal)
Soybeans	7.9 ($S_{20,w}$)	193	12.3 ($S_{20,w}$)	360
Peanuts	8.7 (S_{20})	190	13.2 ($S_{20,w}$)	340
Peas	8.1 (S_{20})		13.1 (S_{20})	398
Garden beans	7.6 ($S_{20,w}$)	140	11.6 ($S_{20,w}$)	340
Broad beans	7.1 ($S_{20,w}$)	150	11.4 ($S_{20,w}$)	328

The 7 S globulins are made of three subunits ($M_r \sim 50{,}000$) which can be identical or different (homo- and heteropolymeric forms). There is little information available on the tertiary and quaternary structure. The subunits can consist of up to three polypeptides (α, β, γ) which are formed from the intact subunit (precursor protein) by proteolysis. Since the amino acid sequences of the α/β (239/240 in Table 16.8) and β/γ (376/377 in Table 16.8) cleavage sites are variable (Table 16.9), intact subunits and subunits with only one cleaved bond are observed, unlike the behavior of the 11 S globulin subunits. Thus, the bond between N (376) and D (377) in vicilin 47k is cleaved, but corresponding ED bonds in other vicilins are evidently not split (cf. Table 16.9).

The amino acid sequences of the 7 S globulin subunits of a number of legumes are known and were mainly derived from the nucleotide sequences of the coding nucleic acids. Table 16.8 shows the sequences of phaseolin from the garden bean (*Phaseolus vulgaris*), vicilin from the pea (*Pisum sativum*), and β-conglycinin (β) from the soybean (*Glycine max*). Sequence homology, which is more pronounced than in the 11 S globulins, exists between the proteins of various legumes. Variable domains are found in the N- and C-terminal regions, but not inside the structure.

The 7 S globulins are glycosylated to different extents. The carbohydrate content is 0.5–1.4% in vicilin from peas, 1.2–5.5% in phaseolin from garden beans, and 2.7–5.4% in β-conglycinin from soybeans. The structures of the oligosaccharide residues are partly known. In β-conglycinin, for example, 6–8 mannose residues are bound to Asn in a branched structure via two N-acetylglucosamine residues.

Under non-denaturing conditions, the 11 S and 7 S globulins exhibit a tendency towards reversible dissociation/association, which greatly depends on the pH value and the ionic strength. According to their behavior, they can be attributed to different types. The 11 S globulins are relatively more stable than the 7 S globulins. They noticeably associate only in the region of the isoelectric point, isoelectric precipitation occurring at low ionic strength (cf. 16.3.1.2.1).

If at pH 7.6, the ionic strength is reduced from $\mu = 0.5$ to $\mu < 0.1$, soybean 11 S globulin dissociates stepwise (α, β: acidic and basic proteins):

$$11\,S(6\,\alpha\beta) \rightarrow 7.5\,S(3\,\alpha\beta) \rightarrow 3\,S(\alpha\beta) \qquad (16.1)$$

Complete dissociation occurs when the disulfide bonds are reduced in the presence of protein-unfolding agents, such as urea or SDS:

$$(\alpha\beta) \rightarrow \alpha + \beta \qquad (16.2)$$

Soybean 7 S globulin has similar properties, as illustrated in Fig. 16.1. Hence, its molecular weight is also strongly dependent on pH and ionic strength.

Table 16.6. Amino acid sequences of the α/β subunits[a] of 11 S globulins, 1) legumin J (*Pisum sativum*) and 2) glycinin A_2B_{1a} (*Glycine max*)

```
  1                 10                  20                  30                  40                  50                  60
1 L ATSSEFDR L ---NQCQLD S INALEPDHR V VSEAGLTET WNPNHPELKC A GVSL I RRTI D
2 - ------LRE Q AQQNECQIQ K LNALKPDNR I ESEGGFIET WNPNNKPFQC A GVAL S RCTL N
  61                70                  80                  90                 100                 110                 120
1 P NGLHLPSF S PSPQLIFII Q GKGVLGLSF P GCPETYEEP R SSQSRQ--E S RQ-------Q
2 R NALRRPSY T NGPQEIYIQ Q GNGIFGMIF P GCPSTYQEP Q ESQ--QRG- R SQ---R--P Q
 121                130                 140                 150                 160                 170                 180
1 Q G--------- ---------- ---------- ---------- --DSHQKVRR F RKGDIIAIP S GIPYWTYNH G
2 - ---------- ---------- ---------- ---------- --DRHQKVHR F REGDLIAVP T GVAWWMYNN E
 181                190                 200                 210                 220                 230                 240
1 D EPLVAISL L DTSNIANQL D STPRVFYLG G NPETEFPET Q EEQQGRHR- Q KHSYPVGRR S
2 D TPVVAVSI I DTNSLENQL D QMPRRFYLA G NQEQEF-LK Y QQQQQG--- ---- G SQSQ K
 241                250                 260                 270                 280                 290                 300
1 G HH-QQEEE S EQQNEGNSV L SGFSSEFLA Q TFNTEEDTA K RLRSPRDE- R S-Q I VRVEG G
2 G K--QQEEE ---NEGSNI L SGFAPEFLK E AFGVNMQIV R NLQGENEEE D SGAIVTVKG G
 301                310                 320                 330                 340                 350                 360
1 L RIIKG--R --T------- ------EE-E K -EQ--SH-- - --SHSHREE K EE-------- -
2 L RVTAPAMR K P----QQ-- - --E-EDDDD E EEQ-P--Q- C VET-DKGCQ ----------- -
 361                370                 380                 390                 400                 410                 420
1 - ---------- ----EEEEE E DEEE------ KQ-RS---- - ------EE-R - -----KNGL E
2 R -----OSK R SR------- ---------- ---------- ---------- ---------- ------NGI D
 421                430                 440                 450                 460                 470                 480
1 E TICSAKIR E NIADAARAD L YNPRAGRIS T ANSLTLPVL R YLRLSAEYV R LYRNGIYAP H
2 E TICTMRLR Q NIGQNSSPD I YNPQAGSIT T ATSLDFPAL W LLKLSAQYG S LRKNAMFVP H
 481                490                 500                 510                 520                 530                 540
1 W NINANSLL Y VIRGEGRVR I RSCELPTNT M FDNKLRKGH L VVVPQNFVV A EQAGEEEGL E
2 Y TLNANSII Y ALNGRALVQ V VNCN-G-ER V FDGELQEGG V LIVPQNFAV A AKSQSDN-F E
 541                550                 560                 570                 580                 590                 600
1 Y VVFKTNDR A AVS-HVQQ- - -VFRATPSE V LANAFGLRQ R QVTELKLSG N RGPM-VH-P -
2 Y VSFKTNDR P -SIGNLAGA N SLLNALPEE V IQHTFNLKS Q QARQVK--N N N-PFSFLVP P
 601                610                 620
1 R -SQSQSH
2 Q ESQ--RRA V A
```

[a] The α-polypeptide ends with Asn (417) and the β-polypeptide starts with Gly (418). The two polypeptides are connected by a disulfide bond between Cys (92) and Cys (424). The two remaining Cys residues of the α-polypeptide (16, 49) probably form an intramolecular disulfide bridge. –: space to maximize homology.

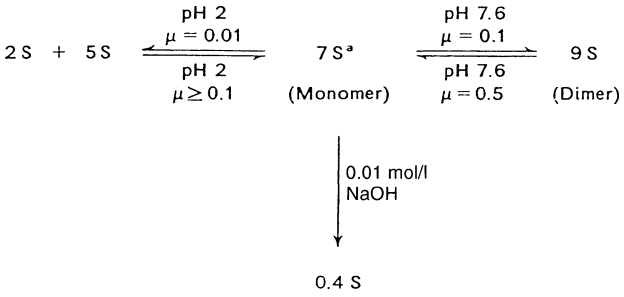


Fig. 16.1. Dissociation and aggregation of the soybean 7 S globulin

[a]Molecular weight: 193 kdal.

Table 16.7. Amino acid sequences in the vicinity of the α/β cleavage site (417/418 in Table 16.6) of sub-units of various 11 S globulins (–: space to maximize homology, ··· sequence not known)

Protein	420
Legumin J (*Pisum sativum*)	– – KNGLEETI CS
Legumin A (*Pisum sativum*)	D– – NGLEETVCT
Glycinin A_2B_{1a}(*Glycine max*)	– – – NGI DETI CT
Glycinin $A_5A_4B_3$ (*Glycine max*)	ETRNGVEENI CT
Glycinin A_3B_4(*Glycine max*)	QTRNGVEENI CT
Glycinin $A_{1a}B_{1b}$(*Glycine max*)	– – – NGI DETI CT
Glycinin $A_{1b}B_2$(*Glycine max*)	. . . NGI DETI CT
Cruciferin(*Brassica napus*)	– – – NGLEETI CS
Legumin β_1(*Vicia faba*)	D– – NGLEETVCT
Legumin B(*Vicia faba*)	– – RNGLEETI CS
Avenin(*Avena sativa*)	– – – NGLEENFCD
Glutelin(*Oryza sativa*)	NGLDETFCT

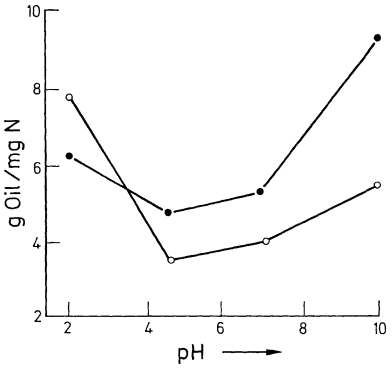


Fig. 16.2. Soybean globulin as an emulsifier. (According to *Aoki* et al., 1980). The capacity of an o/w-emulsion after addition of 11 S globulin (–○–) and 7 S globulin (–●–) is plotted versus pH

The thermal stability of the 11 S and 7 S globulins varies. While 7 S globulin coagulates in a 10% salt solution at 99 °C, 11 S globulin remains in solution. The opposite is true at μ = 0.001. Since dissociated proteins are more easily coagulable thermally than associated proteins, it follows that 11 S globulin is destabilized by dissociation at low ionic stringth, as shown above. Under these conditions, however, the 7 S globulin is stabilized by association.

The amino acid compositions of both major soybean proteins, with the exception of methionine, are very similar (Table 16.10). However, large differences exist in their carbohydrate contents. The 7 S globulin contains 5% carbohydrate and the 11 S globulin less than 1% carbohydrate.

Legume proteins exhibit a marked gelling capacity. The gel properties depend on the protein used and on the production conditions (pH value, ionic species, ionic strength, and temperature). They are suitable for the production of foams and emulsions.

In the pH range of 4–10, the 7 S globulin is a better emulsifier than the 11 S globulin, when the capacity (Fig. 16.2) and the stability of an o/w emulsion are compared. Partial acid hydrolysis improves the emulsifier properties.

16.2.1.2 Allergens

A food allergy is a diseased state caused by immunologic reactions which are induced by the intake of food. All other reactions which are

Table 16.8. Amino acid sequences of subunits[a] of the 7 S globulins, 1) β-conglycinin α′ (*Glycine max*), 2) phaseolin (*Phaseolus vulgaris*) and 3) vicilin (*Pisum sativum*)

```
   1                 10                  20                  30                  40                  50
1[b]- - - DEDEEQDKES QESEG SES QREPRRHKNKNPFHFNSKR- F QTLFKNQ
2 A  TS LREEEE- - - - S QD- - - - - - - - - - - - - - - - - NPFYFNSDNS  WNTLFKNQ
3 -  - - - - - - - - - R- - - - - - - -SDP Q- - - - - - - - - NPFI FKSNK-F QTLFENE
   51                60                  70                  80                  90                  100
1 Y  GH VRVLQRFNKRS QQLQN LRDYRI LEF NSKPNTLLLPHHAD A DYLI VI L
2 Y  GHI RVLQRFDQQS KRLQN LEDYRL VEF RSKPETLLLPQQAD A ELLLVVR
3 N  GHI RLLQKFDQRS KI FEN-QNYRLLEYKSKPHTI FLPQHTDA D YI LVVL
   101               110                 120                 130                 140                 150
1 N  GT AI LTLVNNDDR DSY- N LQS GDA- L - - - - - RVPAGTTFYV  V NPDNDEN
2 S  GS AI LVLVKPDDR REYFF LTQGDNPI F SDNQKI P- - - - - - - - - - - - - - - - - -
3 S  GK AI LTVLKPDDR NSF- N -ERGDT- I - - - - - KLP- - - - - - - - - - - - - - - -
   151               160                 170                 180                 190                 200
1 L  RMI AGTTFYVVNP DNDEN LRMI TLAI P VNKPERFESFFLSS T QAQQSYL
2 -  - - - AGTI FYLVNP DPKED LRI I QLAMP VNNPQ- I HEFFLSS T EAQQSYL
3 -  - - - AGTI AYLVNR DDNEE LRVLDLAI P VNRPGQLQSFLLSG N QNQQNYL
   201               210                 220                 230                 240                 250
1 Q  GF SKNI LEASYDT KFEEI NKVLFGREE GQQ- Q- - - G- - - EE R - - - - LQE
2 Q  EF SKHI LEASFNS KFEEI NRVLF- EEE GQQ- - - - - - - - - - - - - - - - - EEGQQE
3 S  GF SKNI LEASFNT DYEEI EKVLL- EEHEKETQHRRSLKD- K R - QQSQEE
   251               260                 270                 280                 290                 300
1 S  VI VEI SKKQI REL SKHAK SSSRKTI SSEDKPFNLGSRDPI Y S NKLGKLF
2 G  VI VNI DSEQI EEL SKHAK SSSRKS HS - - - KQ- D- - - - NTI - G NEFGNLT
3 N  VI VKL SRGQI EEL SKNAK STSKKSVSSESEPFNLRSRGPI Y S NEFGKFF
   301               310                 320                 330                 340                 350
1 E  I T - QRN- PQLRDL DVFLS VVDMNEGALFLPHFNSKAI VVLV I NEGEANI
2 E  RT - D- N- - - - S - L NVLI S SI EMKEGALFVPHYYSKAI VI LV V NEGEAHV
3 E  I T PEKN- PQLQDL DI FVN SVEI KEGSLLLPHYNSRAI VI VT V NEGKGDF
   351               360                 370                 380                 390                 400
1 E  LVGI K- - - - - - - - - - - - EQ QQRQQQ- - - EEQ- - P- - - - - LEV R KYRAELS
2 E  LVGPK- - - - - - - - - - - - GN - - - - KE- - - - - - - - - - - T - LEF E SYRAELS
3 E  LVGQR- - - - - - - - - - - NEN QQEQRKEDDEEEEQGEEEI NKQV Q NYKAKLS
   401               410                 420                 430                 440                 450
1 E  QDI FVI PAGYP VMVNATS DLNF - F - AFG- - - - - I NAENNQR N FLAGSKD
2 K  DDVFVI PAAYP VAI KATS NVNF - - TGFG- - - - - I NANNNNR N LLAGKTD
3 S  GDVFVI PAGHP VALKASS NLDL - L - GFG- - - - - I NAENNQR N FLAGDED
   451               460                 470                 480                 490                 500
1 N  VI SQI PSQV- - QE - - - LA FPRS AKDI E NLI KSQ- SESYFVD A - - - - QPQ
2 X  VI SSI GRALDGKD VLGLT FSGS GEEVMKLI NKQ- SGSYFVD G HHHQQEQ
3 N  VI SQVQRPV- - KE - - - LA FPGS AQEVDRI LENQ- KQSHFAD A - - - - QPQ
   501               510                 520                 530
1 Q  KE E- GN- - - - - - KGRKGP LSSI LRAF - Y
2 Q  K- - - GS HQQEQQK GRKG- - - - - - - AF V Y
3 Q  RE - RGS - - RE - TR DR- - - LSS V
```

[a] α/β cleavage site: 239/240, β/γ cleavage site: 376/377; -- space to maximize homology.

[b] In order to show the homology clearly, the N-terminal of β-conglycin was omitted in the table. It consists of the following sequence:

V E E E E E C E E G Q I P R P R P Q H P E R E R Q Q H G E K E E D E G E Q P R P F P F P R P R Q P H Q
E E E H E Q K E E H E W H R K E E K H G G K G S E E E Q D E R E H P R P H Q P H Q K E E E K H E W
H K Q E Q K H Q G K E S E E E E E D Q

Table 16.9. Amino acid sequences in the vicinity of the α/β cleavage site (239/240 in Table 16.8) and the β/γ cleavage site (376/377 in Table 16.8) of subunits of various 7 S globulins (–: space to maximize the homology)

Protein	α/β 240		β/γ 377	
Phaseolin (*Phaseolus vulgaris*)	–	–	–	–
Vicilin (*Pisum sativum*)	K	D	E	D
Convicilin (*Pisum sativum*)	R	D	E	D
Vicilin 50k (*Pisum sativum*)	R	D	E	D
Vicilin 47k (*Pisum sativum*)	K	D	N	D
β-Conglycinin α′ (*Glycine max*)	–	–	–	–
β-Conglycinin β (*Glycine max*)	–	–	–	–
α-Gossypulin B (*Gossypium sp.*)	–	–	–	–

Table 16.10. Amino acid composition of 7 S and 11 S globulins from soybeans

Amino acid	g amino acid/100 g protein	
	7 S globulin	11 S globulin
Asx	11.18	13.10
Thr	3.14	3.37
Ser	4.79	4.16
Glx	17.54	18.03
Pro	5.21	5.40
Gly	3.37	3.97
Ala	3.66	3.55
Cys	1.52	1.44
Val	4.68	5.05
Met	0.43	1.84
Ile	4.99	4.69
Leu	8.15	7.17
Tyr	3.51	4.05
Phe	5.55	5.73
His	2.32	2.22
Lys	6.26	4.88
Arg	7.37	7.75

not based on specific immunologic mechanisms are classified as food intolerance. It has been estimated that 1–2% of adults and up to 8% of children suffers from a food allergy. Persons with a genetic predisposition develop a food allergy in two stages. In the first stage, sensitization, the allergen (= antigen, cf. 2.6.3) initiates reactions which lead to the formation of allergen-specific antibodies of the immunoglobulin class E (IgE). This process proceeds without discernible changes in the condition of the affected person. In the second stage, the allergen binds to the IgE molecules, leading to the release of pharmacologically active mediators, e. g., histamine, leucotriens, prostaglandin D_2. These mediators can start inflammations. The specificity of IgE applies to certain structural features of the allergen. Accordingly, the IgE can react in a cross reaction not only with the antigen which generated the IgE, but also with other antigens which have partial structures that correspond with those of the sensitizer. For example, birch pollen, which enters the body via the respiratory tract, produces IgE which can cross react with proteins from apples, hazelnuts, celery or carrots, triggering an allergy.

A region of 5–7 amino acids of the antigen is responsible for the binding to IgE. It is called the epitope. It is either a section of the sequence (linear epitope) or, which is more often the case, amino acid residues which have come together as a result of the folding of the protein (conformational epitope).

Sensitization can occur not only by the inhalation of allergens, but also in the digestive tract. This is the cause of the allergenic effect of milk, egg and fish proteins and of some proteins of plant origin on sensitive persons. Allergens are proteins or glycoproteins occurring in food. Among plant foods, primarily peanuts and other legumes, hazel nuts and other nuts as well as celery and some spices can be allergenic. Examples of some well characterized plant food allergens are listed in Table 16.11. Some allergens, e. g., Mal dl (Table 16.11), show a high degree of correspondence in their sequence with the main allergens of birch pollen, many kinds of stone fruit and pomaceous fruit, celery (Api g1) and carrots. The thermal stability of the allergens varies (Table 16.11). While the allergens of soybeans survive cooking by microwaves (25 min), the allergen Api g1 is so thermolabile that it is no longer detectable in celery after 30 min (100 °C).

16.2.2 Enzymes

Various forms of lipoxygenase (cf. 3.7.2.2) are of interest in food chemistry since they strongly affect the legume aroma.

Urease, which hydrolyses urea,

$$NH_2 - CO - NH_2 + H_2O \rightarrow CO_2 + 2NH_3 \qquad (16.3)$$

Table 16.11. Examples of allergens in plant foods

Food	Allergen	Molecular weight	Characteristics	Stability[a]
Peanuts	Ara h1	$6.3–6.6 \times 10^4$	7 S Globulin (vicilin), glycoprotein	High
	Ara h2	17,000	Glycoprotein	Medium
Soybeans	Glycinin	$3.5–3.6 \times 10^5$		Medium
	β-Conglycinin	156,000	Glycoprotein	Unknown
	2 S Globulin	18,000		Medium
	Kunitz trypsin inhibitor	21,500		Medium
Mustard	Sin a1	14,000	2 S Albumin	High
Rice	16 kDa allergen	16,000	Albumin	Medium
Celery root	Api g1	ca. 16,000		Low
Celery root	Profilin	$1.5–1.6 \times 10^4$	2 Isoforms	Medium
Apple	Mal d1	$1.7–1.8 \times 10^4$		Low
Apple	Mal d3	11,410		High
Peach	Pru p1	9178		High
Apricot	Pru ar3	9178		High
Cherry	Pru av3	9200		High

[a] Thermal stability.

occurs in soybeans in relatively high concentration. Heat treatments of soy preparations can be detected by measuring the activity of this enzyme.

16.2.3 Proteinase and Amylase Inhibitors

16.2.3.1 Occurrence and Properties

Inhibitors of hydrolases, themselves proteins, form stoichiometric inactive complexes with the enzymes and are distributed in microorganisms, plants, and animals. Apart from the thoroughly examined group of proteinase inhibitors, some proteins that inhibit amylases are known.

Of the large number of known proteinase inhibitors, only those compounds found in foods are of interest in food chemistry. These include in particular the inhibitors in egg white, plant seeds, and plant nodules. Table 16.12 shows the most important sources of proteinase inhibitors, which have molecular weights between 6000 and 50,000. The specificity for proteinases varies considerably. Some inhibitors inhibit only trypsin, many act on both trypsin and chymotrypsin, and others inhibit microbial or plant proteinases as well, e. g., subtilisin or papain. Proteinase inhibitors are often located in, but not limited to, the seeds of plants. The seeds of legumes (soybeans ca. 20 g/kg, white beans ca. 3.6 g/kg, chick peas ca. 1.5 g/kg, mungo beans ca. 0.25 g/kg), the tubers of Solanaceae (potatoes ca. 1–2 g/kg), and cereal grains (ca. 2–3 g/kg) contain especially high concentrations. The inhibitor content greatly depends on the variety, degree of ripeness, and storage time.

Often, several different inhibitors are found in plant materials. They differ in their isoelectric points and also in their specificity for proteinases, specific activities and thermal stabilities. For example, in the more than 30 legumes analyzed so far, nine inhibitors have been identified and five partially purified.

Food which contains inhibitors might cause nutritional problems. For example, feeding rats and chickens with raw soymeal leads to reversible pancreatic blistering. A consequence of excessive secretion of pancreatic juice is increased secretion of nitrogen in the feces. Furthermore, growth inhibition occurs which can be eliminated by incorporating methionine, threonine and valine into the diet.

These findings indicate that the poor growth rate might be due to some amino acid deficiencies, which are a result of increased N-excretion. All the possible effects of proteinase inhibitors are not fully understood.

Table 16.12. Proteinase inhibitors of aninal and plant origin

Source/Inhibitor	Molecular weight	Inhibition of[a]						
		T	CT	P	Bs	AP	SG	PP
Animal tissues								
Bovine pancreas								
Kazal inhibitor	6153	+	−	−				
Kunitz inhibitor	6512	+	+	−	−	−	+	
Chicken egg								
Ovomucoid	27–31,000	+	−		−			
Ovoinhibitor	44–52,000	+	+	−	+	+		
Ficin-papain-inhibitor	12,700	−	−	+	−			
Plant tissues								
Cruciferae								
Raphanus sativus[b]	8–11,200	+	±	−	+	+		
Brassica juncea[b]	10–20,000	+	±					
Leguminosae								
Arachis hypogaea[b]	7500–17,000	+	+					
Cicer arietinum[b]	12,000	+	+					
Glycine max								
Kunitz inhibitor	21,500	+	+	−	−			
Bowman–Birk inhibitor	8000	+	+	−	+			
P. coccineus[c]	8800–10,700	+	+					
P. lunatus[c]	8300–16,200	+	+	−	−	±		
P. vulgaris[c]	8–10,000	+	+	−	−			
Pisum sativum[b]	8–12,800	+	+					
Vicia faba[b]	23,000	+	+					
Convolvulaceae								
Ipomoea batatas[b]	23–24,000	+	−	−	−	−		
Solanaceae								
Solanum tuberosum[b]	22–42,000[c]	±	±	−	±	±	±	±
Bromeliaceae								
Ananas comosus[b]	5500	+	+					
Gramineae								
Hordeum vulgare[b]	14–25,000	±	−	−	±	±	±	
Oryza sativa[b]		±	−	+				
Secale cereale[b]	10–18,700	+	+	−				
Triticum aestivum[b]	12–18,500	±	−	−				
Zea mays[c]	7–18,500	+	+	−				

[a] T: trypsin, CT: α-chymotrypsin, P: papain, Bs: Bacillus subtilis proteases, AP: Aspergillus spp. proteases, SG: Streptomyces griseus proteases, PP: Penicillium spp. proteases, +: inhibited, −: not inhibited, ±: inhibited by some inhibitors of the particular source.
[b] The properties of different inhibitors are combined.
[c] Subunits 6–10,000.

16.2.3.2 Structure

Many proteinase inhibitors have been isolated and their structures elucidated. The active center often contains a peptide bond specific for the inhibited enzyme, e. g., Lys-X or Arg-X in trypsin inhibitors and Leu-X, Phe-X or Tyr-X in chymotrypsin inhibitors (Table 16.13, 16.14). In addition, inhibitors are known that inhibit trypsin and chymotrypsin and contain only one trypsin-specific peptide bond at the active center, e. g., *Kunitz* inhibitors from bovine pancreas and soybeans (cf. Table 16.14). Some double-headed inhibitors contain two different active

Table 16.13. Active centers in *Bowman–Birk* type inhibitors

Inhibited enzyme	Active center	Occurrence
Trypsin	Lys-X	Adzuki bean (API II) Chickpea Garden bean (GBI I) Lima bean (LBI IV) Soybean (BBI) Wisteria (inhibitor II)
	Arg-X	Garden bean (GBI II) Soybean (inhibitor C-II) Soybean (inhibitor D-II)
α-Chymotrypsin	Leu-X	Lima bean (LBI IV) Soybean (BBI)
	Tyr-X	Adzuki bean (API II) Chickpea
	Phe-X	Garden bean (PVI 3) Lima bean (LBI IV′)
Elastase	Ala-X	Garden bean (GBI II) Soybean (inhibitor C-II)

Table 16.14. Amino acid sequences in the region of the active centers of proteinase inhibitors

Inhibitor	Sequences at the active center[a]	Inhibited enzyme[b]
Bovine pancreas	18	
Kazal inh.	NGCPRIYNPVCG	T
	15	
Kunitz inh.	TGPCKARIIRYF	T,CT
Soybean	63	
Kunitz inh.	SPSYRIRFIAFG	T,CT
	16	
Bowman–Birk inh.	CACTKSNPPQCR	T
	43	
	CICALSYPAQCF	CT
Lima bean	26	
inhibior IV	CLACTKSIPPQCR	T
	53	
	CICTLSIPAQCV	CT
	60	
Potato, subunit A	PVVGMDFRCDRV	CT
	25	
Corn	GIPGRLPPLZKT	T

[a] Active center underlined.
[b] T: trypsin, CT: α-chymotrypsin.

centers, which, e. g., are both directed towards trypsin or towards trypsin and chymotrypsin. An example of the latter type is represented by the *Bowman–Birk* inhibitors found in legumes (cf. Table 16.14). Their reactive centers are localized in two homologous domains of the peptide chain, each of which form a 29 membered ring via a disulfide bridge (cf. 1.4.2.3.2). In this way, the centers are exposed for contact with the enzyme. An active center can also be exposed by another suitable conformation, as is the case with the *Kunitz* inhibitor from soybeans.

X-ray analyses of the trypsin inhibitor complex show that 12 amino acid residues of the inhibitor are involved in enzyme contact, including the sequence Ser(61)-Phe(66) with the active center Arg(63)-Ile(64).

The double-headed *Bowman–Birk* inhibitor from soybeans was cleaved into two fragments by cyanogen bromide (Met (27)-Arg (28)) and pepsin (Asp (56)-Phe (57)) (cf. Fig. 1.25). Each of these fragments contained an active center and, therefore, inhibited only one enzyme with remaining activities of 84% (trypsin) and 16% (chymotrypsin) compared with the native inhibitor.

Modifications of the active center of an inhibitor result in changes in the properties. For example, Arg (63) of the *Kunitz* inhibitor from soybeans can be replaced by Lys without changing the inhibitory behavior, while substitution by Trp abolishes the inhibition of trypsin and increases the inhibition of chymotrypsin. Indeed, Ile (64) can be replaced by Ala, Leu, or Gly without change in activity, while the insertion of an amino acid residue, e. g., Arg (63)-Glu (63a)-Ile (64), abolishes all inhibition and makes the inhibitor a normal substrate of trypsin.

16.2.3.3 Physiological Function

The biological functions of most proteinase inhibitors of plant origin are unknown. During germination of seeds or bulbs, an increase as well as a decrease in the inhibitor concentration has been observed, but only in a few cases were endogenous enzymes inhibited. It is probable that the inhibitors act against damage to plants by higher animals, insects, and microorganisms. This is indicated by the inhibition of proteinases of the gen-

era *Tribolium* and *Tenebrio* and by the increase in inhibitor concentration in tomato and potato leaves after infection with the potato bug or its larvae. Proteinase inhibitors from the potato also inhibit the proteinases of microorganisms found in rotting potatoes, e. g., *Fusarium solani*.

16.2.3.4 Action on Human Enzymes

Inhibitor activity is normally determined with commercial animal enzymes, e. g., bovine trypsin or bovine chymotrypsin. The evaluation of a potential effect of the inhibitors on human health assumes that the inhibition of human enzymes is known. Present data show that inhibitors from legumes generally inhibit human trypsin to the same extent or a little less than bovine trypsin. On the other hand, human chymotrypsin is inhibited to a much greater extent by most legumes. Ovomucoid and ovoinhibitor from egg white as well as the *Kazal* inhibitor from bovine pancreas do not inhibit the human enzymes. The *Kunitz* inhibitor from bovine pancreas inhibits human trypsin but not chymotrypsin. The data obtained greatly depend not only on the substrate used, but also on the enzyme preparation and the reaction conditions, e. g., on the ratio enzyme/inhibitor. The stability of an inhibitor as it passes through the stomach must also be taken into account in the evaluation of a potential effect (cf. Table 16.15). The *Kunitz* inhibitor of soybeans, for example, is completely inactivated by human gastric juice, but the *Bowman–Birk* inhibitor from the same source is not. The available data show that the average amount of trypsin and chymotrypsin produced by humans per day can be completely inhibited by extracts from 100 g of raw soybeans or 200 g of lentils or other legumes.

Table 16.15. Resistance of inhibitors[a] to pepsin at pH2

Source/Inhibitor	Remaining activity[b](%)
Soybean, *Kunitz* inhibitor	0
Bowman–Birk inhibitor (BBI)	100
extract	30–40
Lima bean, BBI-type inhibitor	70–93
Kidney bean, BBI-type inhibitor	100
Kintoki bean, BBI-type inhibitor	100
Lentil, BBI-type inhibitor	83–100
Chick pea, inhibitors	100
Broad bean, trypsin-chymotrypsin inhibitor	100
Moth bean, trypsin inhibitor	91
Broad bean, trypsin inhibitor	100

[a] Different incubation times.
[b] Against bovine and human trypsin and chymotrypsin.

16.2.3.5 Inactivation

The inactivation of proteinase inhibitors in the course of food processing has been the subject of many studies. In general, the inhibitors are thermolabile and can be more or less extensively inactivated by suitable heating processes. In these processes, both the starting material as well as the process parameters are of great importance (time, temperature, pressure, and water content of the sample) (Table 16.16). Steaming of soybeans for 9 minutes at 100 °C causes an 87% destruction of inhibitors (Table 16.17).
A decrease in the inhibitor activity can also be achieved by soaking. A thermal step can then follow under gentler conditions. Although the processing of soybeans into protein isolates, textured protein, or meat surrogates causes a decrease in the inhibitor activity against trypsin, noticeable activity can still be present (Table 16.18).
Soybeans promote the growth of rats to the same extent as casein when about 90% of the inhibitor activity is eliminated (Table 16.19).

16.2.3.6 Amylase Inhibitors

Relatively thermostable proteins, which have an inhibitory effect on pancreatic amylase, are found in aqueous extracts of navy beans, wheat, and rye. As a result of the high thermostabilty, inhibitor activity is also detectable in breakfast cereals and bread.
The amylase inhibitor of navy beans is instable in the stomach and becomes active only after preincubation with the enzyme in the absence of starch. As a result, it has no measurable influence on the digestion of starch by human beings. Moreover, the average amounts of inhibitor ingested with the food are small compared to the amylase activity present.

Table 16.16. Destruction of trypsin inhibitors by heating

Sample	Process	Destruction (%)
Soy flour	Live steam, 100 °C, 9 min	87
Soy bean	10% $Ca(OH)_2$, 80 °C, 1 h	100
Navy bean	Autoclaving, 121 °C, 5 min	80
	Autoclaving, 121 °C, 30 min	100
	Dry roasting, 196–204 °C, 20–25 s	75
Navy bean	Pressure cooking, 15 min	89
Winged bean	Autoclaving	92
	Soaking + autoclaving	95
Chickpea	Autoclaving	54
Broad bean	Autoclaving, 120 °C, 20 min	90
Horse bean	Autoclaving	100
Black gram	Cooking, 100 °C, 10 min	15
	Autoclaving, 108 °C, 10 min	27
	Autoclaving, 116 °C, 10 min	38
Cow pea	Cooking, 90–95 °C, 45 min	52
	Autoclaving, 121 °C, 15 min	11
	Toasting, 210 °C, 30 min	44
	Toasting, 240 °C, 30 min	22
	Extrusion cooking	19
Peanut	Moist heat, 100 °C, 15 min	100

Table 16.17. Inactivation of soybean trypsin inhibitors by steaming (100 °C)

Steaming (min)	Trypsin inhibitor	
	Concentration (mg/g soy meal)	Inactivation (%)
0	40	0
3	30	25
6	16.5	59
9	5.2	87
12	1.7	96
15	0.9	98

Table 16.18. Inhibition of bovine trypsin activity by some soya products[a]

Product	Extracted with	
	0.125 mol/l H_2SO_4	0.01 mol/l NaOH
Untreated soybean (cv. Caloria)	51.5	33.7
Supro G 10	6.8	15.6
Soyflour	1.1	8.7
TVPU 110 chunks	0	4.1
Flocosoya	0	1.9

[a] A 50% inhibition of mg trypsin/g product; substrate: N^{α}-benzoyl-L-arginine-p-nitroanilide.

Table 16.19. Effect of soybean trypsin inhibitors on the growth of rats

Trypsin inhibitor Amount (mg/100 g diet)	Inactivation (%)	Body weight (g)	Protein efficiency ratio (PER)
887	0	79	1.59
532	40	111	2.37
282	68	121	2.78
119	87	148	3.08
Control (casein)		145	3.35

16.2.3.7 Conclusions

In summary, it can be concluded that many foods in the raw state contain inhibitors of hydrolases. The heating processes normally used in the home and in industry generally inactivate the inhibitors more or less completely, so that damage to human health is not to be expected. As a result of the greatly varying thermal stability of the inhibitors, constant and careful control of raw materials and products is required, especially when new materials and processes are applied.

16.2.4 Lectins

Lectins are sugar-binding proteins which differ from antibodies and enzymes. They are widely distributed in plants, e. g., in more than 600 species of legumes. One method of detection is based on the fact that lectins attach themselves to erythrocytes and cause their precipitation (agglutination). It should be taken into account that some lectins (e. g., those from pinto beans) only agglutinate erythrocytes which have been treated with pronase or trypsin. Alternatively, lectins can be detected by the precipitation of polysaccharides and glycoproteins. The examples in Table 16.20 show that biopolymers which contain N-acetyl galactosamine are preferentially bound, but other sugar specificities also exist.

Most lectins are glycoproteins. In general, they consist of several subunits (Table 16.20), which readily dissociate by a change in pH or ionic strength. A characteristic feature of their amino acid composition is the high content of acidic and hydroxy amino acids and the absence or low content of methionine.

Animal tests have demonstrated that their toxicity often does not parallel hemagglutination activity. Thus, lectins from soybeans and garden beans are toxic, but not those from peas and lentils. These and other observations suggest that it is not the hemagglutination activity but other activities of lectins which are responsible for their toxicity. One toxic effect originates in the, at least partial, resistance of lectins to proteolysis *in vivo*. After reaching the intestinal tract, some lectins attach themselves to the epithelial cells of the intestinal villi and enter the intercellular space, resulting in severe metabolic damage.

After cooking or dry heating, the activities of legume lectins and the associated toxic effects are destroyed. After heating to 100 °C for 10 minutes, e. g., soybeans were free of lectin activity. However, the lectins in some legumes are much more stable.

16.2.5 Carbohydrates

The carbohydrates which are present in legumes are listed in Table 16.21. The major carbohydrate is starch, amounting to 75–80%. The soybean is an exception (Table 16.21), but it contains arabinoxylans and galactans (3.6 and 2.3% respectively). In peanuts, about onethird of the total carbohydrate is starch.

Oligosaccharides in legumes are present in higher concentration than in cereals. Predominant in this fraction are sucrose, stachyose and verbascose (Table 16.21).

After legume consumption, oligosaccharides might cause flatulency, a symptom of gas accumulation in the stomach or intestines. It is a result of the growth of anaerobic microorganisms in the intestines, which hydrolyze the

Table 16.20. Occurrence of lectins in food

Source	Molecular weight (kdal)	Subunits	Glycan-component		Specificity[a]
			% Carbohydrate	Building blocks	
Soybean	122	4	6.0	D-Man, D-GlcNAc	D-GalNAc, D-Gal
Garden beans	98–138	4	4.1	GlcN, Man	D-GalNAc
Jack beans[b]	112	4	0		α-D-Man
Lentils	52	2	2.0	GlcN, Glc	α-D-Man, α-D-Glc
Peas	53	4	0.3		α-D-Man, α-D-Glc
Peanuts	11	4	0		α-D-Gal
Potato	20		5.2	Ara	D-GlcNAc
Wheat	26		4.5	Glc, Xyl, Hexosamine	D-GlcNAc

[a] Precipitates biopolymers that contain the given building blocks (polysaccharides, glycoproteins, lipopolysaccharides).

[b] *Canavalia ensiformis*.

Table 16.21. Carbohydrates in legume flours[a]

Flour	Glucose	Saccharose	Raffinose	Stachyose	Verbascose	Starch
Garden beans	0.04	2.23	0.41	2.59	0.13	51.6
Broad beans	0.34	1.55	0.24	0.80	1.94	52.7
Lentils	0.07	1.81	0.39	1.85	1.20	52.3
Green gram (mungo beans)	0.05	1.28	0.32	1.65	2.77	52.0
Soybean[b]	0.01	4.5	1.1	3.7		0.62

[a] Weight-% of the dry matter.
[b] Defatted flour.

oligo- into monosaccharides and cause their further degradation to CO_2, CH_4 and H_2. Model feeding tests have demonstrated that phenolic ingredients, such as ferulic and syringic acids, inhibit microorganism metabolism and the related flatulency.

16.2.6 Cyanogenic Glycosides

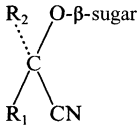
Cyanogenic glycosides (Table 16.22) are present in lima beans and in some other plant foods. Precursors of cyanogenic glycosides are the amino acids listed in Table 16.22. As in the biosynthesis of glucosinolates (cf. 17.1.2.6.5), an aldoxime is initially formed, which is then transformed into a cyanogenic glycoside by means of the postulated reaction pathway shown in Fig. 16.3.

Seeds are ground and moistened in order to detoxify them. This initiates glycoside degradation with formation of HCN (cf. Table 16.23) which, after incubation, is expelled by heating.

The cyanogenic glycoside degradation is initiated by β-glucosidase (Fig. 16.4) which in the cells is separated from its substrate. Once the cell structure is ruptured by seed grinding, the enzyme and the substrate are brought together and the reaction starts.

The substrate specificity of β-glucosidase is governed by an aglycon moiety. Thus, the enzymes present in "*emulsin*", a glycosidase mixture from bitter almonds, hydrolyze not only amygdalin but also other cyanogenic glycosides which are de-

Table 16.22. Cyanogenic glycosides in fruit and some field crops



Glycoside Name	Structure		Sugar	Amino acid precursor	Occurrence (seeds)
	R_1	R_2			
Linamarin	CH_3	CH_3	Glucose	Val	Lima bean Linseed (flax) Cassava
(R)-Lotaustralin	C_2H_5	CH_3	Glucose	Ile	like Linamarin
(R)-Prunasin	Phenyl	H	Glucose	Phe	Prunes
(R)-Amygdalin	Phenyl	H	Gentiobiose	Phe	Bitter almond Apricots Peaches Apples
(S)-Dhurrin	HO–Phenyl	H	Glucose	Tyr	Sorghum sp.

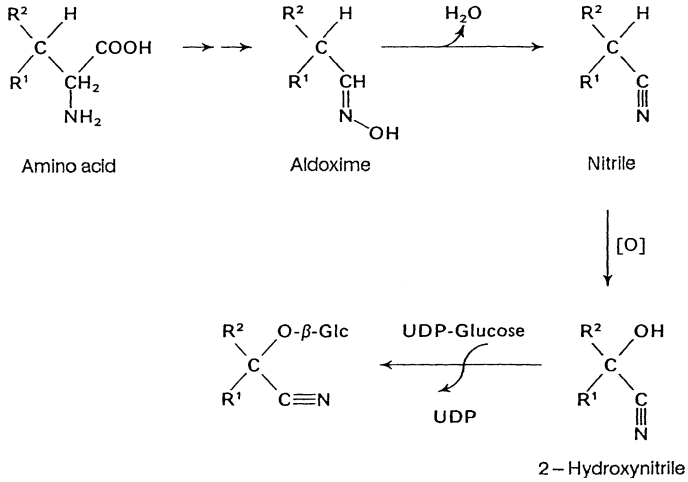


Fig. 16.3. Biosynthesis of cyanogenic glucosides

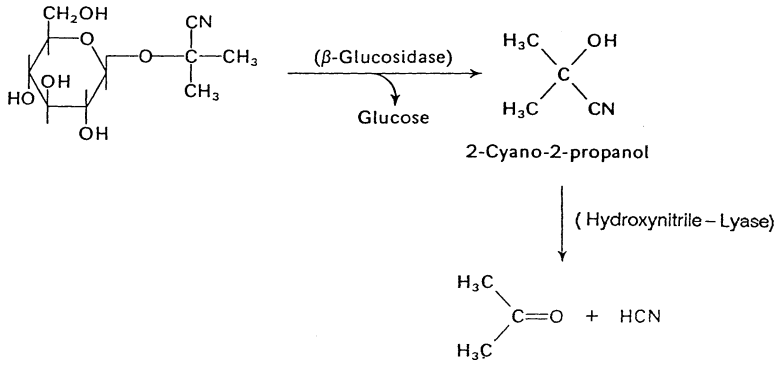


Fig. 16.4. Lima beans: linamarin degradation, resulting in a release of hydrocyanic acid

Table 16.23. Amount of glycoside-bound hydrocyanic acid in food

Food	HCN (mg/100 g)
Lima bean[a]	210–310
Bitter almond	280–310
Sorghum sp.	250
Cassava	110
Pea	2.3
Bean	2.0
Chick pea	0.8

[a] In the United States new cultivars have been developed that contain only 10 mg HCN/100 g seed.

rived from phenylalanine or tyrosine, but not linamarin.

As shown in Fig. 16.4, β-glucosidase hydrolysis produces an unstable hydroxynitrile which slowly degrades into the corresponding carbonyl compound and HCN. However, most legume seeds contain a hydroxynitrile lyase which accelerates this reaction.

16.2.7 Lipids*

With the exception of soybeans and peanuts, the lipid content of most legumes is so low (cf. Table 16.2) that they can not be considered

* The composition of soy and peanut lipids is covered in Chapters 3 and 14.

Table 16.24. Fatty acid composition of legume lipids (weight-%)[a]

Fatty acid	Garden beans	Chick peas	Broad beans	Lentils
14:0	0.22	1.3	0.6	0.85
16:0	21.8	8.9	9.3	23.2
18:0	4.7	1.6	4.9	4.6
20:0	0.53	0.03	0.7	2.3
22:0	2.9	0	0.42	2.7
24:0	1.1	0	0	0.85
16:1 (9)	0.21	0.05	0	0.15
18:1 (9)	11.6	35.4	33.8	36.0
18:2 (9,12)	29.8	51.1	42.1	20.6
18:3 (9,12,15)	27.4	1.7	6.4	1.6
20:1	0.02	0	0.7	1.9

[a] In Table 14.11 the fatty acid compositions are provided for soya oil and peanut butter.

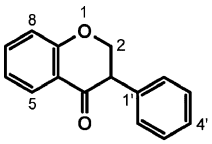
as a source of fats or oils. Examples of their fatty acid composition are listed in Table 16.24.

16.2.8 Vitamins and Minerals

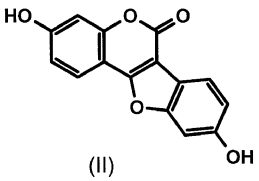
Vitamin and mineral content of some legumes is presented in Table 16.25. In addition to B-vitamins, the two oilseeds are rich in tocopherols.

16.2.9 Phytoestrogens

The isoflavones daidzein (Ia in Formula 16.4), genistein (Ib) and glycitein (Ic) as well as coumestrol (II) together with the lignans (cf. 18.1.2.5.7) are called phytoestrogens because they can dock onto estrogen receptors. Accordingly, they are competitors of endogenous estrogen, but have lower activity. Sources of isoflavones are soybeans and soybean products (Table 16.26). In addition, they occur in traces in many plant foods.



(Ia) Daidzein: 4' - OH, 7 - OH
(Ib) Genistein: 4' - OH, 5 - OH, 7 - OH
(Ic) Glycitein: 4'- OH, 6 - OCH_3, 7 - OH



(II) (16.4)

Table 16.25. Vitamin and mineral composition of legumes[a]

	Soybeans	Peas	Garden beans	Peanuts
Vitamins				
Tocopherols	127	12		202
B_1	8.2	1.2	4.5	9.0
B_2	4.3	0.64	1.6	1.5
Nicotinamide	20.8	9.5	20.8	153
Pantothenic acid	15.9	2.9	9.7	26
B_6	9.9	0.64	2.8	3.0
Minerals				
Na	33	8.0	20	52
K	1.4×10^4	1.2×10^3	1.3×10^4	7.1×10^3
Mg	2.1×10^3	132	1.3×10^3	1.6×10^3
Ca	2.1×10^3	96	1.1×10^3	590
Fe	71	7.4	60.4	21.1
Zn	42	10.6	26	30.7
P	4.9×10^3	432	4.3×10^3	3.7×10^3
Cl	58	160	248	70

[a] Results are given in mg/kg.

Table 16.26. Daidzein (Dai), genistein (Gen), glycitein (Gly) and coumestrol (Cou) in soybeans[a]

Foods	Dai	Gen	Gly	Cou
Soybeans	566	442	28.1	0.015
Soy milk	9.2	18	1.7	0.006
Soybean sprouts	2.7	5.1	0.045	n.n.
Tempeh	69.7	107	5.7	0.006
Tofu	93.4	170	7.3	0.007
Miso	44.2	59	8	0.024
Soybean protein	25.3	59.7	3.1	0.005

[a] values in mg/kg
n.n.: not detected

Table 16.27. Saponin content in foods

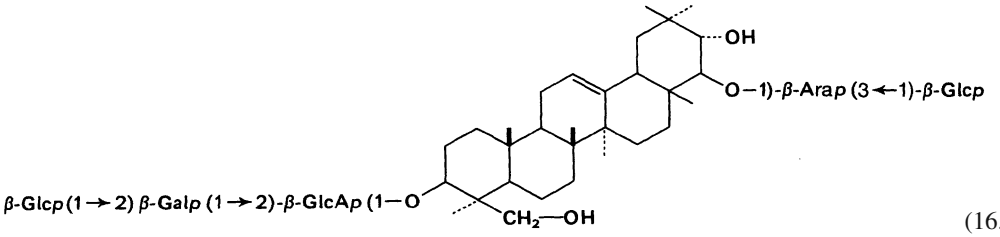
Food	Saponin (g/kg solids)
Chick peas	56
Soybeans	43
Garden beans	4.5–21
Peanuts	6.3
Lentils	3.7–4.6
Broad beans	3.5
Peas	11
Spinach	47
Asparagus	15
Oat bran	1.0

16.2.10 Saponins

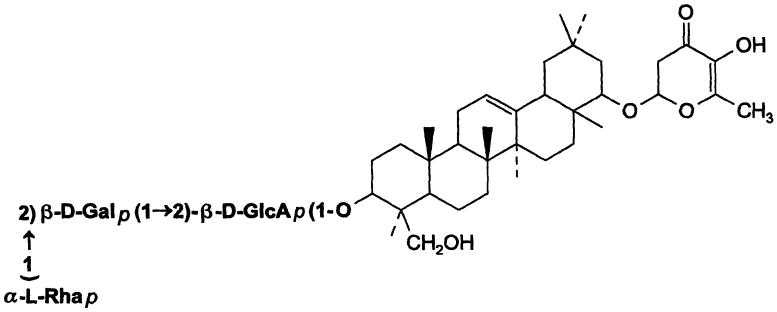
Saponins are surface-active plant constituents, which are broken down into a carbohydrate portion and an aglycone on acid hydrolysis (Formula 16.5). The carbohydrate chain consists of 1 to 8 monosaccharides or uronic acids. It is usually branched and often terminated by a pentose, e. g., arabinose. There are saponins with one carbohydrate chain (mono-desmosides) and with two chains which are independent of each other (bisdesmosides).

$$\text{Saponin} \xrightarrow{H_2O(H^{\oplus})} \text{Sapogenin} + \text{Monosaccharide} \qquad (16.5)$$

According to the structure of the aglycone, there are two groups: pentacyclic triterpenes and steroid sapogenins. The first mentioned group is found in legumes, the main source of saponins in food (Table 16.27). A steroid sapogenin is found, e. g., in oats, which also contain representatives of the first group. The saponins of soybeans have been most intensively studied; more than one dozen have been identified. As examples, the structures of two soybean saponins of the A (bisdesmosides) and B (monodesmosides) series are shown in Formula 16.6 and 16.7. Saponins of the B series contain a 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one residue at C_{22}. (Formula 16.7)



(16.6)



(16.7)

Saponins contribute to the characteristic taste of soybeans and other legumes. They are heat stable in the neutral pH range. Since a considerable portion of the saponins occurs in the seed coat and in the hypocotyl, the taste of soybean products, e. g., tofu, improves when these parts are removed.

A series of saponins are hemolytically active, the aglycone as well as the sugar residue playing a role. Monodesmoside triterpene saponins are more active than the bisdesmosides, a longer sugar residue and a branch weaken the effect.

Steroid saponins and, to a smaller extent, triterpene saponins complex cholesterol, ergosterol and 7-dehydrocholesterol but not vitamin D. Since saponins are very poorly absorbed, their toxic effect is negligible. Even in vegetarians who ingest higher amounts of saponins with their food, no negative symptoms have been observed.

16.2.11 Other Constituents

The meadow pea, *Lathyrus sativus*, which is cultivated in India in periods of drought, contains β-N-oxalyl-α,β-diaminopropionic acid (cf. XXXVII in Table 17.5). Possibly due to its structural similarity to glutamic acid, this compound causes the disease known as neurolathyrism, which is characterized by paralysis of the lower limbs. More than 100,000 cases of this disease were described in 1975 alone. The diaminopropionic acid derivative can be largely eliminated by cooking the seeds in excess water, which is then discarded, or by soaking the seeds overnight, followed by steaming, roasting, or drying in the sun. The flour obtained from dried seeds has 24–28% of protein and a high lysine content. It can be used to make unleavened Indian bread ("chapatis").

The horse bean, *Vicia faba*, contains the glucosides vicin (Formula 16.8, I) and convicin (II).

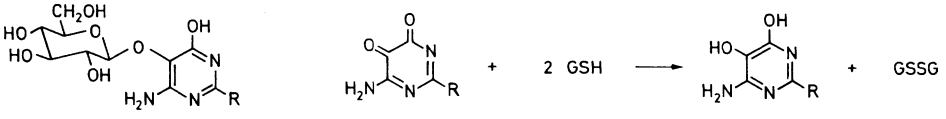
The aglycones of these compounds divicin (III) and isouramil (IV) can be released by the β-glycosidases of the digestive tract. In the oxidized form, they cause quick oxidation of glutathione in erythrocytes (cf. Formula 16.8) which have a hereditary deficiency of glucose-6-phosphate dehydrogenase. Consequently, these erythrocytes are incapable of re-producing reduced glutathione with the help of glutathione reductase for lack of NADPH. The lack of reduced glutathione causes a hemolytic anemia called favism. This genetic defect is found especially in people from the Middle East. Since *Vicia faba* plays a big role in the protein supply of people in this region, attempts are being made to cultivate variants which do not contain these toxic glucosides or to develop suitable methods for its removal (soaking, heating).

16.3 Processing

16.3.1 Soybeans and Peanuts

16.3.1.1 Aroma Defects

Preparation and storage of products from both oilseeds is often inhibited by rancidity and bitter aroma defects caused mostly by volatile aroma active carbonyl compounds, e. g., (Z)-3-hexenal, (Z)-1,5-octadien-3-one and 3-methyl-2,4-nonandione. The rancidity-causing compounds are formed through peroxidation of linolenic acid, accelerated by the enzyme lipoxygenase and/or by hem(in) proteins (cf. 3.7.2.2). Furan fatty acids are the precursors in the case of the dione (cf. 14.3.2.2.5). Lipid peroxidation is also involved in the formation of another very potent odorant, 2-pentylpyridine, which produces grassy aroma defects in soybean products. Defatted soybean protein isolates contained 60–510 μg/kg of this compound, which with an odor threshold



I : R = NH_2
II : R = OH

III (ox.) : R = NH_2
IV (ox.) : R = OH

III (red.) : R = NH_2
IV (red.) : R = OH (16.8)

of 0.012 µg/kg (water), corresponds to aroma values of 5×10^3–4.25×10^4. One way to increase quality is to thermally inactivate enzymes or hem(in) catalysts. Table 16.28 illustrates steam heating of peanuts for a prolonged time in order to inactivate peroxidase activity. Lipoxygenase denaturation, under the conditions given in Table 16.28, occurs after 2 min, but this alone does not yield a satisfactory storage stability. Peroxidase and probably other catalysts should be excluded as well (Fig. 16.5).

The complete removal of lipids is used as an additional precautionary measure in order to obtain an off-flavor-free product, particularly in the case of production of protein isolates. For example, the lipid residue which remains in soy flakes after hexane solvent extraction (cf. 14.3.2.2.1) is removed by extraction with hexane-ethanol 82:18 v/v.

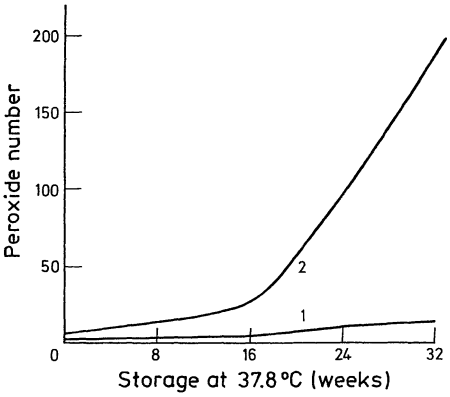


Fig. 16.5. Storage stability of peanut flakes. (according to *Mitchel* and *Malphrus*, 1977) Peanut flakes treated with steam at 100 °C for 30 min (1) and 5 min (2)

Table 16.28. Thermal inactivation of lipoxygenase and peroxidase in peanuts

Heat treatment			*Enzyme activity* (%)	
Type	°C	Time (min)	Peroxidase	Lipoxygenase
Control			100	100
Dry heat	110	60	48	7
Steam	100	2	35	0
Steam	100	6	8	0
Steam	100	30	1	0

16.3.1.2 Individual Products

Protein preparations and milk-like products are processed from soybeans and peanuts. Alone or together with cereals, soybeans are processed into a large number of fermented products in Asia. The following products are made from soybeans.

16.3.1.2.1 Soy Proteins

Figure 16.6 gives an overview of the most important process steps in soybean processing. Soy protein concentrate is usually obtained from the flaked and defatted soy meal that is left after oil extraction (cf. 14.3.2.2.1). The process involves soaking of flakes in water, acidification of the aqueous extract to pH 4–5 (cf. 16.2.1) and separation of the precipitate from solubilized ingredients by centrifugation followed by washing and drying of the sediment collected.

Soy meal isolates enriched in protein are obtained by a preliminary extraction of soluble soy constituents with water or diluted alkali, pH 8–9, followed by protein precipitation from the aqueous extract by adjusting the pH to 4–5. Such protein isolates, texturized and flavored (cf. 1.4.7) are used as meat substitutes. The compositions of protein concentrates and isolates are compared in Table 16.29. For both products, the essential amino acid content corresponds to that of soybeans (cf. Table 16.3). Soy protein is added as an ingredient to baked and meat products and to baby food preparations to raise their protein level and to improve their processing qualities, such as increased water binding capacity or stabilization of o/w emulsions. These properties are required for processing at higher temperatures. The addition of soy protein to beverages at a pH of 3 results in better solubility of beverage constituents. Soy protein market value may be increased by its partial hydrolysis with papain (cf. 2.7.2.2.1).

Table 16.29. Composition of soya protein concentrate and isolate (%)

Product	Protein	Crude fiber	Ash
Concentrate	72	3.5	5.5
Isolate	95.6	0.2	4.0

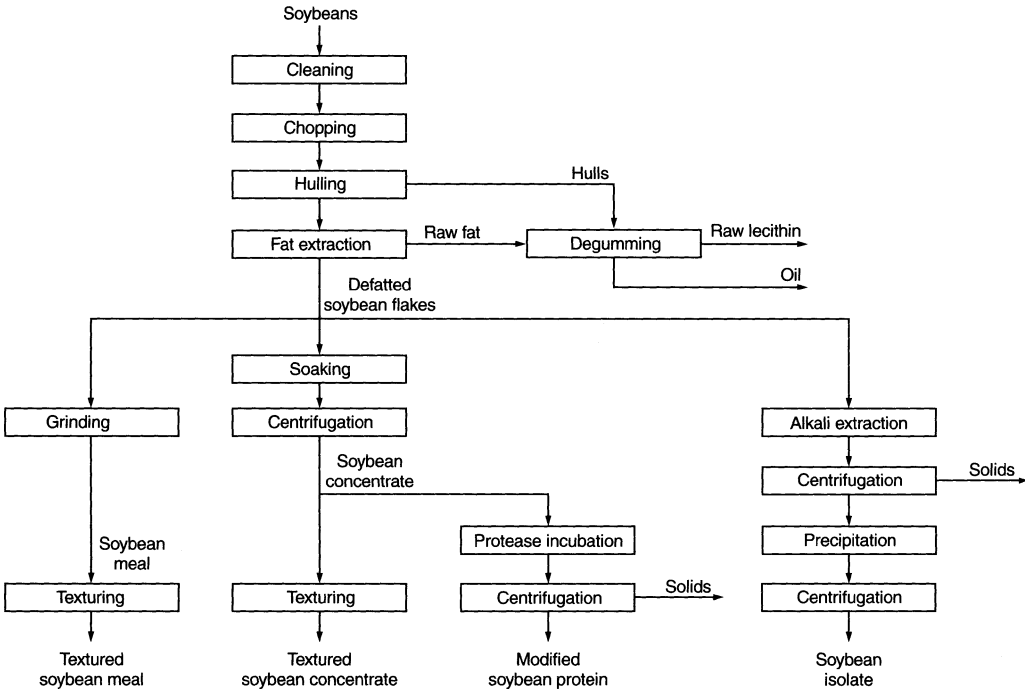


Fig. 16.6. Soybean processing

16.3.1.2.2 Soy Milk

Soybeans are swollen and ground in the presence of a 10-fold excess of water. Heating the suspension close to its boiling point for 15–20 min pasteurizes the suspension and inactivates lipoxygenase enzyme and proteinase inhibitors. A soy milk preparation enriched with calcium and vitamins is of importance in infant nutrition as a replacement for cow's milk, which close to 7% of infants in the USA are unable to tolerate.

16.3.1.2.3 Tofu

When calcium sulfate (3 g salt/kg milk) is added to soy milk at 65 °C, a gel (called soy "curd") slowly precipitates. The curd is separated from excess fluid by gentle squeezing in a special wooden filter box. A washing procedure then follows. The water content of the product is about 88%. Tofu contains 55% protein and 28% fat dry weight. In China and some other Asian countries, tofu is the largest source of food protein. It is consumed fresh or dried, or fried in fat and seasoned with soy sauce.

16.3.1.2.4 Soy Sauce (Shoyu)

Defatted soy meal is used as a starting material in the production of this seasoning sauce (Fig. 16.7). The meal is moistened, then mixed with roasted and crushed wheat and heated in an autoclave for 45 min. The mix ratio in Japan is fixed at 1:1, while in China it varies up to 4:1. Increasing the amount of soy decreases the quality of the endproduct. The mix, with a water content of 26%, is then inoculated with *Aspergillus oryzae* and *Aspergillus soyae*. Initial incubation is at 30 °C for 24 h and then at 40 °C for an additional 48 h. This fermentation starter, called "koji", is then salted to 18% by addition of 22.6% NaCl solution. Inoculation with *Lactobacillus delbrueckii* and with *Hansenula* yeast species results in lactic acid fermentation, which proceeds under gentle aeration in order to prevent the growth

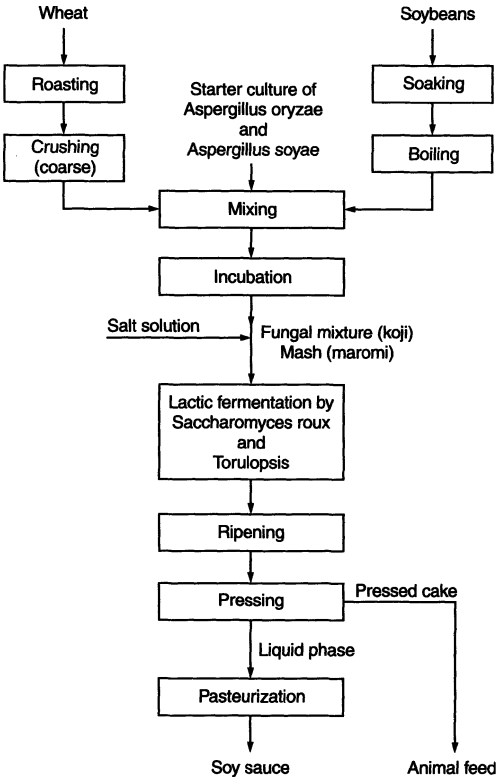


Fig. 16.7. Production of soy sauce

of undesired anaerobic microorganisms. It is a long and tedious fermentation carried out in stepwise fashion: for example, starting at 15 °C for one month, followed by 28 °C for four months, and finishing at 15 °C for an additional month. Highly-valued products ripen for several years. After the fermentation is completed, the soy sauce of pH 4.6 is filtered, pasteurized at 65–80 °C and preserved with benzoic acid for the export market.

During fermentation the microorganisms produce extracellular hydrolases which decompose the main components of the raw material: proteins, carbohydrates and nucleic acids. Soy sauce contains 1.5% N (of which 60% corresponds to amino-N) and 4.4% reducing sugar. The N-containing fraction consists of 40–50% amino acids (glutamic acid predominates at 1.2% of the product), 40–50% peptides, 10–15% ammonia and less than 1% protein. In addition, soy sauce contains by-products of microorganism metabolism, such as ethanol (1.2%) and lactic, succinic and acetic acids.

Soy sauce products of lower quality are blended with spices and are prepared by acid hydrolysis of the above mix of raw materials (cf. 12.7.3.5).

The compound 2(5)-ethyl-4-hydroxy-5(2)-methyl-3(2H)-furanone (EHM3F) is responsible for the sweetish caramel-like aroma note. It is formed by the yeast *Zygosaccharomyces rouxii* from D-sedoheptulose-7-phosphate, which originates from the pentose phosphate cycle. Apart from EHM3F, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HD3F) and 3-hydroxy-4,5-dimethyl-2(5H)-furanone (HD2F) contribute to the aroma. 5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone (EHM2F) is also present, but is of secondary importance because of its lower concentration compared to that of HD2F.

16.3.1.2.5 Miso

Miso is a fermented soybean paste. To produce this substance, rice is soaked, heated, and incubated with *Aspergillus oryzae* at 28–35 °C for 40–50 hours. At the same time, whole soybeans are soaked, heated, and mixed with the incubated rice (60:30) with the addition of salt (4–13%). The mixture is allowed to ferment for several months at 25–30 °C in the presence of lactic acid bacteria and yeasts. The product is then pasteurized and packed. The aroma of miso can be enhanced by the addition of EHMF (cf. 16.3.1.2.4).

16.3.1.2.6 Natto

Various types of natto, a fermented soybean product, are known. For production (Itohiki type), soybeans are soaked in water, boiled and after cooling, incubated with *Bacillus nato*, a variant of *Bacillus subtilis*, for 16–20 hours at 40–45 °C. The surface of natto has a characteristic viscous texture caused by a polyglutamic acid produced by *B. natto*.

16.3.1.2.7 Sufu

Sufu is soy cheese made from tofu. Tofu is cut into cubes (3 cm edge length), treated with an acidified salt solution (6% NaCl, 2.5% citric

acid), heated (100 °C, 15 min) and inoculated with *Actinomucor elegans*. After incubation at 12–25 °C for 2–7 days, sufu is placed in a 5–10% salt solution which contains fermented soybean paste and ethanol, if necessary, and allowed to ripen for 1–12 months.

16.3.2 Peas and Beans

Peas and beans are consumed only when cooked. In order to shorten the cooking time which, even after preliminary soaking in water overnight (preliminary swelling), is several hours, the legumes are precooked or parboiled by the process described in 15.3.2.2.1.

Additionally, seed hull removal provides about a 40% reduction in cooking time which, for peas, involves seed steaming at 90 °C, followed by drying and subsequent dehulling.

The softening of legumes during cooking is due to the disintegration of the cotyledonous tissue in individual cells. This is caused by the conversion of native protopectin to pectin, which quickly depolymerizes on heating. The middle lamella of the cell walls, which consists of pectins and strengthens the tissue, disintegrates in this process.

Conversely, the hardening of legumes during cooking is due to cross linkage of the cell walls. The following reactions which can start even during storage at higher temperatures are under discussion as the cause of cross linkage. Calcium and magnesium phytates included in the middle lamellae are hydrolyzed by the phytase present (cf. 15.2.2.4). Apart from meso-inositol and phosphoric acid, Ca^{2+} and Mg^{2+} ions also released cross link the pectic acids and thus strengthen the middle lamellae. Pectin esterases, which demethylate pectin to the acid, promote the hardening of the tissue. In the case of legumes that are relatively rich in phenolic compounds and polyphenol oxidases, the formation of complexes between proteins and polyphenols should contribute to the strengthening of the tissue.

Similar to soybeans, a number of beans are processed into fermented products in Asia.

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