LITERATURE CITED

Bear, R. S., *J. Amer. Chem. Soc.* **64**, 1388 (1944).
Chirife, J., Karel, M., Flink, J., *J. Food Sci.* **38**, 671 (1973).
Chirife, J., Karel, M., *J. Food Sci.* in press (1973a).
Chirife, J., Karel, M., *J. Food Technol.* submitted for publication (1973b).
Colombo, E. A., Immergut, E. H., *J. Polym. Sci. Part C* No. 31, 137 (1970).
Flink, J. M., Karel, M., *J. Agr. Food Chem.* **18**, 295 (1970a).
Flink, J. M., Karel, M., *J. Food Sci.* **35**, 444 (1970b).
Flink, J. M., Labuza, T. P., *J. Food Sci.* **37**, 617 (1972).
Flink, J. M., Gejl-Hansen, F., *J. Agr. Food Chem.* **20**, 691 (1972).
King, J., *Crit. Rev. Food Technol.* **1**, 379 (1970).
Kuge, T., Takeo, K., *Agr. Biol. Chem.* **31**, 257 (1967).
Lauer, K., Ayer, J. E., *J. Polym. Sci.* **26**, 67 (1957).
Le Maguer, M., "Proceedings of the International Symposium on Heat and Mass Transfer Problems in Food Engineering," Vol. 1, Wageningen, Netherlands, Oct 24–27, 1972, p C1-1.
Maier, H. G., Bauer, A., *Die Starke* **24**, 101 (1972).
Osman-Ismail, F., Solms, J., *Die Starke* **24**, 213 (1972).
Rulkens, W. H., Thijssen, H. A. C., *J. Food Technol.* **7**, 79 (1972).
Russell, J. K., Maass, O., Campbell, W. B., *Can. J. Res.* **15**, 13 (1937).
Solms, J., Osman-Ismail, F., Beyeler, M., *Can. Inst. Food Sci. Technol. J.* **6**, A10 (1973).
Staudinger, H., Inder Birken, K. H., Staudinger, M., *Makromol. Chem.* **9**, 148 (1953).
Takashi, K., Takeo, K., *Agr. Biol. Chem.* **32**, 753 (1968).
Thijssen, H. A. C., Rulkens, W. H., *Ingenieur (The Hague)* **80**, 45 (1968).

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# Inhibition of Cooked Flavor in Heated Milk by Use of Additives

Aldo Ferretti

Four organic thiolsulfonates and three organic thiosulfates effectively inhibited cooked flavor when added to whole milk, prior to heating, at a level ranging between 0.003 to 0.05%. They are: 2-aminoethyl 2-aminoethanethiolsulfonate dihydrochloride; 5-aminopentyl 5-aminopentanethiolsulfonate dihydrochloride; 2-acetamidoethyl 2-acetamidoethanethiolsulfonate; cystine *S*-dioxide; 2-aminoethanethiosulfuric acid; *S*-sulfocysteine; and *S*-sulfoglutathione. Their inhibitory action is based on their ability to react with the sulfhydryl-containing compounds that form by heat denaturation of β-lactoglobulin. The possible implications of sulfhydryls removal with regard to the stale flavor control are discussed.

The appearance of a cooked off-flavor is the first measurable manifestation of chemical changes that occur in heated milk. The origin of the cooked flavor has been the object of much speculation. The consensus is that it is due to the presence of volatile sulfides and thiols that arise from thermal breakdown of serum proteins, primarily β-lactoglobulin, and of the proteinaceous material associated with the fat globule membrane (Hutton and Patton, 1952). That the cooked flavor is largely associated with sulfhydryl compounds is confirmed by its concomitant appearance with titratable (nitroprusside test) mercapto groups and by the fact that the flavor of milk depleted of mercapto groups becomes indistinguishable from that of unheated milk (Josephson, 1954). However, the chemical path, or paths, leading to formation of sulfides and sulfhydryls from sulfur-containing amino acids is still largely obscure. The hypothesis, as elaborated below, that volatile sulfur compounds in milk are byproducts of the Maillard reaction (Ellis, 1959; Maillard, 1912) has never been proposed but deserves consideration.

The occurrence of the Maillard reaction in dairy products has been extensively demonstrated, along with its nutritional and organoleptic consequences (Ferretti and Flanagan, 1971, 1972; Henry *et al.*, 1948; Patton, 1955). Reductones and dehydroreductones are important intermediate compounds in this reaction (Hodge, 1953). The latter are characterized by the presence of an α-dicarbonyl moiety or by the structure $-CO[C{=}C]_nCO-$, where $n$ is zero or an integer. The dehydroreductones are Strecker degradation (Schönberg and Moubacher, 1952) agents, presumably responsible for the formation of many volatile compounds when systems containing amino acids and reducing carbohydrates are heated (Hodge, 1967). When cysteine is involved, hydrogen sulfide is one of the products (Kobayashi and Fujimaki, 1965; Schutte and Koenders, 1972). When methionine is one of the reactants, methanethiol is one of the main products (Ballance, 1961; Schutte and Koenders, 1972). The mechanism of the reaction has been studied in some detail by Schutte and Koenders (1972). Whereas the formation of dimethyl disulfide can be readily rationalized on the basis of methanethiol oxidation, the mechanism of formation of dimethyl sulfide from methionine (Ballance, 1961) is less obvious. One precursor of dimethyl sulfide in heated milk is an *S*-methylmethionine sulfonium salt, possibly originating from plant material (Keenan and Lindsay, 1968). The involvement of methionine and cystine in the production of volatile sulfur compounds in heated milk has been confirmed by Demott and Gibbs (1966) by using $^{35}S$-labeled materials.

In addition to the dehydroreductones that may form by a Maillard-type degradation of carbohydrates, milk has natural constituents which can act as Strecker degradation agents on amino acids resulting from protein breakdown. The most likely candidates for such interaction are vitamins C and K. Milk, immediately after removal from the udder, contains vitamin C in the form of L-ascorbic acid (Hartman and Dryden, 1965), which is a reductone, but is fairly readily converted to the dehydro form. Vitamin K, on the other hand, having a naphthoquinone structure, would be ready to function as a degrading agent.

Gruenwedel and Patnaik (1971) recently reported a quantitative study of the previously known hydrogen sulfide and methanethiol release from L-cysteine and DL-methionine, respectively, by action of pyridoxal catalyzed by metal ions, including iron. Because 70 to 95% of vitamin

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$B_6$ in whole milk is present as pyridoxal (Hartman and Dryden, 1965), and in view of the presence in milk of ionic iron, it is conceivable that the interaction of vitamin $B_6$ with sulfur-containing amino acids could represent another source of volatile sulfur in heated milk.

Diacetyl, another dehydroreductone often present in milk, has been found in traces in raw milk, but its concentration increases dramatically on heating (Scanlan *et al.*, 1968), presumably as a consequence of the Maillard reaction (Hodge, 1967). Sometimes it also is considered a microbial metabolite. Whatever its origin, diacetyl too can function as a Strecker degradation agent.

The relative contribution of the various volatile sulfur compounds (thiols, sulfides, disulfides, and $H_2S$) to the cooked flavor is not known. To the extent that it is due to the thiols, their removal should parallel the elimination of the off-flavor. To our knowledge, realistic methods of cooked flavor control in fluid dairy products by chemical removal of thiols have not yet been developed. The purpose of the present investigation was to explore the possibility of developing such a method.

As early as 1947, Patton and Josephson observed that when skim milk is heated for long times at 95°, the quantity of titratable sulfhydryls diminishes substantially. However, the authors continued, "in the absence of casein (rennet whey) and lactose (dialyzed skim milk), such a diminution did not occur." More recently, Kiermeier and Hamed (1961) reported that the concentration of SH groups, which increases rapidly when milk is heated between 75 and 95° and less rapidly thereafter, declines if the heating temperature is raised beyond 110°. They also concluded that oxidation of the SH groups by atmospheric oxygen is not responsible for the observed trend because the pattern is the same in absence of air. Analogous remarks on the variation of sulfhydryls content with changes in heating temperature were recorded by Demott and Gibbs (1966). The results of these three series of experiments can be rationalized readily if the sulfhydryls formed on heating react with other molecules, specifically with certain intermediates of the Maillard reaction, which becomes more important at higher temperatures. Patton (1955), in a different context, speculated that SH compounds might react with the Amadori rearrangement products. That some intermediates of the Maillard reaction can react with mercapto compounds has been demonstrated by Arnold (1969), who achieved partial control of heat-induced browning by adding small amounts of L-cysteine to raw milk prior to heating at 121°. On the basis of this finding, volatile sulfhydryls, released on heating, might also be expected to behave like L-cysteine. Although direct evidence is lacking, some of the end products might contribute to the development of the stale flavor which invariably appears in processed dairy products during prolonged storage. Significantly, in the case of sterile milks, the distinct cooked flavor present at the time of storage fades away and is replaced by the stale flavor even when the milk is stored in sealed containers where the amount of oxygen present would not be enough to account for the decreased concentration of titratable SH groups and the concomitant fading of the cooked flavor. This observation alone, however, does not prove a cause–effect relationship.

The market demand for a long-lived fluid milk of normal or high concentration has led to the development of high-temperature, short-time (HTST) and ultra-high-temperature (UHT) fluid sterilized milks. The problem of physical stability (gelation) with such products has been solved with additives. Because of flavor problems, however, HTST and UHT products have not been commercialized. These products, if stable at room temperature, would have a sizable market. This assumption provided the incentive for the present work, which is a study of the effectiveness of various organic thiosulfonates and thiosulfates in inhibiting the cooked flavor in heated milk. Their expected effectiveness was based on their abilities to react with thiols according to either of the following schemes.

$$RSH + R'SO_2SR' \longrightarrow RSSR' + R'SO_2H$$

$$RSH + R'SSO_3^- \rightleftharpoons RSSR' + HSO_3^-$$

The reaction between thiols and thiolsulfonates (Schöberl and Wagner, 1955) occurs rapidly and goes to completion even at low temperature (Gilman *et al.*, 1925). The reaction represented by the second scheme is reversible (Distler, 1967), but the equilibrium is favored to go to the right. On the basis of relative reactivity toward the thiols, the thiolsulfonates would be expected to suppress cooked flavor more efficiently than the thiosulfates.

## EXPERIMENTAL SECTION

Four organic thiolsulfonates and three organic thiosulfates were tested as inhibitors of cooked flavor when added to milk prior to heating. They are: 2-aminoethyl 2-aminoethanethiolsulfonate dihydrochloride (I); 5-aminopentyl 5-aminopentanethiolsulfonate dihydrochloride (II); 2-acetamidoethyl 2-acetamidoethanethiolsulfonate (III); cystine *S*-dioxide (IV); 2-aminoethanethiosulfuric acid (V); *S*-sulfocysteine (VI); and *S*-sulfoglutathione (VII).

$Cl^-H_3N^+(CH_2)_2SO_2S(CH_2)_2N^+H_3\ Cl^-$

I

$Cl^-H_3N^+(CH_2)_5SO_2S(CH_2)_5N^+H_3\ Cl^-$

II

$CH_3CONH(CH_2)_2SO_2S(CH_2)_2NHCOCH_3$

III

$HOOCCHCH_2SO_2SCH_2CHCOOH$
($NH_2$ and $NH_2$)

IV

$H_3N^+(CH_2)_2SSO_3^-$

V

$HOOCCHCH_2SSO_3H$
($NH_2$)

VI

$HOOCCH(CH_2)_2CONHCH(CONHCH_2COOH)(CH_2SSO_3H)$
($NH_2$)

VII

**Synthesis of Additives.** 2-Aminoethyl 2-aminoethanethiolsulfonate dihydrochloride (I) (Field *et al.*, 1961), 5-aminopentyl 5-aminopentanethiolsulfonate dihydrochloride (II) (Field *et al.*, 1964), and 2-acetamidoethyl 2-acetamidoethanethiolsulfonate (III) (Field *et al.*, 1961) were prepared by published methods.

Cystine *S*-dioxide (IV) was prepared by performic acid oxidation of L-cystine (Emiliozzi and Pichat, 1959). Recently, chemical analysis by electron spectroscopy has directly and conclusively proved that cystine *S*-dioxide, prepared either by performic or perbenzoic (Toennies and Lavine, 1936) acid oxidation, has a thiolsulfonate structure (Axelson *et al.*, 1967).

2-Aminoethanethiosulfuric acid (V) was purchased from Eastman Kodak Co., Rochester, N. Y., and was recrystallized from water before use.

The sodium salt of *S*-sulfocysteine (VI) ($C_3H_6O_5NS_2Na \cdot 1.5H_2O$) was prepared according to Sörbo (1958).

The barium salt of *S*-sulfoglutathione (VII) ($C_{10}H_{15}O_9N_3S_2Ba \cdot 4H_2O$) was prepared as described by Eriksson and Rundfelt (1968). Just before use it was dissolved in a little water and converted into the sodium form by treatment with an equimolar amount of sodium

**Table I. Comparative Efficiency of Different Compounds in Inhibiting the Cooked Flavor**

| Compound | Mol wt | Mg/100 ml of milk (minimum) | mmoles | $I_e$ = mol wt/mg | Relative effectiveness |
|---|---|---|---|---|---|
| 2-Aminoethyl 2-aminoethanethiolsulfonate dihydrochloride (I) | 257 | 5 | 0.0195 | 51.4 | 6.5 |
| 5-Aminopentyl 5-aminopentanethiolsulfonate dihydrochloride (II) | 341 | 5 | 0.0147 | 68.2 | 8.7 |
| 2-Acetamidoethyl 2-acetamidoethane-*thiolsulfonate (III)* | 268 | 3 | 0.0112 | 89.3 | 11.4 |
| Cystine S-dioxide (IV) | 272 | 10 | 0.0368 | 27.2 | 3.5 |
| 2-Aminoethanethiosulfuric acid (V) | 157 | 20 | 0.1274 | 7.8 | 1.0 |
| S-Sulfocysteine (sodium salt) (VI) | 250 | 20 | 0.0800 | 12.5 | 1.6 |
| *S*-Sulfoglutathione (sodium salt) (VII) | 503 | 50 | 0.0994 | 10.1 | 1.3 |

sulfate. Barium sulfate was centrifuged off and the supernatant used as described below.

**Inhibition of Cooked Flavor.** For tests of cooked flavor inhibition, homogenized whole milk was purchased at the local grocery store. Control samples (100-ml batches) were prepared by heating milk in Erlenmeyer flasks, with stirring, from room temperature to 90° in 4 min, holding the temperature at 90° for 5 min, and finally cooling to 40° with ice-water. By this treatment, milk invariably developed a strong characteristic cooked flavor, which was easily detectable by taste or odor. The efficiency of the different additives in inhibiting the cooked flavor was determined by adding varying amounts, in the crystalline state, to 100-ml batches of milk before heating. The sodium form of *S*-sulfoglutathione was added in water solution (see above).

**Flavor Evaluation.** Treated milks were evaluated organoleptically by a panel of five expert judges, who determined the effect of each additive on the intensity of the cooked flavor. The judges used the rating scale 4 = strong, 3 = distinct, 2 = slight, 1 = questionable, and 0 = no criticism.

## RESULTS

The minimum amount (mg/100 ml of milk) of each additive required for significant suppression of the cooked flavor is shown in Table I. Flavor reduction was considered to be significant when the average intensity rating dropped from 4 to 2 or less in the rating scale. Table I (column five) shows the efficiency index ($I_e$), defined as the reciprocal of the required amount of compound, in millimoles. Column six shows the effectiveness of each compound relative to that of 2-aminoethanethiosulfuric acid (the least effective on a molar basis).

Gravimetrically, the amount of additive required to reduce significantly the cooked flavor is very small, from 0.003 to 0.05%. All products tested in this investigation were effective even when added to milk right *after* the heating–cooling cycle was completed. Poststerilization introduction of the additive in the HTST or the UHT process might reduce the level necessary to achieve the desired results.

None of the compounds investigated imparted any foreign flavor to milk, with the exception of *S*-sulfocysteine, which caused a slight broth-like or methional taste at the concentration indicated in Table I. 2-Aminoethanethiosulfuric acid, if used as obtained from the manufacturer without recrystallization, produced an objectionable burnt flavor.

## DISCUSSION

The results of the present investigation represent additional confirmation of the importance of the sulfhydryls in the cooked flavor in milk and confirm the prediction (see introductory remarks) that the thiolsulfonates would be more effective than the thiosulfates in inhibiting the cooked flavor. I hoped that the addition of a strontium salt would drive the reaction of sulfhydryls with thiosulfates toward completion by precipitating the sulfite ion (Swan, 1957). However, the results with strontium lactate were not encouraging, probably due to the low concentration of sulfite. Factors other than the purely kinetic and mechanistic ones might also influence the overall performance of the products studied in removing thiols from heated milk. With the thiolsulfonates, for instance, inherent stability must be an important element. Previous studies (Field *et al.*, 1964) have shown that the thiolsulfonates, when dissolved in water, undergo a process of cleavage whose rate is a function of their structure. It might not be a pure coincidence that the stability in water (Field *et al.*, 1964) of the first three thiolsulfonates I used roughly paralleled their relative effectiveness in inhibiting the cooked flavor. In an experiment designed to test the survival of 2-aminoethyl 2-aminoethanethiolsulfonate dihydrochloride in milk, twice the amount shown in Table I was added to 100 ml of milk, which was refrigerated for 24 hr before heating; most of the compound's ability to inhibit cooked flavor had been lost.

As for the use of additives for flavor control in the HTST or UHT processes, it is difficult to predict whether larger or smaller amounts of thiolsulfonates and thiosulfates than used in the present work would be needed to attain the same level of control. The HTST and UHT procedures entail faster heating, higher temperature, and a much shorter holding time than used here. These and other factors might result in an increase or a decrease of the efficiency index of each additive. I hope that pilot plant work now in progress will answer these questions and ascertain any effect that the use of organic thiolsulfonates and thiosulfates might have on the evolution of the stale flavor along the line of reasoning discussed in this article.

Whenever food additives are considered for actual use the issue of safety arises. Although a detailed discussion on this topic is beyond the scope of this paper, the following observations are related to safety: aminoalkanethiosulfuric acids have been studied as protective agents against the lethal effects of ionizing radiations, and certain organic thiosulfates are metabolic intermediates in animal and plant tissue. Specifically, *S*-sulfocysteine, believed to be an intermediate in the formation of cysteine in molds and bacteria, has been identified in the urine of an infant deficient in the enzyme sulfite oxidase (Mudd *et al.*, 1967) and in that of the Kenya genet (Crawhall and Segal, 1965). Naturally occurring *S*-sulfoglutathione has been found in calf-lens extracts (Waley, 1959) and in the small intestine of rats (Robinson and Pasternak, 1964). Organic thiolsulfonates too have been studied as potential antiradiation drugs. Most of the thiolsulfonates used in this study probably will not survive the combined effects of the HTST thermal treatment and of prolonged storage (*vide supra*). Whatever remains after reaction with the thiols will slowly undergo disproportionation, with the ultimate formation of the corresponding disulfides and

sulfonic acids according to well known pathways (Bauer and Cymerman, 1950). The necessary data to be collected before approval of any specific thiolsulfonate or thiosulfate as food additive could be obtained might include the identification and pharmacological evaluation of the chemical byproducts of the cooked flavor inhibition and of the products of decomposition of the additive itself.

The findings of the present work with whole milk might be applicable to evaporated skim milk and other heated dairy foods.

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LITERATURE CITED

Arnold, R. G., *J. Dairy Sci.* **52,** 1857 (1969).
Axelson, G., Hamrin, K., Fahlman, A., Nordling, C., Lindberg, B. J., *Spectrochim. Acta Part A* **23,** 2015 (1967).
Ballance, P. E., *J. Sci. Food Agr.* **12,** 532 (1961).
Bauer, L., Cymerman, J., *J. Chem. Soc.* 109 (1950).
Crawhall, J. C., Segal, S., *Nature (London)* **208,** 1320 (1965).
Demott, B. J., Gibbs, A. W., *J. Dairy Sci.* **49,** 1152 (1966).
Distler, H., *Angew. Chem. Int. Ed. Engl.* **6,** 544 (1967).
Ellis, G. P., *Advan. Carbohydr. Chem.* **14,** 63 (1959).
Emiliozzi, R., Pichat, L., *Bull. Soc. Chim. Fr.* 1888 (1959).
Eriksson, B., Rundfelt, M., *Acta Chem. Scand.* **22,** 562 (1968).
Ferretti, A., Flanagan, V. P., *J. Dairy Sci.* **54,** 1769 (1971).
Ferretti, A., Flanagan, V. P., *J. Agr. Food Chem.* **20,** 695 (1972).
Field, L., Owen, T. C., Crenshaw, R. R., Bryan, A. W., *J. Amer. Chem. Soc.* **83,** 4414 (1961).
Field, L., Ferretti, A., Crenshaw, R. R., Owen, T. C., *J. Med. Chem.* **7,** 39 (1964).
Gilman, H., Smith, S. E., Parker, H. H., *J. Amer. Chem. Soc.* **47,** 851 (1925).
Gruenwedel, D. W., Patnaik, R. K., *J. Agr. Food Chem.* **19,** 775 (1971).
Hartman, A. M., Dryden, L. P., in "Fundamentals of Dairy Chemistry," Webb, B. H., Johnson, A. H., Ed., AVI, Westport, Conn., 1965, pp 261–338.
Henry, K. M., Kon, S. K., Lea, C. H., White, J. C. D., *J. Dairy Res.* **15,** 292 (1948).
Hodge, J. E., *J. Agr. Food Chem.* **1,** 928 (1953).
Hodge, J. E., "Symposium on Foods. Chemistry and Physiology of Flavors," Schultz, H. W., Day, E. A., Libbey, L. M., Ed., AVI, Westport, Conn., 1967, pp 465–491.
Hutton, J. T., Patton, S., *J. Dairy Sci.* **35,** 699 (1952).
Josephson, D. V., *J. Agr. Food Chem.* **2,** 1182 (1954).
Keenan, T. W., Lindsay, R. C., *J. Dairy Sci.* **51,** 112 (1968).
Kiermeier, F., Hamed, M. G. E., *Z. Lebensm. Unters. Forsch.* **115,** 322 (1961).
Kobayashi, N., Fujimaki, M., *Agr. Biol. Chem.* **29,** 698 (1965).
Maillard, L. C., *C. R. Acad. Sci.* **154,** 66 (1912).
Mudd, S. H., Irreverre, F., Laster, L., *Science* **156,** 1599 (1967).
Patton, S., *J. Dairy Sci.* **38,** 457 (1955).
Patton, S., Josephson, D. V., *J. Dairy Sci.* **30,** 538 (1947).
Robinson, H. C., Pasternak, C. A., *Biochem. J.* **93,** 487 (1964).
Scanlan, R. A., Lindsay, R. C., Libbey, L. M., Day, E. A., *J. Dairy Sci.* **51,** 1001 (1968).
Schöberl, A., Wagner, A., in "Methoden der organischen Chemie (Houben-Weyl)," Müller, E., Ed., Vol. 9, G. Thieme-Verlag, Stuttgart, 1955, p 72.
Schönberg, A., Moubacher, R., *Chem. Rev.* **50,** 261 (1952).
Schutte, L., Koenders, E. B., *J. Agr. Food Chem.* **20,** 181 (1972).
Sörbo, B., *Acta Chem. Scand.* **12,** 1990 (1958).
Swan, J. M., *Nature (London)* **180,** 643 (1957).
Toennies, G., Lavine, T. F., *J. Biol. Chem.* **113,** 571 (1936).
Waley, S. G., *Biochem. J.* **71,** 132 (1959).

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# Preparation of Aqueous Beef Flavor Precursor Concentrate by Selective Ultrafiltration

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Aqueous beef extract was fractionated by two ultrafiltration systems, batch-type and recirculating thin-channel, equipped with two membranes of different retentive capacities. Ultrafiltration was achieved in 2.5–10% of the time needed for exhaustive conventional dialysis. The influence of rate of stirring, pressure applied, and concentration of solute on ultrafiltration flux was studied in the batch system. Gel permeation chromatograms of beef flavor precursors prepared by conventional dialysis and ultrafiltration show a high degree of similarity beyond the void volume fraction. The three preparations exhibited the same beefy flavor and odor notes. Ultrafiltration is a technique of preparative isolation, fractionation, and purification having the advantages of simplicity, speed, and economy.

Beef flavor precursors are small molecular weight compounds present in raw meat and are the prime source of the characteristic flavor developed upon processing. These compounds are extractable with cold water along with proteins.

Various inorganic salts, organic and inorganic acids are in use to precipitate proteins for the preparation of a protein-free extract. Trichloroacetic acid is a well known protein precipitant (Neuberg *et al.*, 1944). Some of the most commonly used deproteinizing reagents are acetic acid with heat (Flatow, 1928), tungstic acid (Woodward and Fry, 1932), molybdic acid (Hess, 1929), tungstomolybdic acid (Benedict and Gottschall, 1933), metaphosphoric acid (Fujita and Iwatake, 1935), tetrametaphosphate and other polyphosphates (Pennell, 1960), picric acid (Hamilton and Van Slyke, 1943), sulfosalicylic acid (Hamilton, 1962), and perchloric acid (Neuberg *et al.*, 1944). Each one of these reagents has its specific use, advantages, and disadvantages. The use of trichloroacetic acid has been criticized because of the tendency of glutathione to become autoxidized (Fujita and Iwatake, 1935). Furthermore, trichloroacetic acid removal from solutions is laborious and time consuming. The presence of traces of trichloroacetic acid in the preparation of protein-free nucleotides' extract introduces errors in the nucleotides' content determined by ultraviolet absorption techniques (Hutchison and Munro, 1961). Block *et al.* (1966) stated that the recovery of added amounts of aspartic, threonine, glycine, valine, isoleucine, leucine, tyrosine, phenylalanine, and arginine from picric acid deproteinated plasma pool

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