ated meat samples. At lower temperatures, the time required to ebulliate all the mercaptan was too long for convenient routine assay. At higher temperatures, the possibility of inaccurate high values as the result of protein degradation appeared likely.

A series of experiments was run to determine the effect of short storage periods on the volatile methyl mercaptan content of irradiated beef. No significant difference in the amount of methyl mercaptan was noticed within 36 hours after irradiation, if the irradiated samples were stored in the frozen state at −18° C. Thus, as long as the irradiated samples were kept frozen, methyl mercaptan could be quantitatively determined within 36 hours after irradiation.

On the basis of methyl mercaptan determinations on a large number of irradiated-meat samples, the methyl mercaptan content of beef increases directly with gamma-irradiation dosage (Table III). In addition to this dosage effect, there is a difference in the amount of methyl mercaptan formed in different samples of beef during irradiation.

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FOOD TANNINS MEASUREMENT

Determination of Food Tannins by Ultraviolet Spectrophotometry

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The need for simpler and more accurate methods for the quantitative determination of tannins prompted the development of a direct procedure based on the ultraviolet absorption of these compounds. The tannins are extracted with ethyl acetate, and, after evaporation of solvent, are taken up in acidified methanol. The absorbance of the methanol solution is measured at 270 mμ. The method was developed for tea and beer, but may be applicable to other materials as well.

AVAILABLE METHODS for the determination of the heterogeneous group of compounds best described as tannins have been reviewed (*10*), and two of the more popular ones critically compared (*15*). The latter are the permanganate titration procedure of Loewenthal [the official method for tannins in tea, coffee, and spices (*1*)], and the colorimetric Folin-Denis-Pro procedure [official for distilled liquors (*2*)]. The limitations of these methods are well known.

The tannins have a common distinguishing feature—namely, the presence of two (generally) oxygenated aromatic rings, joined by a three-carbon chain of varying oxygen substitution (*5*). The method for determining tannins proposed here, which takes advantage of this common structure, is not subject to interference by aliphatic reducing substances, and is unique among the many methods for estimating tannins.

This procedure involves extracting the tannins from an acidified sample by ethyl acetate, removing the solvent, dissolving the residue in methanol, acidifying the solution, and determining the absorbance at the wave length of maximum absorption (270 mμ for beer, 275 mμ for tea). A prior extraction of the sample with a nonpolar solvent (such as iso-octane) may be necessary with some materials. The tannin content of the sample is calculated from the extinction coefficient of the tannins present. If this value is not known, the foodstuff or other material to be analyzed is extracted as for the determination—but on a scale that allows isolation of the tannins and determination of the extinction coefficient.

Procedure

Reagents and Apparatus. Ethyl acetate, reagent grade, redistilled.
Hydrochloric acid, 1 *N*.
Iso-octane.
Sodium sulfate, anhydrous, reagent grade.
Beckman spectrophotometer, Model DU.

Isolation of Tannins from Hops. Twenty grams of dried hops was homogenized with benzene in a Waring Blendor (*16*), and extracted by decantation through a filter paper with about 3.5 liters of benzene—or to the disappearance of color. The hops were then extracted in a similar manner with 2.5

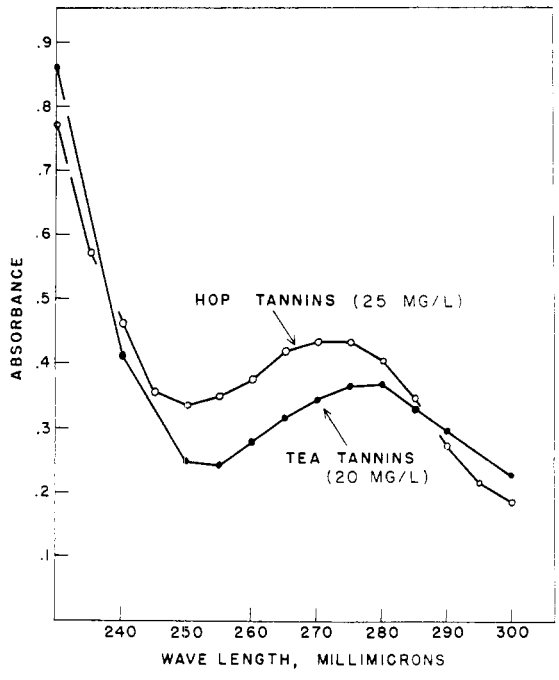

Figure 1. Absorption spectra of tannins

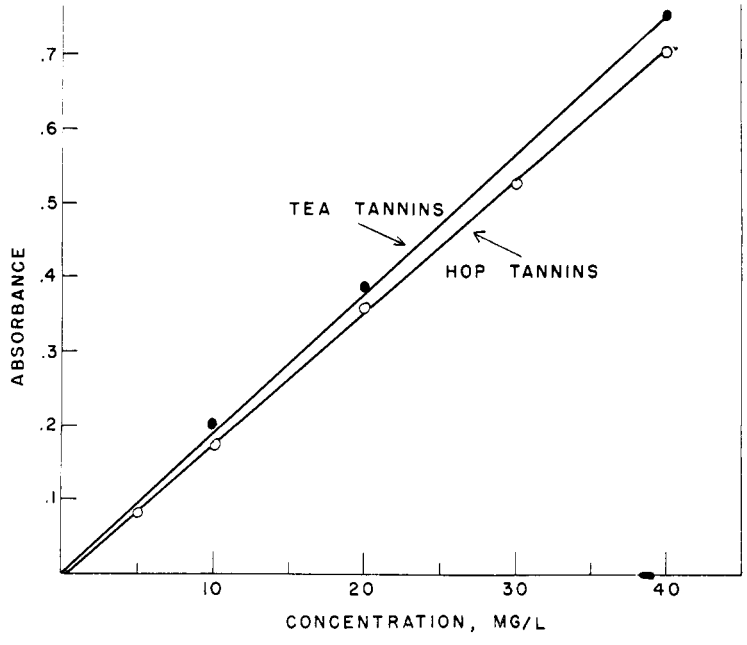

Figure 2 Calibration curve of tannins at 275 mμ

liters of ethyl ether. After being air-dried, the hops were extracted with 5 portions of boiling water, totaling about 700 ml. The water extracts were concentrated to about 100 ml. by vacuum distillation, and then acidified with 1*N* hydrochloric acid to pH 2.0. The acidified solution was then extracted with three 100-ml. portions of ethyl acetate. The ethyl acetate extract was concentrated to exactly 200 ml., and an aliquot was taken for total solids determination. The dry material was dissolved in a known volume of methanol and an ultraviolet absorption curve was taken. The maximum was at 270 mμ (Figure 1). A standard curve was made at this wave length; it obeys Beer's law (Figure 2), and is very reproducible.

Method for Determination of Tannins in Beer. Three milliliters of degassed beer was acidified with 1 ml. of 1*N* hydrochloric acid and made to 25 ml. with distilled water. Iso-octane, 25 ml., was added; the mixture was shaken vigorously for 30 seconds and permitted to stand for 5 minutes. Ten milliliters of the aqueous phase was transferred by pipet to a separatory funnel, and 15 ml. of ethyl acetate was added. The mixture was shaken, and, after clear separation of the two phases, the aqueous layer was extracted twice more with ethyl acetate. After combination of the ethyl acetate extracts, the moisture was removed by sodium sulfate. The extract was then transferred by decantation to a 100-ml. beaker, evaporated, and dried at 80° C. for 30 minutes.

Complete dryness is essential for removal of traces of acetic acid and ethyl acetate. The residue was taken up with three 2-ml. portions of methanol and transferred to a 10-ml. volumetric flask containing 0.5 ml. of 1*N* hydrochloric acid. Methanol was added to the mark and the absorbance of the solution was read at 270 mμ. A solvent blank was run simultaneously. The tannin content was taken from the calibration curve for hop tannins. (The absorption of malt tannins is similar to that of hop tannins.)

Isolation of Tannins from Tea. Two grams of commercial soluble tea was extracted with six 50-ml. portions of boiling methanol. After concentration to 80 ml., the methanol extract was poured into 400 ml. of chloroform. The resulting precipitate was collected on a Büchner funnel. After being washed with 100 ml. of chloroform, the precipitate was sucked almost dry. The precipitate was then dissolved in 50 ml. of water. After remaining traces of chloroform were boiled off, the solution was brought to pH 2.0 with 1*N* hydrochloric acid. The acidified solution was extracted with three portions of ethyl acetate, and thereafter the procedure was exactly the same as that for the hop tannins—except that absorbance was read at 275 mμ.

An infusion of tea leaves may be substituted for the solution of tea solubles used above.

Determination of Tannins in Tea. Exactly 100 mg. of commercial soluble tea was dissolved in 100 ml. of water. Two milliliters of this solution was acidified with 1 ml. of 1*N* hydrochloric acid and made up to 25 ml. with water. Ten milliliters of this solution was then extracted with ethyl acetate. The determination of tea tannins was then carried out in the same way as that for beer tannins, but using the appropriate wave length and calibration curve.

Discussion and Results

The authors have made no attempt to characterize the flavonoids of beer or tea, as other investigators have done this (*6–8, 11, 13, 16*). However, this work shows that the polyphenols of tea are remarkably similar to those of hops in their ultraviolet absorption (Figure 1). Moreover, these absorption curves resemble those derived by investigators (*6, 7, 9*), who utilized ultraviolet absorption as a means for characterizing various tannins or groups of tannins. The curves presented here agree particularly well with those of Bradfield and Penney (*6, 7*), who measured the ultraviolet absorption of catechins, gallocatechins, and catechin gallates. In every case, their compounds showed maxima which lay between 271 and 280 mμ, and strong absorption below 240 mμ. Figure 1 shows a peak for hop tannins at 270 to 275 mμ, and a peak of 275 to 280 mμ for tea tannins.

Besides being similar with respect to the pattern of ultraviolet absorption, when extracted in the manner described, hop and tea tannins, weight for weight, exhibit essentially the same absorption at 270 and 275 mμ, respectively (Figure 2).

Some results for commercial beers are shown in Table I and for commercial soluble teas in Table II. Table II also shows data obtained with the AOAC method for tea (*1*).

To determine if there were interferences from substances normally pres-

Table III. Recovery Studies

System	Material Added	Increment, γ	Total Tannins, γ	Increment Recovered γ	Increment Recovered %
Beer A (3 ml.)	Digallic acid	0	552	...	...
		150	696	144	96
		300	822	270	90
Beer B (3 ml.)	Digallic acid	0	477	...	...
		75	546	69	91
		300	767	290	97
		300	752	275	92
Water	Hop tannins	200	177	177	89
		300	270	270	90
		600	636	636	106
Beer C (3 ml.)	Hop tannins	0	663	...	...
		300	957	294	98

ent in the brewing process, this procedure was applied to a solution containing 5% commercial corn sirup, 1% caramelized sugar, and 5% ethyl alcohol. The absorbance at 270 mμ was 0.025 and 0.019 as compared with 0.023 for a solvent blank.

Acidification of the solution to pH 2 prior to treatment with ethyl acetate is necessary to ensure complete extraction of the undissociated tannin molecules. Such treatment, however, permits formation of traces of acetic acid which may act as a positive interference at 270 to 275 mμ. The residue remaining after evaporation of the solvent is therefore heated at 80° C. to drive off any traces of acetic acid and ethyl acetate which may be present. To show that this treatment is sufficient to drive off any acetic acid which may be present, one drop of a 1% solution of acetic acid was added to duplicate ethyl acetate extracts of beer.

Table I. Tannin Content of Beers

Sample	Tannins, Mg./L.	Av., Tannins, Mg./L.
1	169, 162	166
2	182, 174	178
3	221, 220, 215	219
4	267, 275	271
5	132, 125	129
6	177, 185, 190, 184	184
7	140, 145, 149, 143	144
8	157, 161	159
9	156, 161	159

Table II. Tannin Content of Dried Tea Extracts

(Comparison of two methods)

Sample	Tannins, % Author's method	Tannins, % AOAC method
1	15.6	11.9
2	13.6	12.4
3	17.5	11.9
4	14.3	10.6

These extracts showed essentially the same absorbance at 270 mμ (0.308, 0.330) as did the controls (0.320, 0.318).

In applying this method to other products, one major precaution must be taken—namely, the removal of any extraneous substances which absorb in the ultraviolet and which would be soluble in ethyl acetate. In the case of beer, for example, the isohumulones (*12*) absorb at 275 mμ. It is to remove these substances that the sample is preextracted with iso-octane. Preliminary extraction with iso-octane or another nonpolar solvent may possibly be necessary for other products as well.

Recoveries of hop tannins and commercial tannic acid from water and from beer are shown in Table III.

At least two groups of investigators have pointed out the inadequacy of the methods for tannin determination currently in use. Smit, Joslyn, and Lukton (*15*), in comparing the Loewenthal method with the Pro method, found serious discrepancies, which they ascribe to the "highly empirical nature of the two methods examined." The Loewenthal procedure gave results that were far too low (*3*). The data in Table II support these findings. Moreover, the Loewenthal method gives results which vary with the state of oxidation of the tannins being determined. Barua and Roberts (*3*), therefore, adopted an alkaline permanganate titration in lieu of Loewenthal's procedure. This change still left the method subject to oxidative changes, albeit less so, and did little to simplify it.

Williams (*17*) has pointed out that permanganate reacts only with *o*- and *p*-dihydroxybenzene compounds, and not with monohydroxy phenols nor *m*-dihydroxybenzenes. A more serious limitation of the permanganate method is that catechol takes up less oxygen than pyrogallol, so that the type of flavonoid present may seriously affect the "tannin" values.

While no effort has been made to investigate the influence of oxidizing agents on the determination of tannins by the present procedure, it was established that no changes in results are induced by exposure of aqueous solutions of the tannins to air for 16 to 24 hours.

The colorimetric method, while simple, is open to interference by any substance capable of reducing phosphomolybditungstic acid, among which ascorbic acid is particularly troublesome (*14*).

The complexity and variety of the tannins have been described by Bate-Smith (*4*). Therefore, to single out any one polyphenolic substance, as is commonly done, and use it as the standard when determining tannins seems unrealistic.

The present method utilizes the very substances being determined as the standard, rather than a single arbitrary compound. The technique has been applied to hops and tea, and may be applicable to other plant materials, such as wines, fruit juices, coffee, and cacao.

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