

## Phenolic Characteristics in Brewing. I

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THE EXTENT OF knowledge of the many flavor components of beer has increased greatly in the past few years. The use of the several new systems of physical separations has been the greatest single stimulus to the study of scores of normal beer flavor constituents and to the elucidation of some abnormal flavor components. It has previously been demonstrated that a flavor component may be completely normal when present in a limited concentration but cause an abnormal flavor when present in higher concentration. This paper will report on the initial stages of an investigation of what is apparently a large group of related compounds whose presence in beer in above minimal concentrations causes abnormal flavors.

During the past few years our beer taste panel has occasionally noted the presence of abnormal flavor characteristics which were best described as "medicinal" or "phenol-like." On at least three occasions, in the past two years, this oftaste has been of such intensity as to cause customer complaints and withdrawal of beer from the market. Subsequent investigation of these beers disclosed the presence of abnormal amounts of phenolic- and chlorophenolic-type compounds. A method of analysis developed by us is described later in this paper.

It should be stated at this point that the references in this paper to phenolic compounds pertain only to relatively volatile phenols. Fairly complex and generally nonvolatile polyphenols such as tannins, cyanins, anthocyanogens, catechins, flavonols, [etc. do](#) not fall within the immediate field of study. However, there is at present limited evidence that hydrolysis or decomposition products of these more complex polyphenols do make a notable contribution to the volatile-phenol picture.

There is without doubt a considerable number of phenolic-type compounds that normally occur in wort and beer. Such well-known investigators as McFarlane and associates (5,10), Harris and Ricketts (4), and Preece (8), to mention a few, have published lists of many such compounds that they found in beer and wort. However, these

research people were primarily interested in phenolics from the viewpoint of their influence on the physical stability of beer; therefore brewing literature is almost free of references to any effect on the flavor of beer. The general statement is found that tannins have an astringent taste which is undesirable in finished beer (4). Helm published an article (3) in 1949 in which he reported an unpleasant phenol-like taste in beer caused by certain bacteria, but little further work seems to have been reported.

Phenols and chlorinated phenols have been prominently mentioned as undesirable contaminants of water for many years (9). The U.S. Public Health Service has set a limit of 1 ppb. of total phenols in acceptable water supplies (1). This extremely low figure indicates the very low threshold limits of odor and taste of some of the phenols and chlorophenols.

**TABLE I**  
**Organoleptic Thresholds of Phenols and Chlorophenols in Water**

	PHENOLS		RESPECTIVE (CHLOROPHENOLS)
	ppm.	ppb.	ppb.
Phenol	25		1-5
Cresol		2.5	0.2-1.0
Thymol		50	50
Resorcinol	10		(no odor)
Hydroquinone		(no odor)	(no odor)
Naphthol	7		500
Creosote		125	10

Table I shows the threshold limits of various phenolics in water as determined by Nesmeyanova (7). Attention is drawn to the very large differences in taste be-

tween such closely related compounds as phenol and cresol and to the much lower limits of most of the chlorinated derivatives.

When many of the more pungent compounds were added to samples of normal beer, our taste panel had no difficulty in noting the phenolic or medicinal character which they imparted. The detectable levels in beer are somewhat higher, in most instances, than those in water. For example, 30 ppm. phenol, 20 ppb. o-cresol, and 15 ppb. 4-chloro-2-methyl phenol (chlorocresol) were needed for detection. However, only 12 or 3 ppb. of o-chlorophenol gave a distinct off-character to beer. When the taste panel was asked to indicate *which* phenolic compounds, when added to beer, most closely matched the off-character of a brewery beer, it chose o-chlorophenol and 4-chloro-2-methyl phenol as being close duplicates, with o-cresol and chlorohydroquinone also being quite similar. It is quite possible that other phenols and chlorophenols that we have not checked will also produce similar flavors. The majority of the simpler phenols reported to be present in beer are rather difficult to obtain, particularly in their chlorinated forms.

The desirability of having an analytical method to determine the amounts of these phenolic compounds in beer was obvious. A literature search failed to disclose any

method that had previously been used with beer. Since water chemists have been determining phenols in water and industrial wastes for several years, it was natural to turn there for a method which could be adapted for use with beer. The water chemistry literature disclosed a fairly large number of available methods. For a method to be useful for our purposes, it must be sensitive to a broad spectrum of compounds, accurate with milligram and microgram amounts of compounds, and useful with available laboratory equipment. Several general methods and colorimetric phenol indicators were tested, but a recently published article by Mohler and Jacob (6) on a comparison of analytical methods for phenolic-type compounds influenced the decision to employ 4-aminoantipyrine as the colorimetric indicator. This indicator is also used in one of the phenol methods published by the American Public Health Association (1) and it is this basic procedure which was modified for our use with beer. The basic color reaction is illustrated in Fig. 1. This reaction is applicable to phenols in which the para position is not blocked with an aryl, alkyl, nitro, benzoyl, or carbonyl group. Para-substituted halogens, carboxyl, sulfonic acid, hydroxyl, and methoxyl groups are expelled in the reaction and do not interfere (6). The colors produced by the different phenolic compounds, while having slightly different visible tints, have

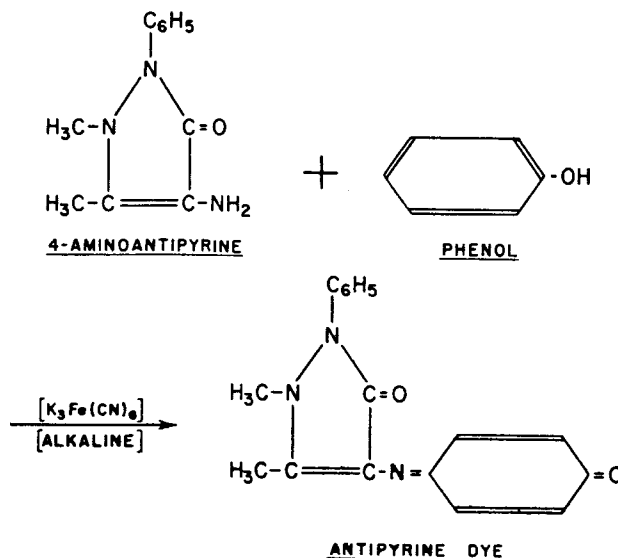


Fig. 1. Phenol color reaction.

their maximum absorbances at the same wave length. From the above it is seen that the method is not all-inclusive, but we trust it does cover the great majority of compounds likely to be encountered.

The following modification of the basic method separates the chlorinated phenols from the nonchlorinated compounds and provides for separate readings of the two types.

#### Method

Apparatus: (1) Distillation apparatus. All glass; 1-liter distillation flask, vertical 400mm. Graham condenser, 500-ml. receiving flask.

(2) Separatory funnels; 1 liter and 250 ml. (3) Spectrophotometer; for use at 460 m $\mu$ .

Reagents: (1) Copper sulfate solution. Dissolve 100 g. CuSO $\cdot$ 5H $_2$ O in distilled water and dilute to 1 liter.

(4) Phosphoric acid solution. Dilute 10 ml. 85% H $_3$ PO $_4$  to 100 ml. with distilled water.

(3) Ethyl ether. Anhydrous, ACS grade. (4) Sodium hydroxide solution, 0.1 N. Dissolve 4 g. NaOH in distilled water and dilute to 1 liter.

(5) Potassium carbonate solution, 0.2 N. Dissolve 27.64 g. K $_2$ CO $_3$  in distilled water and dilute to 1 liter.

(6) 4-Aminoantipyrine solution. Dissolve 2.0 g. 4-aminoantipyrine in distilled water and dilute to 100 ml. Stable for about 5 days.

(7) Potassium ferricyanide solution. Dissolve 8.0 g. K $_3$ Fe(CN) $_6$  in distilled water and dilute to 100 ml. Stable about 2 days.

(8) Ammonium chloride solution. Dissolve 50 g. NH $_4$ Cl in distilled water and dilute to 1 liter.

(9) Chloroform. Spectra grade.

(10) Standard phenol solution. (a) Stock solution. Dissolve 1 g. phenol in distilled water and dilute to 1 liter. Standardize by the following method: Place approximately 100 ml. distilled water and 50 ml. of stock phenol solution in a glass-stoppered 500-ml. flask. Add exactly 10 ml. bromate-bromide solution [to 2.784 g. KBrO $_3$  dissolved in distilled water, add 10 g. KBr and dilute to 1 liter] and then 5 ml. conc. HCl. Stopper the flask and swirl gently. If brown color of free bromine does not persist, add bromate-bromide reagent in 10-ml. portions until color does persist. Stopper and allow to stand for 10 min. and then add 1 g. KI. Prepare a blank in the same manner using distilled water in place of stock phenol solution and only 10 ml. of bromate-bromide solution.

Titrate both blank and sample with 0.025 N sodium thiosulfate solution using starch solution (5 g. soluble starch per liter of distilled water) as the indicator.

Calculate the strength of phenol solution by the formula

$$\text{mg./liter phenol} = [(A \times B) - C] \times 7.835.$$

A = ml. of 0.025 N thiosulfate used for blank;

B = ml. of bromate-bromide solution used for sample; C = ml. of

0.025 N thiosulfate used for sample.

(b) Diluted phenol standard. Dilute 10 ml. of stock solution to 1 liter and then 10 ml. of the diluted stock solution to 100 ml. 1 ml. = 0.001 mg. or 1 mcgm.

Procedure: (a) Preliminary separations. Place 500 ml. of nondecarbonated beer in the distilling flask. Add 5 ml. copper sulfate solution and lower pH to less than 4.0 with phosphoric acid solution. Use pH test paper or methyl orange indicator to check the pH. Connect flask to distilling apparatus and distill over 450 ml. Stop distillation, add 50 ml. distilled water to the distilling flask, reconnect and continue distillation until a total of 500 ml. has been collected. Care must be exercised to prevent scorching or burning of beer on sides or bottom of distilling flask.

To distillate add 1 ml. phosphoric acid solution and 5 ml. copper sulfate solution. Transfer to 1-liter separatory funnel and make three extractions with ethyl ether, using 50 ml. for each extraction. Combine the ether extracts and discard the aqueous phase.

Extract the combined ether extracts in a 450-ml. separatory funnel with three 50-ml. portions of 0.2 N potassium carbonate. Combine the carbonate extracts, which contain the chlorinated phenols.

Extract the nonchlorinated phenols from the ether extract with three 50-ml. portions of 0.1 N sodium hydroxide and combine these hydroxide extracts.

Dilute the carbonate and hydroxide separately to about 250 ml. with distilled water. Heat on a water bath until all the ether has been removed. Cool and dilute each to 500 ml.

Place the diluted extracts in 1-liter distilling flasks, lower pH to less than 4.0 with phosphoric acid solution, add 2 ml. copper sulfate solution, and distill each as directed under "Preliminary separations" above; collect 500 ml. of distillate.

(b) Color development and extraction. Prepare a blank consisting of 500 ml. of phenol-free distilled water. Also prepare a standard phenol solution consisting of 0.005 mg. phenol in 500 ml. distilled water.

To the blank, the phenol standard, and the two sample solutions add 10 ml. ammonium chloride solution, stir, and then adjust with ammonium hydroxide to pH 10.2 = 0.1. Add exactly 3.0 ml. 4-aminoantipyrine solution and mix. Add 5 ml. potassium ferricyanide solution and mix. Allow to stand 3 min. and extract immediately with chloroform, making three serial extractions using 15, 10, and 5 ml. chloroform. Combine the chloroform extracts,

which will have a volume of 41 to 44 ml., filter through a small dry filter paper to remove any water. Carefully wash filter paper with a small amount of chloroform and collect exactly 45 ml. of total filtrate.

(c) Color determination. With the spectrophotometer set at 460 mmu wave length, zero the machine against the blank solution and read the optical densities or absorbances of the phenol standard and the two unknowns. Readings can usually be made in 1-cm. cell, but if very low densities are obtained a 5-cm. cell should be employed.

(d) Calculations.

Calculate the phenol or chlorophenol content of the sample by using the formula.

$$P = \frac{St \times OD_s \times 1000}{OD_{st} \times S}$$

in which

**P** = mcgm./l. phenol or chlorophenol (ppb.);

**St** = mcgm. phenol or chlorophenol in standard;

**OD<sub>s</sub>** = optical density of sample (460 mmu);

**OD<sub>st</sub>** = optical density of standard (460 mmu);

**S** = ml. of sample.

The calculated value from the carbonate extraction solution **will** be the chlorophenol-type compounds and the value from the hydroxide extraction solution will be the phenol-type compounds.

The method is sensitive to less than 1 ppb. of phenol or chlorophenol. The time required for a single determination is about 8 hr. The separation of chlorophenols from phenols by the carbonate and the hydroxide extractions is excellent. When known amounts of phenol and chlorophenol were added to water solutions they were recovered in their proper fraction in essentially 100% amounts.

In order to establish the levels of phenol and chlorophenol-type compounds that would be found in normal-tasting beers, by the use of this method, a series of commercial beers were analyzed. The average value for 15 beers is shown in the upper half of Table II. Beers from small, medium and very large-sized breweries are included in the averages. With one notable exception, the values from the larger brewery beers are below the averages of 7.1 ppb. for chlorophenol and 12.5 ppb. for phenol. In all instances the figures for phenol-type were greater than the chlorophenol-type values, but the ratios vary from 1.1/1.0 up to 4.0/1.0.

Comparable values for 15 beers having

**TABLE II**  
**Phenol and Chlorophenol Values**

	CHLOROPHENOL	PHENOL
	ppb.	ppb.
<b>For beers of normal taste</b>		
High value	11.8	16.9
Low value	3.0	9.6
Average of 15 beers	7.1	12.5
<b>For beers with "phenolic" or "medicinal" tastes</b>		
High value	38.6	71.2
Low value	4.0	19.0
Average of 15 beers	13.9	27.1

abnormal phenolic or medicinal flavors are presented in the lower part of Table II. These 15 beers represent seven different breweries. A comparison of the two sections of this table shows the off-taste beers having average phenol and chlorophenol values about twice as high as those of the normal beers. In only one sample was the chlorophenol value greater than the phenol. We have found the phenol values to vary to a greater extent and to correlate better with taste panel findings than the chlorophenol values. To (late the taste panel has not found a beer with less than 19 ppb. phenols that had this off-taste. With one exception, the panel has found the phenolic taste in beers with above 18.5 ppb. phenols. The exception is a flavorful, hoppy beer that usually has from 19 to a3 pph. phenol and sometimes is criticized by the panel and sometimes passes as clean. Beer samples from three different producers, with phenol values of 71, 38, and 45 ppb., had such disagreeable taste characteristics that remaining supplies were withdrawn from the market.

This type of off-taste has been a continuing problem with a few breweries, while others have had a sudden occurrence of short duration. These facts have led us to the conclusion that several factors may cause the production of excess phenols and chlorophenols in the brewing process. During the investigation of the phenolic-like taste in beer we began, and are continuing, to investigate the biological aspects that may be involved. A wort was obtained from a brewery designated as brewery 1 which has had periodic problems with a medicinal taste in their beer. We inoculated this wort with yeast from the brewery and also with a pure-culture

yeast from our collection. The wort originally had a concentration of chlorophenolic- and phenolic-type compounds of 10.8 and 27.5 ppb., respectively. After fermentation the level of phenolic-type compounds was reduced by about 50% by both yeasts, while the same wort sample, incubated 9 days without yeast, yielded a very high level of these compounds (Table III, Section A). From this information we assumed that these yeasts did not play a role in the development of the

was obtained. To test the effectiveness of the isolate we inoculated the pure culture into sterile wort obtained from a different brewery which we have designated as brewery 2. At the same time wort from this brewery which was not sterilized was allowed to spoil naturally. As Table III, Section C indicates, the wort inoculated with the pure culture shows an increase in chlorophenolic- and phenolic-type compounds. The uninoculated and incubated sample of nonsterile wort shows a tre

**TABLE III**  
**Effect of Yeast and Bacterial Growth**  
**on Brewery Worts**

WORT SAMPLE		CHLOROPHENOLS ppb.	PHENOLS ppb.
<b>Brewery 1, Wort 1</b>			
A	As received	10.8	27.5
	Fermented with brewery yeast	12.2	17.6
	Fermented with laboratory yeast	12.2	15.6
	Incubated 9 days without yeast	80.0	91.0
<b>Brewery 1, Wort 2</b>			
B	As received	16.9	27.1
	Incubated 9 days without yeast	20.0	50.8
<b>Brewery 2, Wort 1</b>			
C	As received	8.0	10.0
	Incubated 9 days without yeast	61.0	117.0
	Sterilized, incubated 9 days with bacteria culture	16.0	42.0
<b>Brewery 3, Wort 1</b>			
D	As received	10.0	20.0
	Incubated 2 days without yeast	10.0	19.0
	Incubated 9 days without yeast	11.0	22.0

medicinal taste, but rather may remove some of the material during fermentation; and secondly, the growth of bacteria during incubation greatly increases the concentration of these compounds.

Further wort samples were obtained from the same source. Some of the samples were incubated for 9 days at 30°C. without yeast fermentation. The amount of phenol- and chlorophenol-type compounds of the incubated sample showed a very large increase over that of the fresh wort (Table III, Section B). A microscopic examination revealed the presence of Gram-negative short rods. Since these appeared to be members of the coli-aerogenes group of wort-spoilage organisms, we decided to isolate a pure culture by inoculating some of the infected wort into lactose broth. Vigorous growth and gas production occurred in 44 hr. at 37°C. By means of this procedure a pure culture

was obtained. The organism found in the uninoculated spoiled wort was a typical Gram-negative short rod which was capable of growth and gas production in lactose broth after 24 hr. of incubation at 37°C.

We have had the opportunity to examine other worts, and although the results with the worts from brewery 1 and 2 point out a relationship between an organism and the production of some chemically detectable substance, we did not find this organism in wort from brewery 3. (Table III, Section D). This wort did contain a Gram-negative short rod, but after 9 days of incubation the organism did not produce the typical off-taste or odor found in the worts from breweries 1 and 4, nor did it produce high levels of phenol- and chlorophenol-type compounds; and, unlike the organisms found in the brewery 1 and 2 worts, it did not produce acid and gas

in lactose broth. Helm (3) has reported that he found a phenolic taste in several continental beers and has related this to the presence of a typical Gram-negative wort-spoilage organism. As he explains in his paper, there are several different organisms which belong to this group but not all cause a phenolic taste. He found that only the indole-negative type produced the flavor. The isolate from brewery 1 which we have been working with is also of the indole-negative type.

Brewery 1 has had a long-term problem with this type of off-taste while brewery 2 has had occasional occurrences. Both plants are attempting to eradicate the wort-spoilage organism from their systems. Brewery 3 also has a phenol-like taste problem, but we believe the cause to be of a different nature. Work has been started and is continuing on other sources of abnormal introduction of phenol and chlorophenol into the brewing process. Water, both raw water supply and condensed steam, are strongly suspected of being contributory factors in some instances. Cleaning compounds accidentally introduced into beer caused a strong medicinal character in a certain pilot-brewery beer. Normal brewing materials, such as malt and hops (when handled in varying manners) cause increases or decreases in analyzable phenol fractions in beer. Improper or poorly handled container coatings are suspected of causing this type of off-taste.

We are also actively engaged in a program to separate and identify the various components which appear in gas chromatograms of phenols and chlorophenols extracted from beer.

#### Summary

An abnormal flavor described as "phenolic" or "medicinal" has been observed by the taste panel in a small number of commercial beers. A colorimetric analytical procedure that determines the volatile phenol-type and chlorophenol-type compounds in beer was studied and modified. Values are presented for a series of normal-tasting beer and another group having the taste-detectable off-flavor. The average chlorophenol and phenol values for the normal beers were 7.1 and 12.5 ppb., respectively. Corresponding values for the abnormal beers were 13.9 and 27.1 ppb.

It is believed that all beers have a small concentration of these classes of compounds but that they only become noticeable or distinctive when produced in abnormal amounts or when different members are introduced into the brewing picture.

A wort-spoilage organism has been isolated from a wort which produces a beer with a "phenolic" taste. This organism, when inoculated into sterile wort, will produce the off-taste. The changes which take place in the wort can be followed by analysis for phenols and chlorophenols. The organism was found to be a natural contaminant in the wort of two breweries. In a third brewery, wort spoilage by a Grant-negative short rod that appeared to be similar to the first isolate did not produce a phenolic taste or high levels of phenol- or chlorophenol-type compounds. This later organism did not produce gas in lactose broth, indicating that it was not a typical member of the coli-aerogenes group. Work is continuing on other causes of excess phenol and chlorophenol compounds in beer and on identifying the various compounds making up these general groups in both normal-tasting and abnormal-tasting beers.

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