l.); 21° showed this statistic at the 8th week (64 μg/l.); and 30° juice showed this statistic at the 2nd week (55 μg/l.). As with canned juice, the highly significant statistic $p < 0.001$ was recorded for essentially the same narrow furfural region (50–70 μg/l.) in glass-packed juice. Extrapolation of furfural values for 10 and 5° stored juices to the 50–70 μg region indicates that these juices would evince a flavor difference ($p < 0.001$) at 34–38 weeks for 10° juice and 88–92 weeks for 5° juice.

The origin of furfural in orange juice is unknown. Huelin (1953) believes that furfural originates from the decomposition of ascorbic acid. Huelin conducted an experiment with a 0.25% ascorbic acid in distilled water at 30° for 2 years and found that the two major decomposition products were furfural and carbon dioxide. With lower pH values there was an increase in furfural formation. Studies by Tatum *et al.* (1967, 1969) on nonenzymic browning of orange powder showed that furfural formed during acid-catalyzed hydrolysis of ascorbic acid. It thus appears from the work of Heulin and Tatum *et al.* that ascorbic acid is a likely candidate for being the precursor of furfural in orange juice.

The present study showed the close relationship between furfural levels and temperature storage and the extent of comparative flavor differences. Furfural levels in juice should be regarded strictly as an index of those substances responsible for flavor change. Furfural, *per se*, does not elicit any flavorful properties even when added to a 5° control juice at the 2000 μg/l. level. It is not necessary, however, for the existence of a direct flavor connection with furfural for accepting furfural as a useful and reliable index of changing flavor properties.

In these experiments, formation of furfural apparently parallels closely the formation of components responsible for flavor changes which have, to date, not been identified. There is the possibility that off-flavors may form at a different rate in a different manner than furfural, depending upon the conditions of the juice; *i.e.*, oxygen content, pH, metal ions, °Brix, and other factors. However, a close correlation of furfural content to flavor change has also been demonstrated with canned and glass-packed grapefruit juices (Randall and Nagy, 1972). Although there is the possibility that furfural may not correlate closely with flavor change, the authors have to date not observed this lack of correlation in several studies on canned and glass-packed orange and grapefruit juices.

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Effect of γ Irradiation and Heating on Proteolytic Activity of Meat Samples

Thomas Losty, Jay S. Roth,* and Gary Shults[1]

Utilizing a ^{14}C-labeled hemoglobin substrate, the total proteolytic activity of ground beef samples subjected to γ irradiation and/or brief heating (blanching) has been determined. γ irradiation alone, in increasing doses from 2 to 6 Mrad, destroys up to 75% of the proteolytic activity but is less effective than blanching, especially when the latter is carried out at 70°. A combination of irradiation at a dose of 4.5 to 5.2 Mrad plus blanching at 65 or 70° may be expected to destroy at least 95% of the proteolytic activity in beef.

Processing of meats with sterilizing doses of ionizing radiation is a useful new method to make cooked meats shelf stable for long periods of time (JCAE, 1968). However, when applied to raw meats, the shelf stability is limited by the activity of the residual proteolytic enzymes. Early works showed that irradiation alone did not inactivate all the proteolytic enzymes of raw meats. Doty and Wachter (1955) found little reduction in the activity of proteolytic enzymes in fresh beef muscles irradiated with 5×10^5 rep of cobalt-60 γ radiation, whereas irradiation at dosages of 1.6×10^6 rep reduced the apparent proteolytic activity (as measured by liberation of tyrosine from casein substrate) of the beef muscles about 50%. Chiambalero *et al.* (1959) determined a time–temperature relationship for heat inactivation of proteolytic enzymes in beef muscles. He found that proteolytic enzymes are inactivated by heating at 71° for 90 sec and at 77° for 17 sec.

It is obviously important to know the amount of the residual proteolytic activity which remains after various treatments, for it may be assumed that the lower the proteolytic activity, the less deterioration there will be in the stored meat.

In this report the effects of various combinations of irradiation and blanching on proteolytic enzyme activity of

Section of Biochemistry and Biophysics, Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268.
[1] Food Laboratory, U. S. Army Natick Laboratories, Natick, Massachusetts 01760.

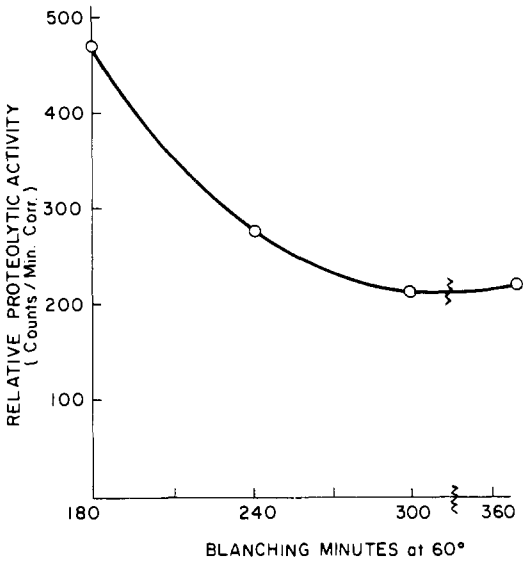

Figure 1. The effect of irradiation with 4.5 to 5.2 Mrad and blanching at 60° on the total proteolytic activity of ground beef. Results are averages of duplicate determinations. Samples were incubated at 37° for 24 hr. The pH of the incubation (3.8) prevented bacterial growth.

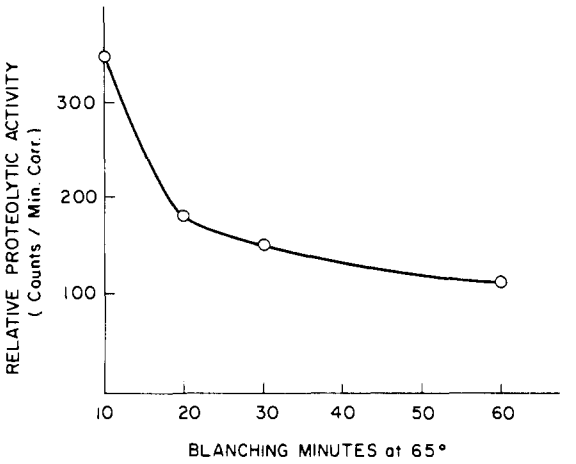

Figure 2. The effect of irradiation with 4.5 to 5.2 Mrad and blanching at 65° on the total proteolytic activity of ground beef. Results are averages of duplicate determinations. Samples were incubated at 37° for 24 hr. The pH of the incubation (3.8) prevented bacterial growth.

meat are determined using an Anson type proteolytic assay (Anson, 1938) but with a ^{14}C-labeled hemoglobin as substrate (Roth *et al.*, 1971). By using the labeled substrate, the sensitivity and specificity of the assay are considerably increased and these are important, since some of the meat samples have very low activities which probably could not be detected by standard methods.

MATERIALS AND METHODS

Assay for Proteolytic Enzyme Activity. The method has been described in detail (Roth *et al.*, 1971). It depends on the use of ^{14}C-labeled hemoglobin which is incubated with the sample in buffered solution. Protein and larger polypeptides are precipitated with trichloroacetic acid, and the acid-soluble radioactivity is determined by liquid scintillation counting.

Meat Samples. The meat samples were prepared by the U. S. Army Natick Laboratories, vacuum packed in 404 × 200 metal cans, 280 g per can. The samples described herein were all ground beef, either *longissimus dorsi* or *semimembranosus* muscle of U. S. Choice grade beef.

The samples, when applicable, were irradiated with γ rays in a 780,000-Ci cobalt-60 irradiation facility at the U. S. Army Natick Laboratories in Natick, Mass., at a rate of 38,500 rad per min. The cans were opened and pieces were taken from two to three places within the can. One gram of meat and 2 ml of glass-distilled water were homogenized for 30 sec in a Waring blender, followed by approximately 20 sec of homogenization in a Potter-Elvehjem homogenizer with a Teflon pestle. Homogenizations were carried out in the cold room (4°). The reaction mixture consisted of 1.6 ml of 0.2 *M* acetate buffer, pH 3.8, 0.8 ml of substrate, and 1.28 ml of tissue. The mixtures were incubated in a shaking water bath at 37° (for 24 hr and aliquots were withdrawn at 0.5 and 24 hr, and precipitated with trichloroacetic acid as described) (Roth *et al.*, 1971). Duplicate reaction mixtures were run for each sample. The results are based on the 24-hr incubation values which are calculated by subtracting the zero time value from the 24-hr value. There was no change in the substrate blank on 24-hr incubation.

Table I. Effect of Irradiation Dose and Irradiation Temperature on the Total Proteolytic Activity of Samples of Ground Raw Beef

Irradiation dose, Mrad	Relative activity		
	Temperature of irradiation, °C		
	0 to 4°	−30 ± 10°	−80 ± 10°
0	5445[a]		
2.0–2.3	5080[b]	5420	6184
4.0–4.6	2485	3954	3601
6.0–6.9	1359	2725	3005

[a] Nonirradiated sample. Values are net radioactive counts/min, corrected for blanks in the acid-soluble supernatant fraction. Average of duplicate determinations. [b] Stored for 1 week at room temperature before assay.

RESULTS

The Effect of Irradiation with 4.5–5.2 Mrad and Blanching at Three Different Temperatures. The meat samples in this experiment were blanched at either 60 or 65°, vacuum packed, irradiated at the temperature of liquid nitrogen (−180 ± 16°), then stored in a frozen state (at −29°) for 1 year prior to delivery to the laboratory. The irradiation dose was 4.5 to 5.2 Mrad.

The results are shown in Figures 1 and 2 and the following observations are made.

Blanching at 65° is much more effective than 60°. Ten minutes at the higher temperature is just as effective as 210 min at the lower temperature.

At 65°, 60 min of blanching gives little more reduction in proteolytic activity than 30 min; the values approach a plateau. The same is true for blanching at 60°; a plateau is reached at 300 min, above which time little further decrease in activity can be expected.

Seventy-degree blanching was used for 5 and 10 min with two ground beef samples and followed by irradiation with 4.5 to 5.2 Mrad. Both of these samples showed a relative proteolytic activity of 100 net counts, approximately the value reached after 60 min of blanching at 65°. Thus, blanching at 70° rapidly destroys all but a trace of proteolytic activity. These counts represent an extremely low level of proteolytic activity. For comparison, fresh ground beef muscle incubated for 24 hr would be expected to release between 4000 and 10,000 counts of activity. The meat samples have, therefore, less than 5% of the activity of unheated muscle and possibly as little as 1%. The significance of the remaining proteolytic activity in the irradiated and blanched meat samples to the integrity of the meat cannot be assessed. The results shown in Figures 1

and 2 were confirmed in a much more extensive experiment (Roth and Losty, 1968).

The Effect of Increasing Irradiation Dose and Irradiation at Different Temperatures. Samples were irradiated with 0 (nonirradiated/control), 2.0–2.3, 4.0–4.6, or 6.0–6.9 Mrad at 0 to 4, −30 ± 10, or −80 ± 10°, and were stored frozen until delivered to the laboratory. These samples were not blanched. The results are shown in Table I.

Three major conclusions may be drawn from the data in Table I. There is a marked decrease in total proteolytic activity with increasing radiation dose. With respect to destruction of proteolytic activity, irradiation at 0 to 4° is considerably more effective than irradiation at −30 or −80° (±10°). Irradiation alone (without blanching) does not destroy all proteolytic activity and is, in actuality, less effective than blanching.

It has also been determined (not illustrated) that storage of these irradiated samples at −16° or room temperature (21–25°) for up to 4 weeks does not significantly change the total proteolytic activity.

DISCUSSION

The methods presented in this report make it possible to determine the optimum conditions for the destruction of the total proteolytic activity of meat samples by blanching alone or blanching and γ irradiation. It is clear that of the two methods blanching is more effective but only when carried out at temperatures of 70°. Even 4.5–5.2 Mrad of γ radiation will destroy only about 75% of the proteolytic activity of beef samples. A combination 4.5–5.2 Mrad γ radiation and blanching at 70° for 5 min may be expected to destroy 95 to 100% of the total proteolytic activity of meat. Such samples would probably undergo little further enzymic change even when stored at room temperature for long periods of time.

Although it is possible that there is some latent proteolytic activity in the meat samples tested, this seems unlikely for several reasons. First of all, the method of preparing the samples for assay by homogenization in water would probably release any lysosomal bound cathepsins. Secondly, assays on samples, both irradiated and blanched, at intervals after up to 8 weeks of storage at room temperature indicated that no significant changes in proteolytic activity occurred.

By use of isolated cathepsins as well as crude tissue homogenates, it may be possible to define more precisely conditions under which complete destruction of proteolytic enzyme action may be expected.

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On the Photodecomposition of Chlorophyll *in vitro*

Marvin M. Morris, Yoong Park, and Gordon Mackinney*

Chlorophyll solutions can be irradiated to yield variable amounts of reddish intermediates and a virtually colorless supernatant. Precipitated after brief irradiation, the red intermediates retain a nitrogen–phytol–magnesium ratio of 4:1:1. After longer exposures, the nitrogen–magnesium ratio remains unchanged but the phytol is lost. The supernatant is evaporated to dryness and the residue is extracted successively with petroleum ether, water, and acetone. The petroleum ether extract is colorless with a weak blue fluorescence. It contains a phytyl ester and 10 to 15% of the original nitrogen. The water extract exhibits a whitish turbidity and a strong blue fluorescence. It contains a substantial fraction of the original nitrogen and magnesium. The original supernatant has negligible absorbance in the visible, but some reddish precipitate is formed on concentration. This is soluble in acetone and is devoid of phytol.

The decomposition of chlorophyll in solution in the presence of light and oxygen can be arrested and red intermediates can be precipitated, provided the solution contains a high percentage of a nonpolar solvent such as petroleum ether (Jen and Mackinney, 1970a). Under the most favorable conditions, yields of red intermediate have never exceeded 75%, and in view of a considerable uptake of oxygen, it is doubtful if 50% of the original chlorophyll has been accounted for.

Department of Nutritional Sciences, University of California, Berkeley, California 94720.

This warrants further examination of both the precipitates and the supernatant solutions, and this paper is concerned primarily with the fate of the phytol, the nitrogen, and the magnesium as a result of the photooxidation of the chlorophyll.

MATERIALS AND METHODS

Solvents and Chlorophylls. The necessary solvents and procedures for preparing the pure chlorophylls have already been described (Jen and Mackinney, 1970b).

Phytol Determination. Phytol was detected by gas–liquid chromatography (glc) in a Varian Aerograph, Model