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Chemical Basis for Biological Effects of Acrylamide

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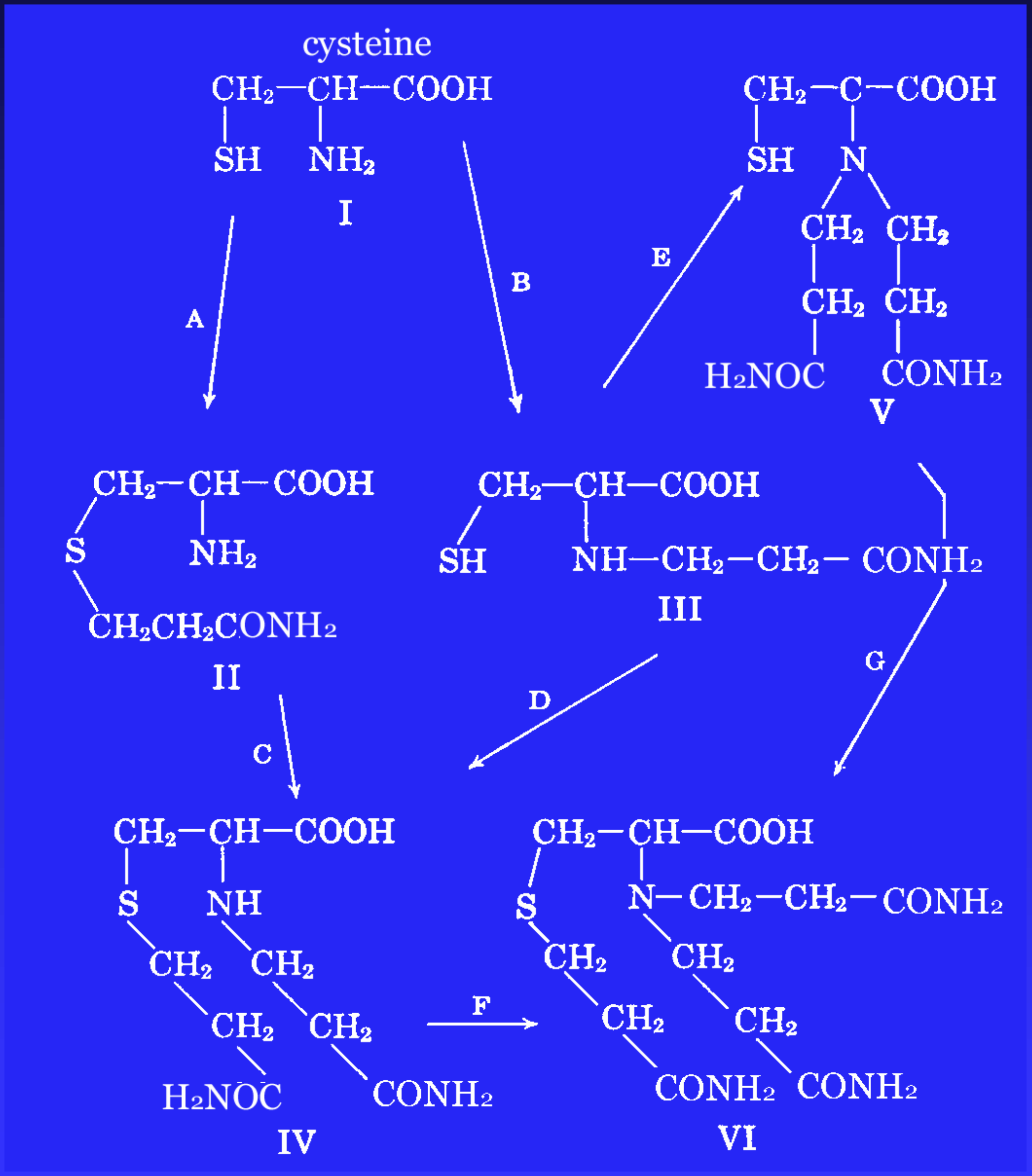
Outline

1. Reactions of protein functional groups with acrylamide and related compounds.
2. Concurrent formation of other potentially toxic compounds during heating of food: mutagenic browning products and heterocyclic amines.
    - Are the effects additive, synergistic, antagonistic?
    - Significance for risk assessment.
    - How much is actually formed compared to what is measured?
    - Reactions in food: polymerization; reaction with other food ingredients such as lysine and histidine.
3. Mechanism of action of acrylamide in vivo: acrylamide and glycidamide. Comparison with aflatoxin.
4. Possible sources of acrylamide in food.
5. Does browning prevention by sulfur amino acids also concurrently prevent the formation and/or reduce levels of acrylamide in food?
6. Since numerous other browning-induced compounds are present in food, are published toxicity data for pure acrylamide relevant to acrylamide in food?

Terminology – Nomenclature

Acrylamide (CH_2=CH-$CONH_2$) is a member of a class of compounds known as: α,β-unsaturated compounds; conjugated vinyl compounds; electrophiles (electron-loving). Acrylamide avidly participates in nucleophilic addition reactions with nucleophiles (electron-rich) compounds which include protein functional groups such the SH group of cysteine, N-terminal NH_2 groups, ε-NH_2 groups of lysine and the NH group of the imidazole ring of histidine. Other members of the group include acreloin (CH_2=CH-CHO), acrylonitrile (CH_2=CH-CN), methyl acrylate (CH_2=CH-CO_2CH_3), methyl vinyl sulfone (CH_2=CH-SO_2CH_3), and vinyl pyridine. Relative reaction rates are governed by the ability of the $CONH_2$ group of acrylamide to withdraw electrons from the double bond compared to the CN group, etc.

Competitive-consecutive reactions



$$S—CH_2—CH(NH_3^{+})COO^{-}$$

$$|$$

$$CH_2—CH_2—R$$

I: R = CN

II: R = $CONH_2$

III: R = CO_2CH_3

IV: R = CO_2H

(after hydrolysis)

Reaction Products of the e-NH_2 Group of Lysine with Acrylonitrile and Acrylamide

ε-NH_2-$(CH_2)_4$-$CH(NH_2)$-$COOH$
L-**lysine**

ε-$NH(CH_2CH_2CN)$-$(CH_2)_4$ -$CH(NH_2)$-$COOH$
e**-N-(**b**-cyanoethyl)-L-lysine (acrylonitrile)**

ε,ε-$N(CH_2CH_2CN)_2$-$(CH_2)_4$-$CH(NH_2)$-$COOH$
e**,**e**-N-*bis*(**b**-cyanoethyl)-L-lysine (acrylonitrile)**

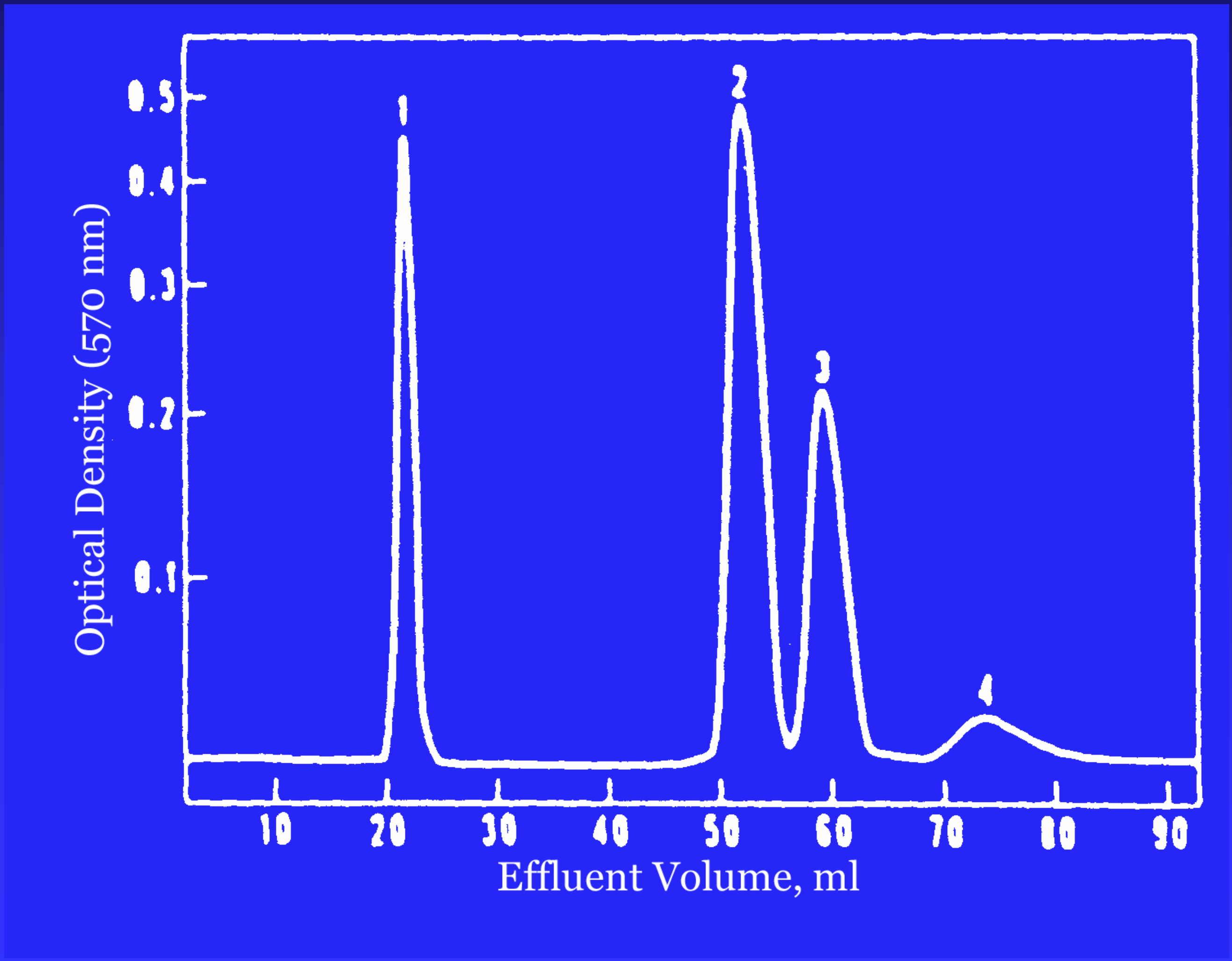
ε-$NH(CH_2CH_2CONH_2)$-$(CH_2)_4$-$CH(NH_2)$-$COOH$
e**-N-(**b**-carbamidoethyl)-L-lysine (postulated with acrylamide)**

ε,ε-$N(CH_2CH_2CONH_2)_2$-$(CH_2)_4$ -$CH(NH_2)$-$COOH$
e**,**e**-N-*bis*(**b**-carbamidoethyl)-L-lysine (postulated with acrylamide)**

ε-$NH(CH_2CH_2COOH)$-$(CH_2)_4$ -$CH(NH_2)$-$COOH$
e**-N-(**b**-carboxyethyl)-L-lysine (hydrolysis product)**

ε,ε-$N(CH_2CH_2COOH)_2$-$(CH_2)_4$-$CH(NH_2)$-$COOH$
e**,**e**-N-*bis*(**b**-carboxyethyl)-L-lysine (hydrolysis product)**

Elution positions of (1) ε-*N*,*N*-bis(β-cyanoethyl)-L-lysine (2) L-lysine, (3) ε-*N*-(β-carboxyethyl)-L-lysine, and (4) ammonia

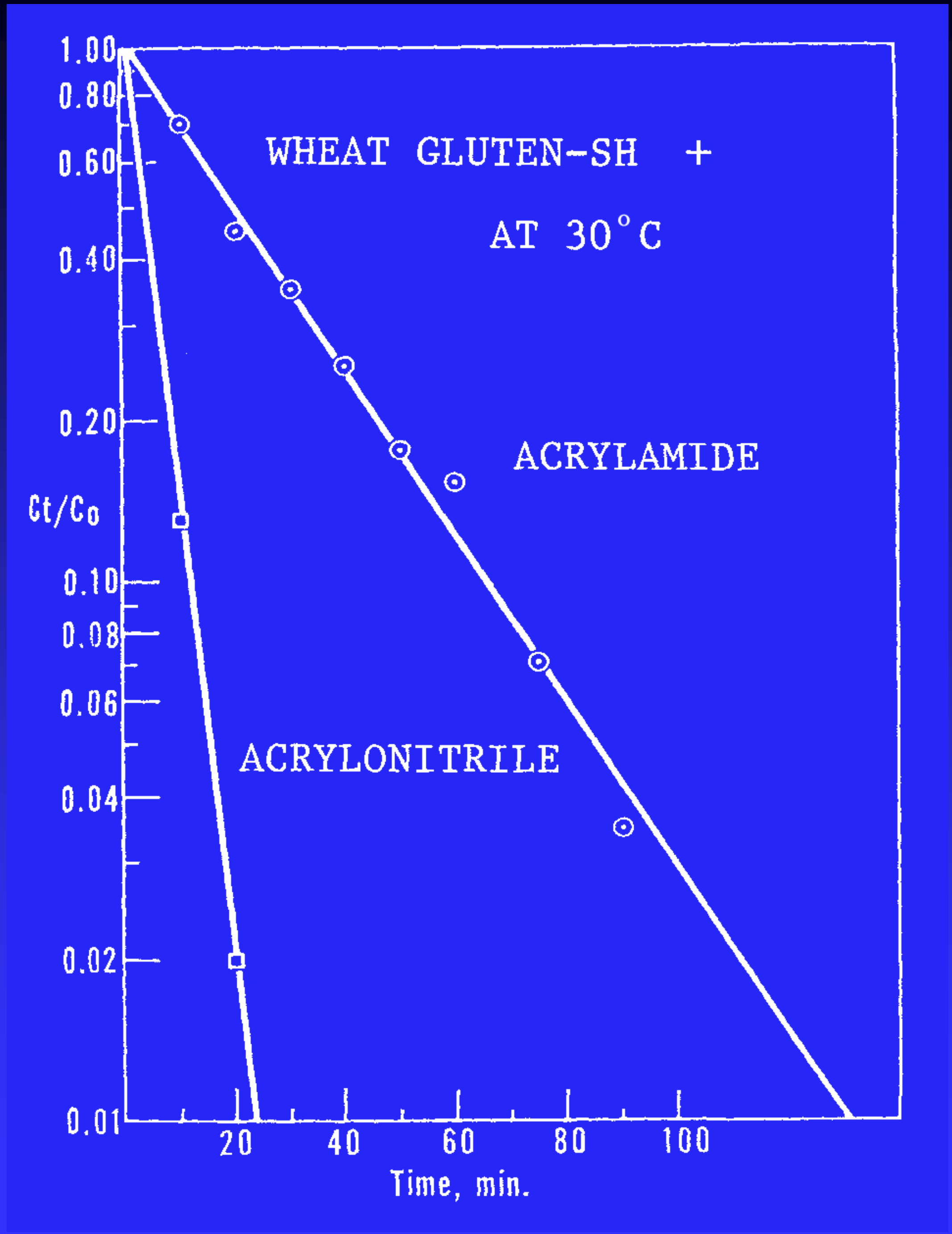


Second-order rate constants (k_2 X 10^4 in l./mole sec) for reaction of amino groups with a,b-unsaturated compounds at 30°

	k_2, pH 8.4	
Compound	diglycine	glycine
CH_2=$CHCONH_2$ (Acrylamide)	1.3	0.49
CH_2=$CHCON(CH_3)_2$	0.21	0.072
CH_2=$CHCN$	9.5	3.9
CH_2=$CHSO_2CH_3$	62.7	

Second-order rate constants for the reaction of the S^- group in $^-S-CH_2CH_2COO^-$ and the NH_2 group in $NH_2-CH_2COO^-$ at pH 8.1 and 30°

Vinyl compound	$k_2(S^-)$ X 10^2	$k_2(NH_2)$ X 10^5	Ratio of rates (S^-/NH_2)
Acrylonitrile	2.70	20.4	132
Methyl acrylate	11.0	76.0	145
Acrylamide	0.46	2.60	178


WHEAT GLUTEN-SH +
AT 30°C
ACRYLAMIDE
ACRYLONITRILE
Ct/Co
1.00
0.80
0.60
0.40
0.20
0.10
0.08
0.06
0.04
0.02
0.01
20
40
60
80
100
Time, min.

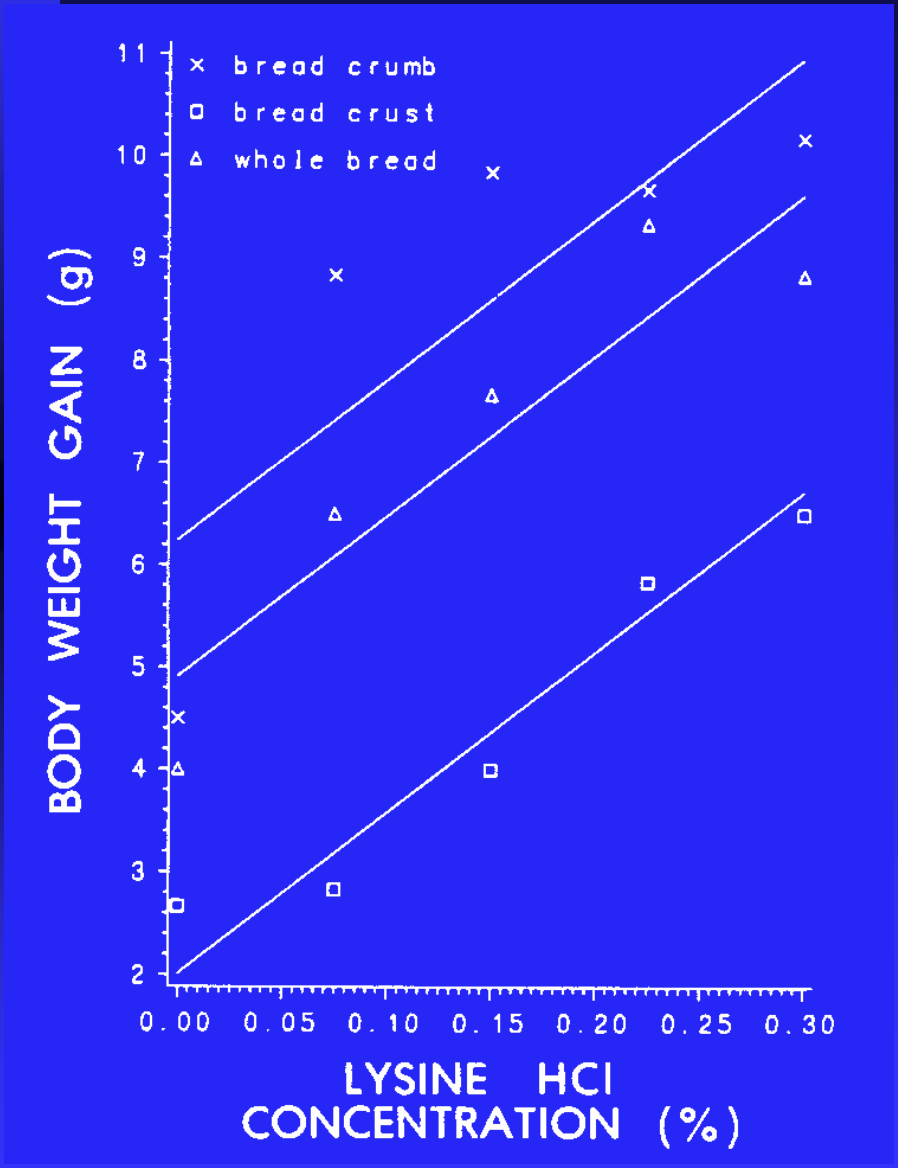
Relative amino acid composition of alkylated wheat gluten

Amino acid	Whole gluten	Gluten and methyl acrylate 10 min	Gluten and acrylonitrile 30 min	Gluten and acrylamide 90 min
	moles amino acid/ moles alanine			
Lysine	0.269	0.280	0.280	0.282
Histidine	0.490	0.512	0.508	0.496
Arginine	0.658	0.667	0.653	0.670
Aspartic acid	0.723	0.761	0.751	0.786
Threonine	0.865	0.875	0.872	0.865
Serine	2.07	2.19	2.17	2.16
Glutamic acid	12.1	12.3	12.3	12.5
Proline	5.42	5.50	5.31	5.36
Glycine	1.71	1.75	1.76	1.72
Alanine	1.00	1.00	1.00	1.00
Half-cystine	0.643	0.000	0.000	0.000
Valine	1.35	1.36	1.38	1.37
Isoleucine	1.12	1.15	1.13	1.14
Leucine	2.21	2.18	2.21	2.19
Tyrosine	0.744	0.784	0.770	0.766
Phenylalanine	1.24	1.26	1.23	1.25

Relative amino acid composition of alkylated bovine serum albumin

Amino acid	Whole BSA	BSA and methyl acrylate 10 min	BSA and acrylonitrile 30 min	BSA and acrylamide 90 min
	moles amino acid/ moles alanine			
Lysine	1.36	1.36	1.36	1.36
Histidine	0.337	0.342	0.334	0.322
Arginine	0.472	0.482	0.471	0.479
Aspartic acid	1.19	1.18	1.19	1.18
Threonine	0.725	0.742	0.732	0.744
Serine	0.629	0.608	0.624	0.625
Glutamic acid	1.77	1.78	1.77	1.79
Proline	0.606	0.613	0.686	0.678
Glycine	0.341	0.351	0.344	0.353
Alanine	1.00	1.00	1.00	1.00
Half-cystine	0.437	0.000	0.000	0.000
Valine	0.730	0.787	0.787	0.784
Isoleucine	0.271	0.288	0.279	0.283
Leucine	1.26	1.32	1.34	1.32
Tyrosine	0.364	0.379	0.381	0.339
Phenylalanine	0.535	0.556	0.573	0.556

Nutritional Improvement of Bread



Weight gain in mice after 14 days fed whole bread, bread crumb, and bread crust supplemented with lysine before baking (cobaked) (Friedman and Finot, 1990)

Does added lysine react with acrylamide in the crust?

Prevention of browning in commercial fruit juices by sodium bisulfite and *N*-acetyl-L-cysteine (Molnar-Perl and Friedman, 1990). Does browning prevention by SH-containing amino acids also prevent the formation of acrylamide?

Inhibition (%)

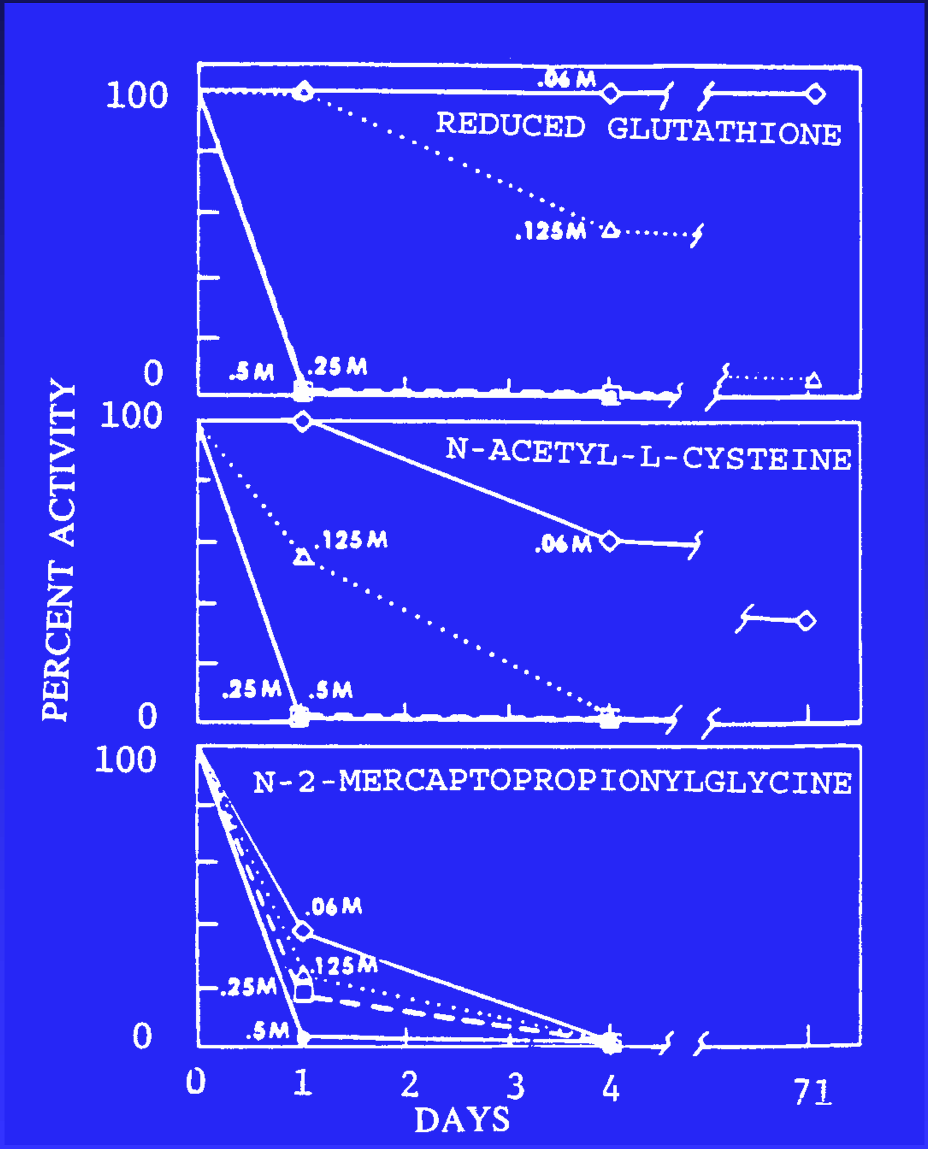
	Sodium bisulfite (mM)					*N*-acetyl-L-cysteine (mM)				
Juice	**1.0**	**2.0**	**4.0**	**8.0**	**16.0**	**2.5**	**1.0**	**25**	**50**	**100**
Grape	10	25	69	72	100	6	18	35	79	100
Apple	42	50	59	93	100	1	36	52	87	100
Apple	10	40	49	100	100	5	40	55	92	93
Pineapple	11	39	68	102	105	10	35	54	76	100
Grapefruit	18	25	39	63	100	32	61	75	87	93
Orange	13	49	69	92	108	3	69	66	72	107

Prevention of browning in protein-containing foods by sodium bisulfite and *N*-acetyl-L-cysteine (Molnar-Perl and Friedman, 1990). Does browning prevention by SH-containing amino acids also prevent the formation of acrylamide?

Inhibition (%)

	Sodium bisulfite (mM)					*N*-acetyl-L-cysteine (mM)				
Protein source	**2.5**	**25**	**50**	**100**	**200**	**25**	**6.2**	**125**	**250**	**500**
Casein	**4**	**12**	**44**	**82**	**100**	**0**	**25**	**42**	**101**	**101**
Barley flour	**3**	**43**	**61**	**98**	**95**	**36**	**42**	**79**	**96**	**104**
Soy flour	**10**	**27**	**80**	**98**	**102**	**19**	**38**	**84**	**99**	**101**
Nonfat dry milk	**3**	**23**	**44**	**94**	**104**	**19**	**43**	**78**	**98**	**101**
Isomil	**7**	**29**	**72**	**88**	**100**	**7**	**43**	**65**	**93**	**109**

Effect of thiol concentration on time of inactivation of mutagenic activity of aflatoxin B_1 by reduced glutathione, N-acetylcysteine, and *N*-2-mercaptopropionylglycine (Friedman et al., 1982)



Biochemistry of Acrylamide

1. **Metabolic transformation of acrylamide to glycidamide.**
2. **Chemical modification (alkylation) of:**
   a. **Non-protein SH groups (cysteine, homocysteine, glutathione).**
   b. **Protein SH groups (enzymes and structural proteins).**
   c. **N-terminal NH_2 group of valine residue of hemoglobin.**
   d. **ε-NH_2 groups of lysine residues?; NH of imidazole ring of histidines?; di-alkylation reactions?**
   e. **NH_2 groups of guanine and other nucleic acids.**
3. **Non-covalent interactions with tryptophan residues of proteins resulting in quenching of tryptophan fluorescence.**

Toxicology of Acrylamide

- Genotoxicity
- Clastogenicity (chromosome-aberrations in germ cells)
- Epigenetic activity
- Carcinogenicity
- Neurotoxicity
- Is the toxicology of pure acrylamide relevant to acrylamide in food?

Research Needs

1. Does prevention of food browning by sulfur amino acids and peptides also prevent acrylamide formation?
2. Do low-lysine proteins (wheat gluten, zein) produce more acrylamide than high-lysine proteins (casein, soy protein, meat proteins)? If so, why?
3. Will replacement of corn meal with high-lysine corn meal result in less acrylamide in potato chips and tortillas?
4. Can we change the composition of the diet to minimize acrylamide formation?
5. Do heat-induced Maillard browning products and other food ingredients affect the safety of acrylamide after consumption? Significance for risk assessment.
6. What is the rate-determining step in acrylamide formation in food and can we control it?

Basic Studies on Reactions of Amino Acids and Proteins with Acrylamide, Related Vinyl Compounds, and Processing-Induced Changes in Food

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