oxidation which can be transported through the vapor phase (and, in the case of water-soluble compounds, undoubtedly also through the aqueous phase). Furthermore, these breakdown products can react through either the well-known nonenzymatic browning reactions or a free-radical mechanism analogous to that postulated for hydroperoxides (Schaich and Karel, 1976).

Some possible reactions, in addition to those known for nonenzymatic browning between proteins and carbonyl compounds, resulting from lipid oxidation, may include those indicated below.

$$V\cdot + PH \rightarrow P\cdot + VH \quad (1)$$

or

$$VH + PH \rightarrow [V\cdots PH] \rightarrow P\cdot + V\cdot + NR \quad (2)$$

or

$$VOOH + PH \rightarrow [VOOH\cdots PH] \rightarrow P\cdot + VO\cdot + H_2O \quad (3)$$

where VH = volatile breakdown product of hydroperoxides, PH = protein, and NR = nonradical fragments.

The protein cross-linking may involve direct incorporation of volatile (eq 4) or recombination of protein free

$$V\cdot + 2P\cdot \rightarrow P\text{-}V\text{-}P \quad (4)$$

radicals (eq 5). The mechanism in eq 5 seems most

$$P\cdot + P\cdot \rightarrow P\text{-}P \quad (5)$$

plausible, given the similarity of products observed when LYS is irradiated with γ rays in the *absence* of lipids to those obtained in the presence of either hydroperoxides or their products.

As expected, high water activities promote cross-linking by facilitating free-radical recombination.

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Lysinoalanine Formation in Yeast Proteins Isolated by Alkaline Methods

Jayarama K. Shetty* and John E. Kinsella

The treatment of disrupted yeast cells with high concentration of alkali at elevated temperatures for the extraction of proteins with low nucleic acid content caused the destruction of amino acids and the formation of lysinoalanine. The generation of lysinoalanine was greater (i.e., 3.59 g/16 g of nitrogen) during the isolation of yeast proteins by a high-alkali, low-temperature (pH 12.5, 65 °C, 2 h) process than when isolated by a low-alkali, high-temperature (pH 10.5, 85 °C, 4 h) process (i.e., 0.49 g/16 g of nitrogen).

Because of their productivity on a variety of substrates, ease of production, and high protein content, microbial sources, particularly yeast, provide attractive supplementary sources of food protein (Tannenbaum and Wang, 1975; Kinsella and Shetty, 1978). However, it is not possible to exploit these sources in significant amounts unless the rigid indigestible cell wall is removed and the nucleic acid content is reduced to permissible levels (Kihlberg, 1972; Edozien et al., 1970; Miller, 1968; Waslien et al., 1970). Several methods have been proposed to reduce the nucleic acid content in yeast (Kinsella and Shetty, 1978). The method commonly recommended for the preparation of yeast protein isolate low in nucleic acid requires the treatment of ruptured cells with alkali at temperatures >60 °C (Cunningham et al., 1975; Hedenskog and Mogren, 1973; Lindblom, 1974; Newell et al., 1975; Vananuvat and Kinsella, 1975a). While this treatment is effective in reducing the nucleic acid, it results in denaturation of proteins and impairs functional properties (Vananuvat and Kinsella, 1975b). Furthermore it may

Institute of Food Science, Cornell University, Ithaca, New York 14853.

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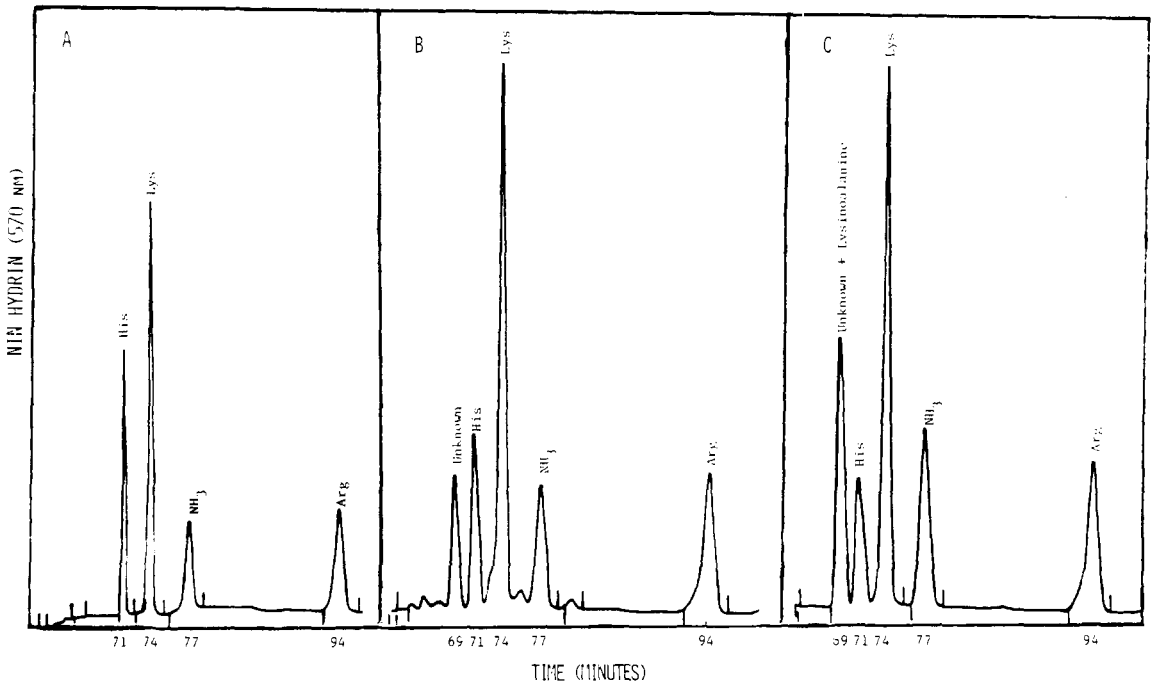

Figure 1. Chromatographic elution profile of basic amino acids of the yeast protein isolated by different methods: (A) yeast protein isolated by succinylation procedure, (B) yeast protein isolated by low-temperature, high-alkali process (65 °C, pH 12.5, for 2 h), (C) method B + 20 nmol of standard lysinoalanine.

cause the destruction of essential amino acids and the formation of lysinoalanine (LAL).

EXPERIMENTAL SECTION

Brewer's yeast (*Saccharomyces carlsbergensis*) obtained from Genesee Brewing Company, Rochester, NY, was washed three times with distilled water and disrupted in a Braun Mechanical Cell homogeneizer (Braun–Melsung, West Germany) as described earlier (Shetty and Kinsella, 1979). Disrupted cells were lyophilized and stored at −10 °C.

Protein Isolation. The isolation of yeast proteins with reduced nucleic acid level was carried out by two different methods, i.e., alkaline extraction with controlled temperature (Newell et al., 1975) and by a succinylation procedure (Shetty and Kinsella, 1979). In the alkaline extraction, the homogenized yeast cells were extracted in distilled water (1:20, w/v) at pH 9.5. The cell wall debris and insoluble materials were separated by centrifugation. The soluble fraction which contained protein and nucleic acid was subjected to either a high-temperature, low-alkali (HTLA) process (pH 10.5; temperature 85 °C for 4 h) or a low-temperature, high-alkali (LTHA) process (pH 12.5; temperature 65 °C for 2 h) to hydrolyze the nucleic acids. The protein was then precipitated at pH 4.5 by adding 6 N HCl, washed once with water (pH 4.5), and lyophilized.

In the succinylation procedure, succinic anhydride was added continuously to the aqueous suspension of homogenized cells (5% concentration), while the pH was maintained at 8.5 with 3 N NaOH. Enough succinic anhydride was added to modify more than 80% of avaliable ϵ-NH_2 groups in the protein. Upon completion of the reaction, i.e., stabilization of the pH, the insoluble cell wall was removed by centrifugation (28000*g* for 45 min at 5 °C). The pH of the supernatant was adjusted to 4.2 and the precipitated protein was separated, dissolved in water (pH 9.0), dialyzed against distilled water, and lyophilized.

Amino Acid Analysis. The proteins, extracted by two methods, were hydrolyzed by using 6 N HCl at 110 °C for 24 h in an evacuated tube, and the amino acids were analyzed by using a Beckman amino acid analyzer, Model 119 CL, using a single column Moore-Stein ion-exchange chromatography method with Beckman W_3 resin; buffer, pH 3.25, 3.95, and 6.40; column, 6 × 460 mm; analysis time, 90 min; temperature of 50–65 °C. The color constant of lysinoalanine was determined using pure lysinoalanine. Norleucine was used as the internal standard.

Other Measurements. Nitrogen was determined by the micro-Kjeldahl method (AOAC, 1975). The extent of succinylation of ϵ-NH_2 groups in the modified yeast proteins was determined by the trinitrobenzenesulfonic acid method (Shetty and Kinsella, 1979).

RESULTS AND DISCUSSION

The amino acid analyses of the yeast proteins isolated by alkaline extraction and the succinylation procedures revealed lower concentrations of all amino acids in the protein isolated by the former method (Table I). In addition, an unusual amino acid was observed in the protein hydrolysate isolated by either the LTHA (Figure 1B) or HTLA process but not by the succinylation procedure (Figure 1A). The unusual amino acid was eluted with the basic amino acids, emerging from the column immediately before histidine (Figure 1B). The unusual amino acid obtained from alkali-treated yeast protein had an R_f identical with that of pure lysinoalanine added to the protein hydrolysate of succinylate yeast proteins. A known amount (20 nmol) of pure LAL was also added to the protein hydrolysate of alkali-treated protein (Figure 1C) and analyzed on the amino acid analyzer. The LAL eluted coincident with the unknown amino acid, causing an increase peak height of the unknown amino acid (Figure 1C). These data indicated the formation of LAL during alkali treatment. With the exception of the alkali labile amino acids, the destruction of most amino acids was greater in the protein isolated by the HTLA method (Table I). Cystine was totally destroyed in both the treatments. The LAL content was highest (3.59 g/16 g of nitrogen) in the protein isolated by the LTHA process, whereas it was only 0.49 g/16 g of nitrogen with the HTLA process.

LAL is formed during the alkaline treatment of proteins (Bohak, 1964; Cheftel, 1977, Friedman, 1978; Patchornik

Table I. Amino Acid Composition of Yeast Protein Isolate Obtained by Different Methods (Expressed as g/16 g of Nitrogen)

aa	yeast protein isolated by: succinylation method[a]	alkaline extraction: high temp, low alkali[b]	alkaline extraction: low temp, high alkali[c]
Asp	10.14	8.02	9.18
Thr	4.28	3.59	3.42
Ser	3.87	3.64	2.85
Glu	12.41	9.38	10.83
Pro	4.19	3.35	3.56
Gly	3.47	2.95	3.33
Ala	5.05	4.56	4.80
$^1/_2$-Cys	1.48	0	0
Val	6.08	5.12	5.82
Met	2.09	1.15	1.43
Ile	5.44	3.75	4.68
Leu	7.94	6.18	7.36
Tyr	4.08	3.13	4.05
Phe	4.93	3.79	3.88
His	2.42	1.59	1.83
Lys	7.65	5.01	5.17
NH_3	2.05	0.30	0.25
Arg	5.27	3.94	3.90
LAL		0.49	3.59

[a] Shetty and Kinsella (1979). [b] 85 °C, pH 10.5, 4 h. [c] 65 °C, pH 12.5, 2 h.

and Sokolovsky, 1964; Woodard and Short, 1973). Bohak (1964) concluded that LAL was produced by the reaction of the ϵ-NH_2 group of lysine residue with the double bond of dehydroalanine which is formed by the β-elimination of crystine and serine residues in proteins. Our data (Table I) indicated a decrease in serine, cystine, and lysine in the protein isolated by the alkali method.

Since mild alkali treatment of proteins is an established technology in the food industry, much interest has centered on the nutritional inpact of LAL (deGroot and Slump, 1969; O'Donovan, 1976; Steinberg et al., 1975; Van Beck et al., 1974). Alkali-treated proteins containing LAL resulted in reduced in vivo and in vitro digestibility (de Groot and Slump, 1969; Finot et al., 1977; Provansal et al., 1975). Alkali treatment of soy proteins induced nephrotoxic properties which were correlated with the appearance of LAL (Woodard and Short, 1973). Proteins containing as little as 0.3 g of LAL/16 g of nitrogen induced kidney changes in rats (Karayiannis, 1976). de Groot et al. (1976) found that only synthetic LAL or protein hydrolysate containing free LAL induced the renal changes in rats and failed to detect any renal changes when rats were fed with the diet containing protein-bound LAL. The renal changes caused by the LAL were shown to be species specific to rats because mice, hamsters, Japanese quail, dogs, rabbits, and monkeys failed to exhibit renal changes when fed synthetic LAL (de Groot et al., 1976). Hence, an unequivocal conclusion of the toxicity of LAL cannot be drawn on the basis of information currently available. In conclusion, the alkali process reduces the nucleic acid level in the yeast protein isolate, but in addition causes chemical changes which may affect the functional properties, nutritive value, and safety of the product.

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