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Studies on Proteins and Amino Acids Exposed to Supercritical Carbon Dioxide Extraction Conditions

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ABSTRACT

Lysozyme was treated with humid supercritical carbon dioxide and nitrogen (300 bar, 80 °C and room temperature, 6 and 2 h). No alterations could be demonstrated by amino acid analysis and assays of TNBS-reactive lysine. Independent of the gas used, digestion experiments and SDS-PAGE indicated an unfolding, a partial oligomerization and some fragmentation of the protein molecules in samples treated at 80 °C. Such alterations are caused by heating proteins in the presence of water as shown elsewhere.

Furthermore, L-*glutamic acid,* L-*glutamine,* L-*methionine,* L-*leucine,* L-*alanine, β-alanine and* L-*lysine were treated with humid supercritical carbon dioxide (300 bar, 80 °C, 6 h). Automated amino acid analysis demonstrated a loss of 15–23% only with glutamine and a loss of 10% with glutamine exposed to nitrogen under the same reaction conditions, while glutamine treated at room temperature remained unaltered. This loss was caused by conversion of glutamine to 2-pyrrolidinone 5-carboxylic acid, identified by ion-exchange and thin-layer chromatography and hydrolysis to glutamic acid.*

Protein alterations to the extent observed here, as well as the formation of pyrrolidinone carboxylic acid, do not negatively influence food quality under the reaction conditions used in supercritical carbon dioxide extraction of foods.

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INTRODUCTION

Supercritical gases, especially supercritical carbon dioxide, were proposed by Zosel (1964) in a basic patent for extraction, among others, of foods and food constituents. Since that time various applications have been patented (see review by Randall, 1982, for references). The decaffeination of coffee and extraction of hops by supercritical carbon dioxide have also been introduced into the food industry. Although carbon dioxide was chosen for food extraction because of its unique properties (i.e. its critical point is relatively accessible, it is comparatively non-toxic and it is moderately unreactive), there has been some discussion of possible reactions of carbon dioxide with food constituents under these conditions. Proteins, amino acids and amines are known to react with carbon dioxide under physiological and/or very specialized experimental conditions (see Weder, 1980, for a broader discussion of this topic).

In a preceding paper (Weder, 1980) the influence of supercritical carbon dioxide on ribonuclease was studied as a model protein system. Only minor alterations could be demonstrated (i.e. unfolding of the protein molecule and some oligomerization and fragmentation). In order to further assure generalization of these results, a second protein, lysozyme, was exposed to supercritical humid carbon dioxide. In addition some amino acids differing in isoelectric points were included in this study. The results with lysozyme, glutamic acid, glutamine, methionine, leucine, alanine, β-alanine and lysine are presented in this paper.

MATERIALS AND METHODS

Supercritical carbon dioxide treatment

Samples of 100 mg of lysozyme (chicken egg white, Serva 28 260) were mixed in a test tube (15 × 98 mm) with 1 ml of distilled water. Each sample was placed in a 1-litre autoclave equipped with a thermometer and pressure gauge and containing an additional 30 ml of water. The autoclave was filled with liquid carbon dioxide (food quality, Rud. Buse Sohn, Bad Hönningen, FRG) up to 300 bar. The temperature was kept at either room temperature or 80 °C by a thermostatted water bath. Exposures to nitrogen (Stickstoff 4.6, Messer Griesheim, Düsseldorf, FRG) were performed in a similar manner. After 2 or 6 h treatment, the samples were lyophilized.

Samples, 500 mg each, of L-glutamic acid, L-glutamine, L-methionine, L-leucine, L-alanine, β-alanine and L-lysine (all biochemical grade, Merck) were exposed to humid carbon dioxide at 300 bar and 80 °C for 6 h. L-Glutamine and L-lysine were also treated with carbon dioxide and nitrogen at room temperature; L-glutamine was also treated with nitrogen at 80 °C.

Amino acid analyses

Samples of about 0·4 mg were hydrolyzed in 2 ml of 6N HCl for 22 h at 110 °C in a vacuum desiccator. Analyses of lysozyme samples and both hydrolyzed and unhydrolyzed treated amino acids were performed on a Multichrom B Analyzer (Beckman) according to the manufacturer's manual.

Tryptophan

Tryptophan was estimated with *p*-dimethylaminobenzaldehyde and sodium nitrite after hydrolysis with barium hydroxide (Blackburn, 1968), according to Spies & Chambers (1948).

TNBS-reactive lysine

Reactivity of ε-amino groups of L-lysine residues with 2,4,6-trinitrobenzene sulphonic acid (TNBS, Sigma) was estimated according to Kakade & Liener (1969).

Tryptic digestibility

This was done as described earlier (Weder, 1980) with 40 mg samples of lysozyme, 2·5 mg of trypsin (bovine, Merck 8213), and 0·01 N NaOH as a titrant.

Polyacrylamide gel electrophoresis with sodium dodecyl sulphate (SDS)

Electrophoresis with and without 2-mercaptoethanol in gels of about 10% acrylamide was performed according to Weber & Osborn (1969).

Chemically oligomerized lysozyme was prepared as described by Wolf *et al.* (1970).

Ninhydrin reaction

Samples of 0·2 μmole amino acid per ml were each mixed with 1 ml of the ninhydrin reagent (10 g of ninhydrin, Merck 6762, plus 1·5 g of hydrindantin dihydrate, Merck 4510, dissolved in 500 ml of methylglycol/4 N sodium acetate buffer (3:1, v/v), pH 5·5, Moore & Stein, 1954) and heated in a boiling water bath for 15 min. The samples were immediately cooled and diluted with 5 ml ethanol/water (1:1, v/v) and the absorbance was read at 575 nm against a reagent blank.

Thin-layer chromatography of treated glutamine

Samples of 5 μl (2 mg sample material dissolved in 1 ml of water) were run on TLC-plastic sheets (silica gel 60), pre-coated (Merck 5748) using the system A of Drawert & Barton (1974), methanol/water (7:3, v/v). Detection was made first by spraying with ninhydrin (1 g of ninhydrin dissolved in a mixture of 85·5 ml of *n*-butanol, 9·5 ml of water and 5 ml of 2,4,6-collidine) and heating for 30 min at 110 °C and then by exposure to chlorine for 5 min (produced by mixing equal volumes of 1·5 % $KMnO_4$ and 10 % HCl according to Brenner *et al.*, 1965), ventilating in a current of air at room temperature by means of a hair-dryer for 2 h and finally spraying with a mixture of 1 % starch–1 % KI solution (CSI, chlorine–starch–iodide procedure of Rydon & Smith, 1952).

Ion-exchange chromatography of carbon dioxide-treated glutamine

A quantity (500 μg) of L-glutamine treated with humid supercritical carbon dioxide was dissolved in 2 ml of 0·2 N sodium citrate buffer (pH 2·2) and applied to an Amberlite CG 120-II column (10 × 400 mm) equilibrated with 0·2 N sodium citrate buffer (pH 3·42). The column was eluted with pH 3·42 buffer and fractions of 1 ml were collected. Each fraction was mixed with 1 ml of 5 N NaOH, treated in a boiling water bath for 15 min, diluted with 2 ml of 30 % acetic acid, and 1 ml of the resulting solution was submitted to the ninhydrin reaction as described above. L-Glutamic acid, L-glutamine and 2-pyrrolidinone 5-carboxylic acid (Serva) were used to calibrate the column.

RESULTS AND DISCUSSION

Alterations of lysozyme exposed to humid supercritical carbon dioxide

Following our study of ribonuclease (Weder, 1980), lysozyme was chosen as a second model protein to study the influence of supercritical carbon dioxide, because extensive studies have been reported on the influence of temperature, heating time, and water content with this protein (Weder & Scharf, 1982; Scharf & Weder, 1983, 1984; Weder & Sohns, 1983). Lysozyme (LSH) was treated in the autoclave with humid supercritical carbon dioxide at 80 °C and 300 bar for 2 and 6 h (samples were denoted LSH/CO_2-300/80-2 and LSH/CO_2-300/80-6, respectively). These reaction conditions were chosen because they correspond to the parameters used for food extraction as compiled in Table 1. One sample of LSH was

TABLE 1
Examples of Supercritical Carbon Dioxide Food Extraction Conditions

Sample	*Conditions*	*References*
Coffee, green	40–80 °C/80–120 bar/wet	Zosel, 1973
roasted	45 °C/330 bar/dry (aroma)	Roselius *et al.*, 1972
	45–50 °C/250–350 bar/wet (caffeine)	
Tea	40–100 °C/300–400 bar/dry (aroma)	Vitzthum & Hubert, 1977
	40–100 °C/200–250 bar/wet (caffeine)	
Hops	40–50 °C/150–400 bar/dry	Vitzthum *et al.*, 1976
Spices	40–50 °C/150–400 bar/dry and wet	Vitzthum & Hubert, 1976, 1978

treated under these conditions at room temperature (LSH/CO_2-300/RT-6). In a parallel set of samples LSH was exposed to nitrogen under the same conditions of pressure, temperature and time (LSH/N_2-300/RT-6, LSH/N_2-300/80-2, and LSH/N_2-300/80-6).

The results of the amino acid analyses of these samples are given in Table 2 and are compared with that of the commercial LSH. The percentage values obtained for the individual amino acids in the treated samples were similar to the untreated LSH. Calculating the deviation from the means for each amino acid, a coefficient of variation (cv) below 2·5 % was obtained for most of the amino acids. Cysteine and methionine exhibited a cv of 3·4 and 3·6 %, respectively, which are well within the usual cv of 3–8 % (Ambler, 1981). Furthermore, not even traces of

TABLE 2
Amino Acid Composition[a] of Lysozyme Samples Exposed to Carbon Dioxide and Nitrogen under Pressure at Different Temperatures

Amino acid	*LSH*	*LSH/CO_2-300*			*LSH/N_2-300*		
		RT-6	*80-2*	*80-6*	*RT-6*	*80-2*	*80-6*
Asp[b]	17·36	17·35	17·33	17·20	17·15	17·19	16·91
Thr	5·26	5·14	5·13	5·20	5·05	5·11	5·16
Ser	6·23	6·06	6·12	6·12	6·07	6·00	6·07
Glu[b]	4·93	4·82	4·79	4·81	4·79	4·91	4·93
Pro	1·56	1·46	1·42	1·50	1·63	1·84	1·73
Gly	5·07	4·99	4·98	5·02	5·00	4·94	5·02
Ala	6·24	6·22	6·21	6·18	6·17	6·11	6·14
Cys[b]	4·85	5·17	5·23	5·39	5·33	5·10	5·11
Val	3·73	3·73	3·73	3·77	3·73	3·86	3·91
Met[b]	1·44	1·50	1·58	1·59	1·50	1·50	1·56
Ile	4·31	4·20	4·23	4·31	4·25	4·28	4·35
Leu	6·64	6·62	6·60	6·61	6·56	6·62	6·52
Tyr	3·48	3·42	3·39	3·44	3·52	3·31	3·31
Phe	3·37	3·26	3·27	3·26	3·38	3·28	3·26
His	1·09	1·07	1·10	1·06	1·11	1·06	1·04
Lys	5·56	5·57	5·57	5·43	5·54	5·49	5·51
Arg	12·11	12·21	12·25	11·99	12·16	12·16	12·15
Trp	6·79	7·19	7·08	7·13	7·07	7·25	7·32
TNBS-Lys[c]	1·55	1·65	1·42	1·44	1·51	1·56	1·51
Protein (%)	90·3	89·7	93·0	92·9	93·1	90·4	86·6

[a] Per cent amino acid residue, means of four values.
[b] Including asparagine, glutamine, cysteic acid, and methionine sulphoxide, respectively.
[c] TNBS-reactive lysine, extinction coefficient 10^7 cm^2 $mole^{-1}$, means of three values.

atypical amino acids (e.g. lysinoalanine or lanthionine) could be detected as reaction products of the amino acid residues most sensitive to processing. These atypical by-products are often found in such studies as indicators of protein alterations ('hot spots', according to Mauron, 1975). Proline, due to its low content of less than 2% in LSH, exhibited a cv of 9·6%.

Overall, no deleterious effect on amino acid composition could be

detected. In the range studied, values lower as well as higher than in untreated LSH were obtained for the individual amino acids. No positive or negative correlation could be found with temperature, time or gas used. The same is true of the protein content calculated from amino acid composition. Maxima of +3·1 and −4·1 % relative to the protein content of untreated LSH failed to demonstrate a significant loss in protein due to the treatment. In order to detect acid-labile reaction products of the ε-amino groups of lysyl residues, the content of free amino groups reactive to TNBS was estimated. The results are included in Table 2. Experimentally, no decrease could be demonstrated as a result of treatment. The cv of 5·1 % relative to the mean is within the analytical error (Kakade & Liener, 1969). These results are therefore in agreement with the previously published results for ribonuclease exposed to supercritical carbon dioxide (Weder, 1980), where no measurable effects on amino acid composition or ε-amino group content were demonstrated.

Reaction of carbon dioxide with the ε-amino groups of lysine residues, as well as acid-unstable crosslinks that have previously been demonstrated in heated proteins (Weder & Scharf, 1981), should theoretically reduce protein digestibility by trypsin. As can be seen from Fig. 1, the opposite effect was observed. Untreated LSH as well as LSH treated with the two gases at room temperature (LSH/N_2-300/RT-6 and LSH/CO_2-300/RT-6) were slowly digested due to the tertiary structure of the molecule, the extent of digestion being equivalent to about one bond split. Most of the lysine residues are buried in the native molecule. During treatment with carbon dioxide or nitrogen at 80 °C, LHS unfolding occurred. These samples were more completely digested and the extent of digestion increased with exposure time. The extent of digestion was fairly independent of the type of gas used in the procedure. Maximum cleavage corresponded to maximum theoretical digestibility of LSH. The amount of digestion observed was similar to that observed with LSH heated at 80 °C with about 15 % water for either 2 or 6 h at normal pressure (Weder & Scharf, unpublished work). On the contrary, heating dry LSH at the same temperature for 2–6 h increased tryptic digestibility by 30–60 %, and even after 24 h digestibility was only doubled. Thus, as previously demonstrated for ribonuclease (Weder, 1980), the increase in LSH digestibility is due to the heating in the presence of water and not to the treatment with supercritical gases investigated.

Studies with both wet and dry heated proteins have shown that it is possible to distinguish protein alterations at the beginning of the reaction

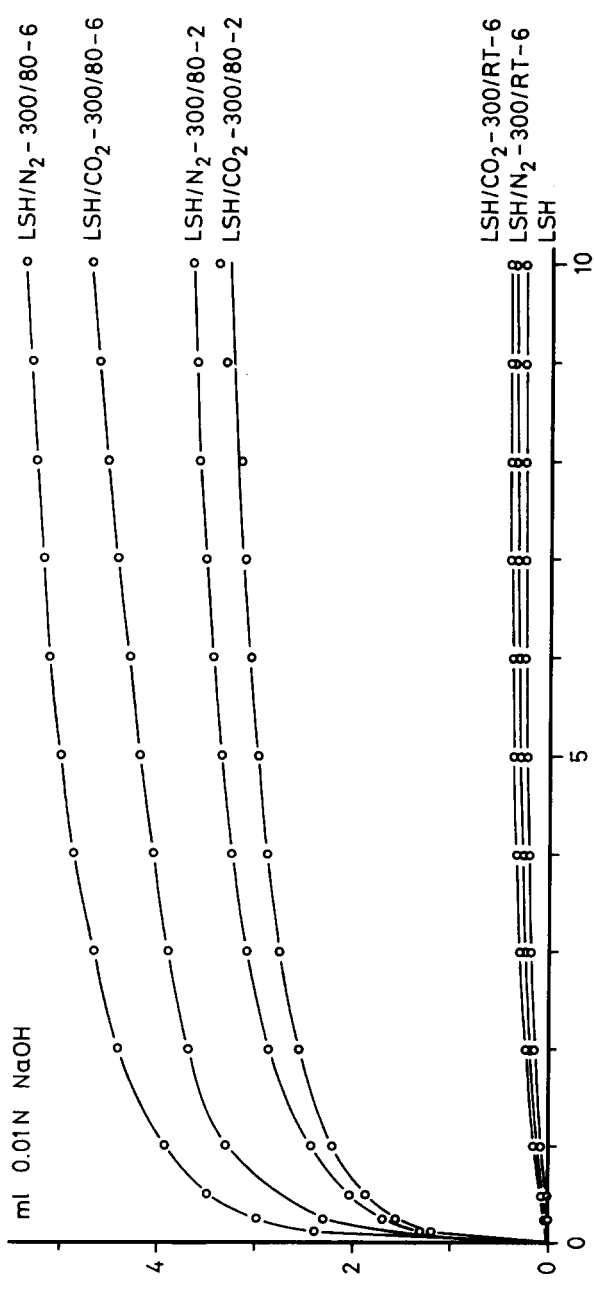

Fig. 1. Tryptic digestion of various treated lysozyme samples. The samples (40 mg) were digested with 2·5 mg of trypsin and the consumption of sodium hydroxide was recorded as a function of digestion time. See Results and Discussion section for explanation of sample notation.

by molecular weight determinations. This is especially true for SDS-polyacrylamide gel electrophoresis, whereas other techniques fail to detect the changes (Weder & Sohns, 1978; Weder & Scharf, 1982). Figure 2, b–g, presents electrophoretic results obtained with LSH exposed to carbon dioxide and nitrogen under pressure. For comparison, a column with untreated LSH is shown on the left (a), while the column on the right shows the separation pattern of chemically oligomerized LSH (h). The

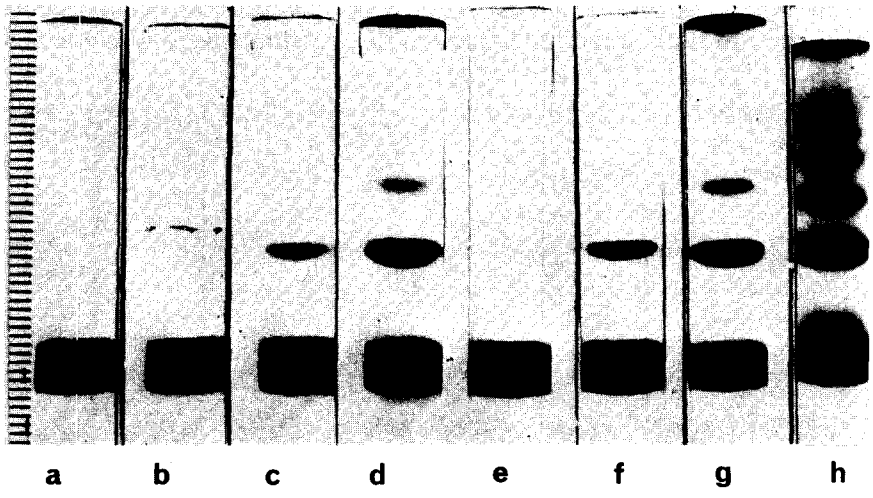

Fig. 2. SDS-polyacrylamide gel electrophoresis of various treated lysozyme samples. Samples (73 μg) were applied to each gel and stained with Coomassie brilliant blue R-250 after electrophoresis, anode at the bottom. a, Untreated commercial LSH; b, LSH/CO_2-300/RT-6; c, LSH/CO_2-300/80-2; d, LSH/CO_2-300/80-6; e, LSH/N_2-300/RT-6; f, LSH/N_2-300/80-2; g, LSH/N_2-300/80-6; h, chemically oligomerized LSH (see Results and Discussion section for explanation of sample notation).

oligomers containing up to six LSH units are clearly visible, and a polymeric fraction with a molecular weight of about 200 000 daltons remains at the top of the column. Samples b and e, which were treated at room temperature, do not differ from untreated LSH (sample a). In samples treated at 80 °C for 2 h with the two gases (samples c and f) the presence of only the dimer can be demonstrated. After 6 h under the same conditions oligomers up to the hexamer were formed (the higher oligomers being only faintly visible on the original gels), as well as a polymeric fraction. Furthermore, traces of non-stoichiometric products were visible, indicating peptide chain fragmentation.

Electrophoretic separations of the same samples under reductive conditions (treated with 2-mercaptoethanol) are shown in Fig. 3. The higher oligomers have disappeared and the amount of dimer has diminished. In addition the proportion of non-stoichiometric products has increased and other fragments with molecular weights below that of the LSH monomer have appeared. This shows that the oligomerization demonstrated here is mainly due to the formation of intermolecular disulphide bridges formed by disulphide interchange, as has been shown for the oligomerization of LSH during wet heating (Weder & Scharf, 1982). In addition, some reduction-stable crosslinks were also formed.

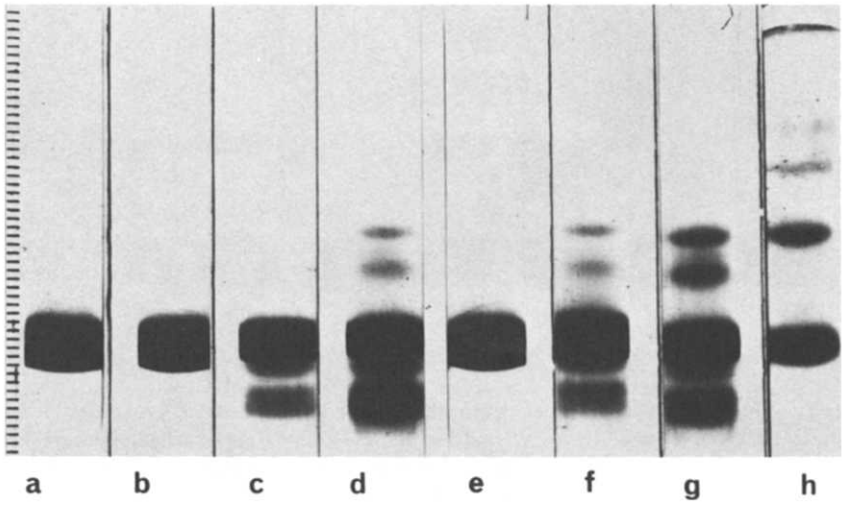

Fig. 3. SDS-polyacrylamide gel electrophoresis of various treated lysozyme samples after reduction. Samples (70 μg) were applied to each gel after reduction with 2-mercaptoethanol and run as in Fig. 2; a–h, conditions as in Fig. 2.

These may be the isopeptides aspartyl-lysine or glutamyl-lysine, since acid-stable crosslinks could not be detected by our amino acid analysis. Furthermore, some fragmentation is demonstrated. Obviously, the fragments are incorporated in oligomer formation and/or the bond split occurs within a disulphide loop of the native molecule. Again, the same alterations could be demonstrated in wet heated LSH under normal pressure.

Altogether, the results presented here for LSH confirm the previous results obtained with ribonuclease. No alterations could be detected by

amino acid analysis and TNBS-reactive lysine reaction. Digestion by trypsin and SDS-polyacrylamide gel electrophoresis indicated an unfolding of the protein molecules, a partial oligomerization and the cleavage of some peptide bonds. The latter reactions were caused by heating in the presence of water and not by the treatment with supercritical carbon dioxide or nitrogen. In particular, no chemical reactions of the proteins with carbon dioxide could be observed.

Alterations of free amino acids by humid supercritical carbon dioxide

Parallel to the studies with proteins, we also investigated the influence of supercritical carbon dioxide on some amino acids. The results are given in Table 3, with the amino acids arranged according to their isoelectric points. Quantitative ninhydrin reaction in test-tubes showed no significant differences for treated amino acids compared with untreated amino acids with the exception of L-glutamine.

The same samples were applied to amino acid analysis by an automated amino acid analyzer both before and after hydrolysis with 6 N HCl as done for the hydrolysis of proteins. Again, no major differences could be detected, except for L-glutamine. Without hydrolysis, the other samples each exhibited one peak at the position of the corresponding amino acid in the amino acid calibration mixture. L-Glutamine exhibited a loss of 15–23% for samples treated with carbon dioxide at 80 °C (Gln/CO_2-300/80-6) and a loss of 10% for the sample treated with nitrogen at the same temperature (Gln/N_2-300/80-6), and additional ammonia was produced. After hydrolysis, the other amino acids displayed similar results. As expected, L-glutamine was converted into glutamic acid and ammonia. A quantitative evaluation of the results showed that the amount of glutamic acid detected was equivalent to the total amount of glutamine treated with carbon dioxide. This means that the reaction product formed from glutamine is unstable to acid hydrolysis.

The conversion of glutamine to 2-pyrrolidinone 5-carboxylic acid (PCA, also called 2-pyrrolidone 5-carboxylic acid, 5-oxo 2-pyrrolidine carboxylic acid, pyroglutamic acid, 5-oxoproline) is well-known from the literature (Vickery *et al.*, 1935; Archibald, 1945). In order to establish the identity of the glutamine reaction product, the samples were first applied to thin-layer chromatography. Using silica gel and methanol/water (7:3) the compounds in question, PCA, glutamine and glutamic acid, were satisfactorily separated (R_F values 0·83, 0·65 and 0·72, respectively) and the spots were selectively stained with ninhydrin and the CSI reagent.

TABLE 3
Amino Acids Exposed to Carbon Dioxide Under Pressure

Sample	*pI*	*Ninhydrin reaction*[a]		*Amino acid analysis*[b]			
		UN[c]	*TR*[d]	*UN*	*TR*	*UN-H*[e]	*TR-H*[f]
L-Glutamic acid	3·22	0·698	0·702	7·41	7·33	6·86	7·10
L-Glutamine[g]	5·65	0·618	0·614	6·28	4·82	6·92	6·89
		0·649	0·676	6·08	5·18	7·37	7·15
			0·698		5·07		7·15
Gln/CO_2-300/RT-6[h]			0·704		6·09		7·34
Gln/N_2-300/80-6[h]			0·698		5·45		7·09
Gln/N_2-300/RT-6[h]			0·695		6·14		7·16
L-Methionine	5·75	0·609	0·618	6·57	6·41	6·26	6·51
L-Leucine	5·98	0·620	0·623	8·17	7·81	7·54	7·40
L-Alanine	6·02	0·614	0·604	10·61	10·39	10·90	10·96
β-Alanine	6·90	0·512	0·530	7·07	7·00	6·32	6·68
L-Lysine	9·74	0·494	0·494	4·31	4·04	4·84	4·68
Lys/CO_2-300/RT-6[i]			0·494		4·24		4·72
Lys/N_2-300/RT-6[i]			0·482		4·14		4·46

[a] $E_{575}^{1\,cm}$ of 0·2 μmole amino acid after reaction with ninhydrin according to the Materials and Methods section; means of four values.
[b] μMole amino acid per mg of sample; means of two values.
[c] UN, untreated.
[d] TR, treated with CO_2 at 300 bar and 80 °C for 6 h, if not indicated otherwise.
[e] UN-H, untreated but hydrolyzed prior to amino acid analysis.
[f] TR-H, treated and hydrolyzed prior to amino acid analysis.
[g] Three experiments, with two different lots of L-glutamine, have been performed.
[h] L-Glutamine treated with CO_2 or nitrogen at 300 bar and room temperature or 80 °C for 6 h, respectively.
[i] L-Lysine treated with CO_2 or nitrogen at 300 bar and room temperature for 6 h.

Samples of Gln/CO_2-300/80-6 were separated into glutamine (detectable with ninhydrin and CSI) and slightly differing amounts of PCA (detectable only with CSI). Gln/N_2-300/80-6 led to the same result with slightly smaller amounts of PCA, while in glutamine treated with carbon dioxide or nitrogen at room temperature no PCA could be detected. Subsequently, one of the Gln/CO_2-300/80-6 samples (glutamine loss 23 %) was applied to anionic exchange chromatography. The column was calibrated with L-glutamine, L-glutamic acid and PCA. Two peaks were eluted from an Amberlite CG 120-II column with a pH 3·42 buffer. One

of these peaks was detectable by ninhydrin only after hydrolysis with sodium hydroxide. Elution volumes and properties demonstrated that this compound was PCA. The second peak was unreacted glutamine. Quantitative experiments show a glutamine loss of 23% and a corresponding gain of 26% PCA. Thus, the loss of glutamine can be adequately explained by its conversion to PCA and ammonia.

Comparing the results obtained with samples treated at 80 °C (Gln/CO_2-300/80-6 and Gln/N_2-300/80-6) with those of samples treated at room temperature clearly demonstrates that the loss in glutamine is caused mainly by heating for 6 h at 80 °C. The conversion to PCA may be influenced by the presence of carbon dioxide reacting with the free ammonia released by this process. Vickery *et al.* (1935) have shown that glutamine is converted, practically quantitatively, to PCA and ammonia by heating solutions of glutamine for 2 h at 100 °C at pH 6·5 and various anions (among them bicarbonate) have been shown to accelerate this conversion (Gilbert *et al.*, 1949). The inability to detect the loss in glutamine by the manual ninhydrin reaction can be explained by the release of an equivalent amount of ammonia formed during this reaction, since ammonia also reacts with ninhydrin.

CONCLUSIONS

Proteins exposed to supercritical humid carbon dioxide undergo unfolding and partial oligomerization, these two processes being caused mainly by the effects of heat and water. These effects are shown with lysozyme in this study and with ribonuclease in a previous study (Weder, 1980). These alterations are not deleterious to the proteins as nutrients. On the contrary, the proteins are better utilized by digestive enzymes, as was shown here and elsewhere. Some protein denaturation may also improve storage stability of foods by reducing deteriorative enzyme activities, as could be demonstrated for peroxidase activity by Christianson *et al.* (1984) with corn germ flour obtained after extraction of corn oil by supercritical carbon dioxide. Also, critical reactions with free amino acids which do occur as constituents of foods in varying amounts could not be detected. The amino acids examined in this study, covering isoelectric points from 3·2 to 9·7, do not react with carbon dioxide. Even with glutamine, carbon dioxide at most accelerates a reaction occurring also without carbon dioxide due to heating. The

resulting PCA is a compound occurring naturally in different foods at varying amounts, e.g. in wheat 5·6–17·4 mg% (Moriguchi *et al.*, 1961*a*), soybeans 100–300 mg% (Moriguchi *et al.*, 1961*b*), shoyu 271–643 mg per 100 ml (Okamoto, 1977), carrots 5·5–11·3 mg% (Bibeau & Clydesdale, 1975), grape must 40–70 mg per 100 ml (Schormüller & Clauss, 1967), sugar beet molasses 3·2–4·3% (Reinefeld & Gosch, 1969). In addition, PCA is an intermediate in the γ-glutamyl cycle of normal mammalian metabolism (van der Werf & Meister, 1975). From the results discussed here, deteriorations to proteins and amino acids as food constituents should not occur under the conditions likely to be employed for supercritical carbon dioxide extraction of foods.

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