Benzoate ice in fish preservation.

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Introduction

Food preservation was a problem long before man first dried his grain and smoked his meat for the winter months. Today, in a fast moving and scientific world, further research has been stimulated not so much by fear of famine as by economic factors. With the increase in population and improved transportation facilities, greater markets are open to the food producer. It is no longer a question of providing perishable food products to the immediate vicinity alone, but rather one of preserving the products in their original condition so that they may be sent to the consumer great distances away.

Perhaps outstanding among the highly perishable foods that must be shipped great distances is fresh fish. Ice until recently has been the sole means of preservation of fresh fish. However, it is effective for a short time only and due to this fact quick freezing methods have greatly supplanted its use. Nevertheless, the use of ice during transportation is still of considerable importance.

Fishing vessels, due to the exhausted fish supply in home waters, sail great distances for their catch. They depend on crushed ice to keep the fish in good condition until port is reached. Due to the time consumed in reaching the fishing grounds, and the relative inefficiency

of ice, the actual time employed in fishing has been reduced and more frequent trips are necessary.

The benzoates have been used with some success as food preservatives. If these compounds were incorporated into ice, its relative efficiency might be enhanced. The use of benzoate ice would possibly extend the distance to which fish may be transported and also the period that vessels may remain at the fishing grounds.

Object

The four important objects of this investigation were (1) to investigate the literature on benzoate preservatives, (2) to manufacture ice containing benzoic acid or its salts, (3) to determine the distribution of the benzoate in ice cakes, and (4) to ascertain the preserving action of benzoate ice on fresh haddock fillets.

Historical Review on Chemical Preservatives as Fish Preservatives

Definite facts concerning the spoilage of fish have been established. Fellers (1926), working on raw salmon spoilage, states that the flesh of freshly caught fish is sterile. Bacterial contamination sets in from the surface of the skin, gills and intestines. The handling of the flesh after catching is an additional cause of bacterial infection.

Gibbons and Reed (1929) have shown that the products of autolysis are responsible for the rapid growth of the organisms, using them almost as fast as they are produced. "By twenty-four hours either their numbers have sufficiently increased or enough enzymes have been elaborated that a very rapid breakdown of the proteins result." Brown (1918) also found that autolysis rather than bacteria plays the most important part in the initial decomposition of fish. This is supported by the findings of Falk and McGuire (1919), Robinson and Rettger (1918) and Sperry and Rettger (1915).

Sanborn (1929) revealed that there are two types of bacterial action on fish tissue. One, proteolytic, may take place at relatively low temperatures, and is somewhat related to autolysis, resulting in the softening and structural breakdown of the flesh. The other is microbial decomposition described as putrefactive, taking place at ordinary temperatures. He declared that marine bacteria are constantly present on fresh, smoked, and frozen fish and are active at relatively low temperatures. They also initiate many proteolytic processes in fish muscle.

In order to better preserve fresh fish for longer periods of time by the use of ice, the bacteria must be controlled. The dead fish muscle is a very favorable medium for bacterial growth. If bacterial growth could be prevented or inhibited to a great degree, and since autolysis proceeds only slowly

at low temperatures, fish would be preserved for longer periods.

Berube (1938) describes the essential properties of a fish bactericide as harmless, rapid acting, easily soluble, simple to handle, and capable of improving the appearance of the fish as well as killing the bacteria present.

Chen and Fellers (1926) first used such a bactericide frozen in ice. They found sodium hypochlorite ice to be effective although the flesh became discolored. Berube (1938) states that boric acid ice has been used with fair success. Griffiths (1935) reports no effective preservation of fish fillets with chloramine-T or Katadyn silver ice. Chlorinated sawdust was also shown to be ineffective.

Literature Survey of the Benzoates

1. The Basic Principles of Benzoate Disinfection

Held (1915) points out that the germicidal action of benzoic acid is entirely due to the undissociated benzoic acid molecules, not the benzoate or hydrogen ions. He explains that disinfectants acting on the microbial cell must first permeate the membrane, described as rich in lipoid material. Substances such as iodine, mecuric chloride, alcohol and cresol are known to be lipoid soluble and easily penetrate the cell membrane, causing a chemical or chemo-physical change in the protoplasm.

Overton's Law states that if a foreign substance enters from a water solution into the cell, its absorption by the cell depends on the coefficient of distribution between water and fat. This law is used as the basis of this assumption.

Meld believes that the antiseptic action of compounds insoluble in lipoids such as salts, alkalis and inorganic acids, is due to their ability to dissolve the cell membrane or precipitate the protein therein. This phenomenon is attributed to the high degree of dissociation of these substances, explaining the germicidal value of strong acids and alkalis.

It was noted, however, that some organic acids possess greater disinfection properties than could be explained by their degree of dissociation. Finding that a phenol solution was more effective when salts decreasing its solubility were added, by raising the coefficient of distribution in favor of the membrane lipoids, Meld upholds Overton's assertion that undissociated molecules of ether soluble acids are soluble in lipoids and easily pass through the cell membrane into the protoplasm. It was further noticed that benzoic acid is more effective in the presence of a stronger acid, where it remains almost completely undissociated.

Vermast (1921) likewise attributes the action of the benzoates to the undissociated benzoic acid molecule.

Basing his investigation on Overton's Law, he found that changes in pH and total benzoic acid content little affected the antiseptic properties if the undissociated benzoic acid content remained constant. As soon as a definite value of undissociated benzoic acid is reached, 42.6 to 44.9 mg. per liter, disinfection always occurs in complete agreement with the Lipoid Theory of Overton. He believes that the salts of organic acids possess as such no antiseptic action in an acid medium and is doubtful if they have antiseptic qualities in an alkaline medium, although disinfection does occur with increased hydroxyl-ion concentration and almost constant benzoate radicle concentration. This may be due to partial saponification of the cell membrane.

McDougal (1928) states that although the structure of the cell membrane as outlined by Overton is known to be impossible in the behavior of colloids, the lipoids do seem to exercise a profound effect on its permeability.

Bleeker (1919) describes 1.0 percent benzoic acid as a prompt and efficient devitalizer of bacteria, although no experimental evidence is offered. Herter,(1910) found that benzoic acid was twice as effective as its sodium salt. The addition of calcium carbonate to the cultures containing sodium benzoate aided growth.

Waterman and Kuiper (1924) report that Dooremans and Dordrecht found the antiseptic action of aqueous solutions

of sodium benzoate on Penicillium glaucum to be less effective than solutions of benzoic acid. Working with the same organism, these investigators found it to be greatly depressed in growth by 0.05 to 0.1 percent benzoic acid at pH 4.0 to 4.4. They observed that the anions concerned had little checking action on the growth, since much larger concentrations of sodium benzoate were without influence. An increase in the antiseptic action of benzoic acid by the addition of other acids led to the conclusion that it may be due to a diminution of the dissociation of the benzoic acid and an increase in the distribution number for the oil-water solution.

Cruess and Irish (1932) report that concentrations of weak acids, such as benzoic and salicylic, required to prevent the growth of typical fruit juice organisms are much greater at or near neutrality than at pH 2.5 to 3.5. They state that the preserving effect of sodium chloride and formaldehyde are little affected by pH and conclude that the undissociated weak acids, not their ions, are the preserving agents. Cruess (1932) demonstrates that fruit juices pH 7.0 require 4.0 percent sodium benzoate to prevent the growth of most fermentative organisms while at pH 2.3 to 2.4, 0.02 to 0.03 percent is sufficient. Kuroda (1926) found the antiseptic action of benzoic acid to be strongest below pH 4.5 and above pH 10. This last is probably due to the strong concentration

of hydroxyl ions as suggested by Vermast.

Perry and Beal (1920) found that disinfection by sodium benzoate is due to the formation of benzoic acid and that low pH itself is ineffective. The individual toxicity of benzoic acid was later demonstrated by Janke and Beran (1933) who found that it required 4 normal acetic acid five minutes at pH 5.0 to kill a fungus while 1/20 normal benzoic acid at the same pH duplicated the reaction.

Cruess and Richert (1929) found the retarding value of sodium benzoate on the rate of multiplication of Sacch. ellipsoideus to be much stronger at pH 2.5 to 4.5 than pH 5.0 to 9.0.

Perrier (1913) reports that in alkaline media, molds, yeast, and bacteria are able to assimilate benzoic acid and its potassium salt as nutrient material, in some cases the benzoates affording better nourishment than sucrose. The preservative action of the benzoates is attributed to the acid media. Liese (1934) suggests that food spoilage organisms may use the benzoates as a source of carbon since investigation showed satisfactory growth of Penicillium glaucum and Penicillium brevicaule in both a 0.1 percent benzoic acid and 0.2 percent sodium benzoate medium.

Cruess (1931) found a growth of Penicillium mold on a 10.0 percent sodium benzoate solution at pH 7.5 after 15 months' storage.

Evidence points conclusively to the undissociated benzoic acid molecule as the active agent of disinfection, and the quantity present is controlled by the pH of the medium.

The benzoate salts, however, present a more complex problem. Their efficiency in media of high acidity and inefficiency in neutral and alkaline media seem to point to the formation of benzoic acid as the cause of disinfection. Lucas (1909) states that crystals of benzoic acid often appear in strongly acid fruit juices preserved with sodium benzoate. Although no literature could be found on the subject, it seems probable that the total acidity as well as the pH of the medium plays an important part in the effectiveness of the salts. The quantity of benzoic acid converted from the salt must depend on the concentration of the converting acid already present in the medium. The dissociation constants of the reacting substances and the buffering action of the medium act as controlling factors in establishing equilibrium. Temperature is another factor in establishing the value of the benzoate salts as well as that of the acid, for in addition to having an effect on growth of organisms present, it would have a marked bearing on the chemical equilibrium, particularly in solutions not well buffered.

Cruess, Richert and Irish (1931) found that sodium benzoate tends to buffer solutions at pH 5.4 - 5.5. The

critical point of efficiency seems to be pH 4.5 as sharp increase in concentration are necessary to prevent growth above that point.

Influence of the Benzoates on Different Organisms

Different organisms under similar conditions have been found to react differently to the benzoates. Tanner and Strauch (1926) show that marked differences exist in the resistance of pure yeast cultures, growth in some cases taking place in the presence of 1.0 percent sodium benzoate. Castan (1924) also reported that some yeasts were resistant to the benzoates whose antiseptic action, as a whole, was relatively low.

Bornand (1925) found 0.05 percent benzoic acid was sufficient to prevent the development of Escherichia coli and Bacillus subtilis in beef extract; but 0.15 percent was needed to prevent the growth of Sacch. cereviseae.

Cruess, Richert, and Irish (1931) found that 0.02 - 0.04 percent sodium benzoate at pH 4.5 and 0.1 - 0.2 percent at pH 7.0 stimulated fermentation.

Herter (1910) reports that sodium benzoate may reduce gas formation among the fecal organisms without completely suppressing vegetation. He finds the cocci particularly resistant and in some cases were actually stimulated. The investigations of Smith demonstrated that Salmonella paratyphi and Eberthella typhi are more sensitive to the benzoates than Esch. coli.

Cruess (1932) reports the acid tolerant organisms to be less sensitive to sodium benzoate than the acid intolerant species, and in addition finds differences of susceptibility within each group. Vermast (1921) remarks that the acid tolerance of the organism must be considered in determining the efficiency of benzoates and that the pH below this level should be taken into consideration.

Held (1915) reports that a concentration of benzoic acid may be reached that permits the growth of Clostridium botulinum while completely inhibiting the production of toxin. This value is so close to the toxic concentration that it is without particular significance, except to point out a possible protoplasmic reaction.

Cruess (1932) found that the same organism grew and produced a fatally potent toxin with 0.8 percent sodium benzoate at pH 7.43 while at pH 4.7, 0.1 percent prevented growth and toxin production.

The Effect of the Composition of the Media

The constitution of the media was found to affect the efficiency of benzoates. Tanner and Strauch (1926) declare that preservation of cider with these compounds depends on the species of apples used. They also found fungi better able to tolerate a solid media containing sodium benzoate than a liquid one.

Held (1915) states that in a medium containing protein, 80 percent of the benzoic acid is bound by the protein, allowing only 20 percent to act as the disinfectant. If the acid-binding power of the protein is satisfied by another acid such as sulfuric and tartaric, the necessary concentration for disinfection may be lessened. Woodward, Kingery and Williams (1935) agree that the fungicidal activity of benzoic acid is reduced in the presence of proteins. Kaufman (1919) points out that due regard must be had to possible neutralization or precipitation of benzoic acid by alkalies or albumen in the media under examination, and only that remaining uncombined acid be taken into consideration. He found a low concentration of benzoic acid in water vapor to show a bactericidal action similar to phenol.

Macht (1926) demonstrates that certain fluorescent dyes affect the action of sodium benzoate, a 1 - 100,000 concentration of esculin decreases the inhibitory action while eosin potentiates it. In addition, 1 to 1,000 and 1 to 2,000 concentrations of sodium benzoate were 100 times more effective in retarding fermentation in direct sunlight than in the dark. The rays of short wave length, about 3,000 Angtstrom units, are thought to have this photo-sensitizing effect.

The acid of the media may also influence the action of sodium benzoate as shown by Cruess (1932). By reducing

the pH of avacado pulp to 4.6 by the addition of 1.0 percent acetic acid, it was found that 0.06 percent sodium benzoate prevented growth; while the use of citric acid to obtain pH 4.5 made necessary a concentration of 0.15 percent to obtain the same result. This was interpreted to be caused by the indivdual antiseptic action of acetic acid.

Cruess (1932) shows that the use of a heavy syrup (57° Balling) required much less benzoate, 0.1 percent, to preserve a relatively non-acid product, which when merely acidified with citric acid required 0.18 percent sodium benzoate for preservation. He demonstrates that in the preservation of different foods as described later, the quantity of benzoate necessary at a given pH varies with the product. Whether this is due to a difference in the species of organisms normally found in different foods, the composition of the food itself or to a combination of both factors, is a problem yet to be undertaken.

Food Preservation

The ease and speed with which the benzoates may be added to food products, requiring none of the expensive machinery used in other methods of food preservation, has made their use favorable to some producers.

However, it will be noticed that the factors already

mentioned determine their applicability to food preservation.

Fellers (1929) states, "The part of the U.S. Food Inspection Decision which supercedes Decisions 76 and 89 relating to benzoates, reads as follows:- 'It having been determined that benzoate of soda mixed with food is not deleterous or poisonous and is not injurious to health, no objection will be raised under the Food and Drugs Act to the use of benzoate of soda provided that such food is plainly labeled to show the presence and amount of benzoate of soda'."

Although no regulation concerning the maximum quantity that may be used is specified by the Food and Drugs Act, the usual concentration is 0.1 percent, established by habit, the taste thresholds, and legislation in some states. Arneus (1933) reports the average taste threshold of sodium benzoate is 320 p.p.m. in aqueous solutions, 440 p.p.m. in syrup and 1350 p.p.m. in fruit juices.

1. Natural Occurrence

Prunes and cranberries are reported to contain 0.05 and 0.06 percent benzoic acid respectively by Radin (1914). Nestler (1910) attributes the resistance of cranberries to spoilage to the benzoic acid content. This is disputed by Clague and Fellers (1934); who, by a study of 24 varieties of mature cranberries varying in benzoic acid content from 0.02 to 0.098 percent, found no relationship between the

benzoic acid and keeping qualities.

2. Fruit Juices

Fruit juices are foods of low pH whose flavors are easily altered by heat treatment. Because of this, benzoates should be ideal for their preservation.

Osterwalder (1929) found acetic acid bacteria far less sensitive to sodium benzoate than yeasts and does not recommend the use of benzoates for the preservation of fruit juices.

Gore (1908) reported that 0.03 - 0.1 percent sodium benzoate inhibited fermentation in cider but had no effect in the control of acid producing organisms. Yeasts grow well in the absence of air and are able to produce a sound palatable fermented beverage, whereas benzoated cider when fermented never becomes alcoholic and supports the growth of Acetobacter. Pie, Meehan and Lincoln (1929) offer similar results. Scott and Will (1921) likewise found that 0.02 percent sodium benzoate prevented any appreciable amount of alcohol formation, but 0.2 percent did not prevent acid production.

Lucas (1909) found that pure apple cider containing 0.1 percent sodium benzoate developed mold growth in ten days while commercial benzoated cider under similar conditions remained unchanged. Brooks (1925) prefers sodium benzoate to pasteurization, stating that any slight change

in flavor due to the chemical is to be preferred to the cooked flavor and loss of esters caused by heat treatment. He emphasizes the use of a high grade benzoate since even 99 percent (U.S.P.) sodium benzoate may contain enough contaminating impurities, such as benzaldehyde, to give a "medicinal" flavor to the beverage. It is also advisable to make a saturated solution of the preservative in a small amount of cider and add this to the main batch in proper quantities, rather than adding the solid directly. This prevents possible settling out of the benzoates.

Rice and Markley (1921) found that the condition of the cider before sodium benzoate is added has a marked effect in the preservative action. If fermentation had already started 0.5 percent sodium benzoate was required to inhibit further growth.

Vandecaveye (1929) innoculated cider with yeast cultures and allowed it to incubate at the optimum temperature until evolution of carbon dioxide showed alcohol production. He then heated the cider to 115° F. to precipitate the protein and passed it through a germ-proof filter to remove the organisms. This process was repeated and it was found that the twice innoculated cider was adequately preserved with 0.04 percent sodium benzoate while 0.1 percent was ineffective in the untreated juice. This was attributed to the removal of the nitrogen and phosphorous from the solution. Widmer (1926) observed that juices from which microorganisms

are filtered require less preservative than unfiltered juices. Juices stored in glass containers were observed to keep better than those in barrels. A combination of 0.05 - 0.06 gram of sodium benzoate and 0.2 gram of potassium acid sulfite per liter is recommended as a good fruit juice preserver.

Alvarez (1927) states that the fermentation of cane juice was prevented for 24 hours by 0.01 percent sodium benzoate and for 48 hours by 0.05 percent. With increasing quantities of sodium benzoate, the tendency toward fermentation decreased. Fermentation was delayed one month by 0.11 percent and longer by 0.12 - 0.14 percent; but the juices became bitter. The percentage of glucose rose and the purity of the juice decreased. Orange juice was preserved 11 - 14 days by 0.01 - 0.05 percent sodium benzoate, but in three weeks cloudiness and altered taste indicated decomposition. Juices containing 0.1 percent presented a normal appearance but showed an altered taste in a month.

Joslyn and Marsh (1934) report sodium benzoate to have no effect on the iodine-reducing value of orange juice in storage. Mrak and Cruess (1929) found that copper dissolves more rapidly in lemon juice containing 0.1 percent and 0.5 percent sodium benzoate. This may be of significance in the use of lemon juice in the manufacture of other products.

3. Other Fermentable Liquids

Herter (1910) found that in a beer wort medium containing brewer's yeast, 0.15 percent sodium benzoate inhibited fermentation, and allowed the formation of a considerable amount of alcohol. McCune and Thurston (1920) state that 0.2 percent sodium benzoate is required to temporarily prevent fermentation in draught near-beer. Bernard (1925) reports that the development of molds in a beer wort medium can be prevented by 0.25 percent benzoic acid.

4. Meat and Meat Products

Tjaden (1930) explains that preparations containing benzoic acid or sodium benzoate can be used with fresh meats since they do not remove evidences of decay nor inhibit bacterial growth already at its height. The addition of the benzoates tends to prevent the development of bacteria and appears to be a harmless practice. Mezzer, Jesser and Hepp (1913), on the other hand, condemn the use of benzoic acid as deceptive and dangerous. Experiments show that bad color due to spoilage can be restored to good appearance, while decomposition is not inhibited, and meat with a bad odor can be made to look fresh.

Eichler and co-workers (1929) made a collaborative study of the effects of incorporating the benzoates in the preserving salt used for sausages. Results showed that

0.06 percent benzoic acid doubles the keeping quality of the sausages. The red color of the meat is not enhanced and in some cases is slightly diminished; the growth of all types of bacteria is retarded although no types were entirely destroyed.

Behr and Segin (1907) report that 0.08 percent benzoic acid aided the preservation of beef juices but caused a change in color. Sodium benzoate could be used in large quantities.

5. Fats and Oils

De Conno and co-workers(1925) declare that benzoic acid has a powerful stabilizing action for oils that are oxidizable, such as olive oil. This preservative action is attributed to the carbxyl group. Similar action was obtained with 0.5 percent acetic acid. Husa and Husa (1926) conversely find benzoic acid of no use in preventing rancidity in lard. Fierot (1930), however, declares that experiments show that rancidity in lard may be bacterial as well as chemical. Benzoate, in a concentration of 1.0 percent, mixed with a neutral soap and emulsified with the lard gave it pronounced keeping qualities over an unbenzoated control.

6. Milk

The addition of sodium benzoate and benzoic acid retards the formation of acid in milk according to Mohorcic

(1917). However, so much acid is eventually formed that it curdles. Higher acidity was often found necessary to curdle preserved milk than raw milk.

7. Tomato Products

Peterson (1927) found that 1.0 percent acetic acid,, 5.0 percent sodium chloride and 0.2 percent sodium benzoate had similar potency in preventing the growth of 32 bacteria and 2 yeast cultures isolated from spoiled tomato products. Cruess (1932) reported that 0.1 percent sodium benzoate prevented the spoilage of tomatoes by microorganisms, but enzymatic activity softened the fruit so as to make it unfit for salad use.

8. Miscellaneous Foods

Cruess (1932) records the pH and concentration of sodium benzoate necessary to obtain the optimum preservation of several foods:-

1. Melon Preserves

pH 2.5 - 3.1	0.02 percent
" 3.3 - 4.0	0.05 "
" 4.5	0.10 "
" 6.0	2.0 "
" 7.6	5.0 "

2. Ripe olives in 3 percent brine

pH 5.0	0.6 percent
" 4.0	0.1 "
" 3.8	0.05 "

3. Maraschino Style Grapes

.05 percent citric acid and .05 percent

4. Artichokes in 3 percent brine

pH 3.4 - 3.6	0.1 percent

5. Avacado Pulp

pH 5.2 -	0.3 percent
" 4.5 -	0.15 "
" 3.8 -	0.06 "

citric acid used to lower pH

6. Prune Pulp

non acid	0.3 percent
(acidified with	
(citric acid	0.18 "
57° Balling	0.1 "

7. Carbonated Beverages

Using syrup pH 3.5	.08 gram per 100 cc.
" 2.0	.02 " " 100 cc.

8. Asparagus Juice

pH 8.0	-	8.0	percent
" 6.7	-	0.8	"
" 6.0	-	0.8	"
" 4.9	-	0.4	"
" 3.5	-	0.03	"
" 3.0	-	0.03	"

It is impossible to preserve string beans and peas with moderate amounts of sodium benzoate.

Lockhead and Farrell (1930) found that sodium benzoate inhibits the fermentation of 80 percent honey.

Bornard (1925) reports that paper impregnated with benzoic acid used for wrapping preserves was found to be without action.

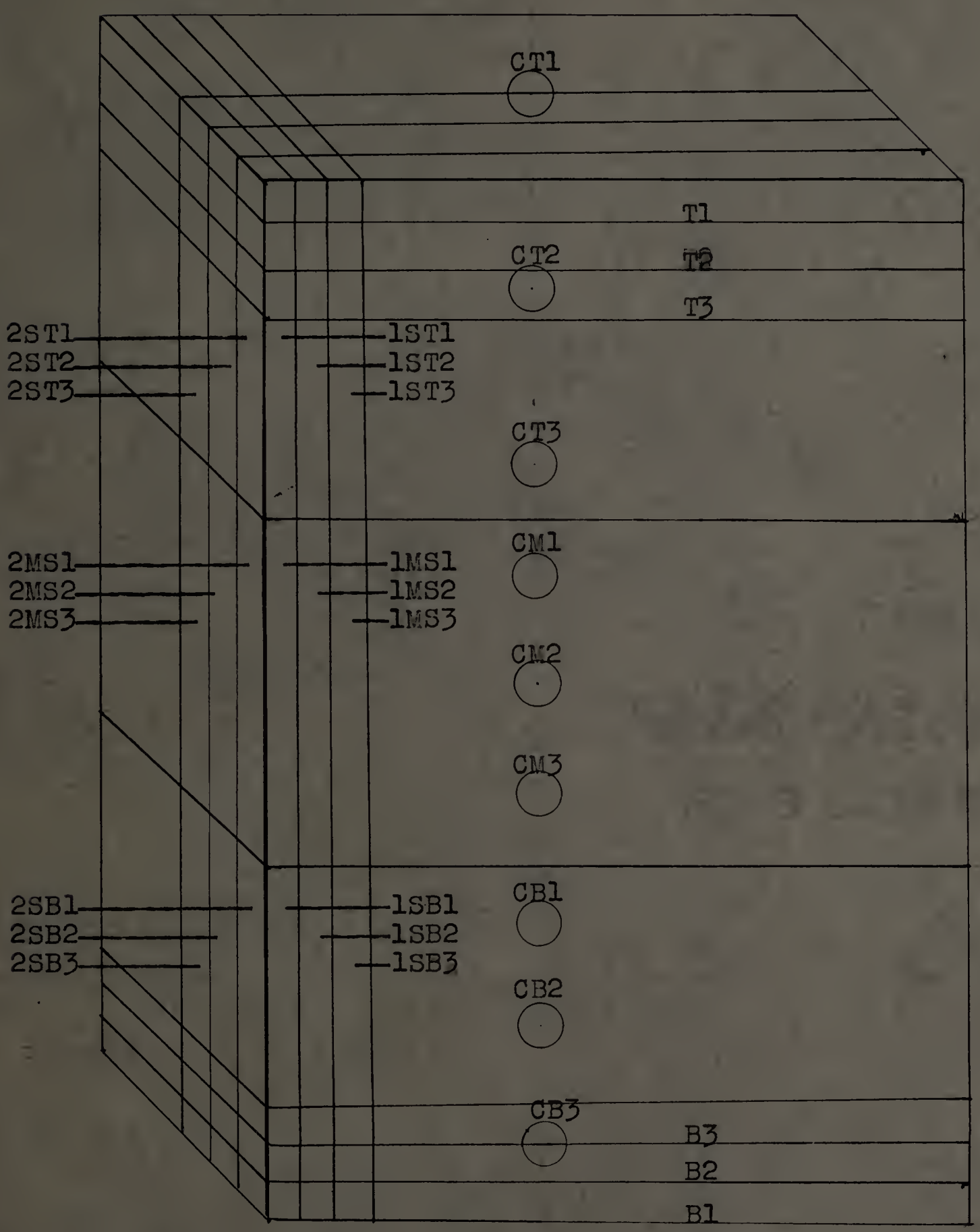
Studies on Benzoate Ice

Experimental Procedure

Four, 100 pound portions of tap water containing 0.05, 0.10, 0.15 and 0.20 percent benzoic acid, respectively, were prepared in rectangular metal ice cake boxes. The metal boxes were immersed in brine at 0° F. The surface level of the solution was below that of the brine but exposed to the air above it. The resulting cakes of ice were removed and from each of the four cakes approximately 100 gram samples were taken. Care was exercised to remove each sample from as near the same position in each cake as possible. This was accomplished by cutting each block of ice into three sections, top, middle, and bottom. By means of a carpenter's chisel, three successive layers were removed from the top (T_1, T_2, T_3), narrow side ($1ST_1$, $1ST_2$, $1ST_3$) and broad side ($2ST_1$, $2ST_2$, $2ST_3$) of the top portion, along with three portions from the core, at the top (CT_1), center (CT_2), and bottom (CT_3). In a similar manner samples were taken from the narrow ($1MS_1$, $1MS_2$, $1MS_3$) and broad ($2MS_1$, $2MS_2$, $2MS_3$) sides of the middle portion, and from the core (CM_1, CM_2, CM_3) in the same relative position as those of the top portion. From the bottom portion similar samples were taken from the narrow ($1SB_1$, $1SB_2$, $1SB_3$) and broad ($2SB_1$, $2SB_2$, $2SB_3$) sides, the core (CB_1, CB_2, CB_3) and the bottom (B_1, B_2, B_3). To prevent confusing the relative positions of these samples when examining this code in Tables I and II,

Diagramatic Sketch of the Original Positions of the Benzoate Ice Samples Taken for Analysis.

Figure 1.



it may be added that the samples of the sides, top, and bottom are numbered from the outside toward the center and the core from the top of the whole cake towards the bottom, CB_3 related to B_1, and CT_1 to T_1.

The distribution of benzoic acid throughout the ice cake was determined by allowing the samples to melt, and titrating 25 cc. portions with 0.05 normal sodium hydroxide using phenolphthalin as the indicator.

Similar ice cakes were prepared and sampled in the same manner, substituting equivalent amounts of sodium benzoate for the benzoic acid.

The method of determining the sodium benzoate content of the samples follows that described in the United States Dispensatory, 22d Ed. Twenty-five cc. portions of the melted ice samples were evaporated to dryness on a steam bath, 75 cc. of ether was added to the residue and the whole was titrated with 0.05 normal hydrochloric acid with methyl orange indicator.

Fifty-pound ice cakes were manufactured as previously described, each containing one of the following compounds:- sodium, magnesium, calcium, and ammonium benzoate, 0.5 and 1.0 percent; benzoic acid 0.2 percent and Antimol - a commercial benzoate preparation composed of so-called "tri-sodium benzoate" plus benzoic acid - 0.5 percent. Due to the low solubility of Antimol and benzoic acid, larger con-

centrations could not be used. The amount of preservative in each cake refers to the benzoate radical; Antimol, being of unknown composition, was computed as sodium benzoate. The cakes were then reduced to large flakes by a mechanical grinder, stored at 0° F., and used as needed. The pH of each ice cake was taken at its melting point.

Haddock fillets, weighing approximately 0.75 pound each were obtained at the local market and initial bacterial counts taken. They were then stored in open No. 10 cans, the bottoms of which were perforated to allow drainage, and each completely covered with one of the benzoate ices for eleven days in an atmosphere of 40 - 42° F. At frequent intervals the fish was removed, examined organoleptically and bacterial counts made to determine the progressive effect of the preservative.

Samples of the flesh for bacterial counts were removed by a sterile cork borer of suitable diameter for removing a 1 to 2 gram specimen. This was pushed into a weighed dilution bottle containing broken glass, with a glass rod. The weight of the sample was determined by difference, and the counts computed accordingly. This method was found to give a uniform sample. The dilution bottles were shaken by hand until the samples were completely shredded. Aseptic technic was used as far as possible in handling the fillets. All glassware was sterilized at 350° F. for 2 hours; media and dilution

bottles were subjected to 15 pounds steam pressure for 15 minutes as outlined in Standard Methods for Water Analysis of the American Public Health Association (1933). The cork borer and board were placed in boiling water for five minutes and the glass rod flamed before the removal of every sample.

The samples were plated on Bacto-Nutrient Agar (Difco) and incubated at room temperatue for 48 - 60 hours. Griffiths (1935) reported higher counts at an 18 - 20° C. incubation temperature than those from the same sample of fish tissue incubated at 25° C. and 37° C., 25° C. giving a higher count than 37° C.

After eleven days the fish was analyzed for benzoate radical retention. The method outlined in the Methods of Analysis of the Association of Official Agricultural Chemists (1935) was used. The fillets were ground in a fine meat chopper and a 50-gram representative sample of each was placed in a 500 cc. volumetric flask. A saturated solution of sodium chloride was added, the mixture made alkaline to litmus with 10 percent sodium hydroxide, diluted to the mark with more sodium chloride solution, and allowed to stand with frequent shaking for at least two hours. It was then filtered and a 200 cc. aliquot placed in a separatory funnel. The solution was made acid to litmus with 1 to 3 hydrochloric acid, followed by an excess of 5 cc. Extractions were

made with successive portions of 70, 50, 40 and 30 cc. of chloroform. The chloroform was distilled off to within 25 percent the original volume and the remainder dried at room temperature. Fifty cc. of ethyl alcohol and 10 cc. of water were added and the solution titrated with .05 normal sodium hydroxide, phenolphthalein as the indicator.

Because the whole fillet was used to give a uniform sample for the determination of benzoate retention, other fillets were employed to establish the taste threshold of the benzoates in fish. Fillets were immersed in 1.0, 0.5, 0.2, 0.1 and 0.05 percent solutions of sodium benzoate and stored at 40° F. for five days. They were then removed, fried and tasted.

Interpretation and Discussion of Results

1. Concentration of the Benzoates in Ice.

If dilute aqueous solutions are frozen in uniform containers, allowing equal freezing on all sides, the heaviest concentrations should result in the center of the ice cake. This is due to the fact that as the solution is cooled below the freezing temperature of water, it becomes saturated with respect to ice, which crystalizes out in a pure state. As this process progresses, the remaining liquid becomes more and more concentrated with a corresponding lowering in its freezing point, until it is saturated with

respect to the salt as well as to the ice. Ice and salt then freeze out in the same proportion in which they were present in the saturated solution according to Timm (1930).

Analysis of the sodium benzoate ice gave higher concentrations than benzoic acid ice for the same samples as seen in Tables I and II. There appears to be more salt present in the ice than was present in the solution before freezing. This is probably due to the fact that a smaller sample of sodium benzoate was used than specified in the method of analysis which may have led to high results.

The ice cake containing 0.2 percent sodium benzoate is radically different in solute distribution than the others. The low concentrations in the upper two-thirds of the cake and abnormally high concentrations in the lowest bottom layers indicate that the salt had not completely dissolved before the solution froze, and had precipitated to the bottom. It is interesting to note that even in this case, there is a slight resemblance to the solute distribution in the other cakes of ice.

The tendency for the ice under examination to follow this course may be understood by glancing at Tables I and II. The highest concentration is found in the core and the lowest concentration in the outside layer of each cake. A 0.2 percent benzoic acid solution is just below saturation at 0° C. The ice containing this amount of benzoic acid

Table I. Distribution of Benzoic Acid in Ice

Original Concentrations Added to Water			
0.05%	0.10%	0.15%	0.20%
Concentrations in Each Sample of Ice			
Sample	Percent	Percent	Percent	Percent
T1	.02	.03	.08	.15
T2	.01	.11	.11	.21
T3	.02	.12	.11	.20
1ST1	.02	.04	.09	.19
1ST2	.01	.10	.19	.20
1ST3	.03	.10	.08	.21
2ST1	.02	.06	.13	.18
2ST2	.04	.09	.14	.17
2ST3	.03	.09	.14	.18
CT1	.10	.12	.16	.18
CT2	.11	.13	.20	.25
CT3	.08	.10	.17	.17
1MS1	.05	.10	.15	.21
1MS2	.04	.09	.16	.19
1MS3	.05	.08	.16	.20
2MS1	.04	.10	.16	.19
2MS2	.04	.08	.15	.18
2MS3	.04	.10	.15	.20
CM1	.07	.10	.16	.17
CM2	.05	.10	.19	.20
CM3	.06	.09	.17	.19
1SB1	.03	.10	.15	.20
1SB2	.04	.09	.14	.20
1SB3	.06	.10	.10	.19
2SB1	.04	.10	.15	.21
2SB2	.05	.10	.14	.20
2SB3	.05	.10	.14	.19
CB1	.07	.14	.15	.24
CB2	.08	.19	.26	.31
CB3	.05	.12	.15	.22
B1	.04	.10	.14	.18
B2	.05	.11	.15	.28
B3	.05	.12	.15	.22

Table II. Distribution of Sodium Benzoate in Ice

Original Concentrations added to Water			
.05%	.10%	.15%	.20%
Concentrations in Each Sample of Ice			
Sample	Percent	Percent	Percent	Percent
T1	.05	.10	.13	.16
T2	.05	.09	.17	.18
T3	.02	.11	.16	.21
1ST1	.06	.10	.16	.17
1ST2	.04	.10	.16	.17
1ST3	.04	.13	.15	.18
2ST1	.05	.10	.14	.18
2ST2	.04	.10	.15	.18
2ST3	.06	.11	.15	.18
CT1	.10	.14	.17	.18
CT2	.09	.13	.15	.18
CT3	.09	.11	.18	.19
1MS1	.05	.11	.13	.22
1MS2	.05	.11	.15	.18
1MS3	.06	.13	.16	.19
2MS1	.05	.10	.16	.19
2MS2	.06	.10	.16	.18
2MS3	.06	.11	.17	.18
CM1	.07	.12	.19	.18
CM2	.07	.15	.16	.21
CM3	.07	.14	.18	.20
1SB1	.06	.11	.14	.19
1SB2	.05	.12	.15	.19
1SB3	.06	.13	.18	.19
2SB1	.05	.10	.15	.18
2SB2	.05	.12	.15	.18
2SB3	.06	.13	.16	.18
CB1	.07	.15	.20	.25
CB2	.06	.18	.25	.46
CB3	.05	.15	.23	.28
B1	.05	.10	.17	.22
B2	.05	.12	.22	.31
B3	.08	.16	.25	.34

has a more even distribution than the others since the solution became saturated quickly and froze out as a eutectic before the conditions under which it was freezing could influence the reaction.

The conditions under which the ice was made are probably the cause of the deviation from the theoretical to a large degree. The greater surface area per volume at the bottom than at the top of the freezing tanks caused the bottom to freeze first. The surface of the solution which was open to the relatively warm air above the brine was the last point to freeze. This probably accounts for the extra heavy concentration of solute in the core about three inches from the top. Also, the open surface may have started convection currents that disturbed any equilibrium already under way. The shutting down of the refrigeration machinery and brine agitator at night undoubtedly had a marked effect on the even freezing of the solution because of the subsequent rise and unequal distribution of the temperature of the brine.

These factors influencing the freezing of solutions may vary in their effect at different times. It appears upon further examination of Tables I and II that due to this fact, identical distribution in all cakes of the same total solute concentration is unlikely.

The difficulty in removing specimen for analysis from exactly the same position in each cake, due to the size

and brittle nature of the ice is responsible for some differences found among similarly located samples.

It may be said, therefore, that under the given conditions a solution may be frozen into an ice cake with the following general characteristics.

The solute will be concentrated most highly in the core extending towards the center to a point eight inches from the top and bottom. The lower third of the ice cake will as a whole contain more solute than any other portion. The ice containing the lowest concentration of solute will be found as the outside layer of the cake. The top third as a whole, excluding the core, contains the least amount of solute.

As a whole, however, the distribution of the solute is sufficiently even to afford practical use of the ice if well flaked.

2. The Use of Benzoate Ice as a Fish Preservative

Tables III, IV and V exhibit the relative efficiency of the benzoate ice. Note must be taken of the original condition of the fish. The pH of the ices is lower in the case of the salts than expected due to the chlorine and minerals present in the water supply.

Benzoic acid ice proved the best bactericide; but the fact that its pH is below the isoelectric point of fish protein, causing coagulation of the flesh, makes it

Table III. Bacterial Counts per Gram and Organoleptic Tests of Fillets Stored in Benzoate Ice at 40°F.

Days in storage	0.2% Benzoic Acid	0.5% Calcium Benzoate	0.5% Antimol	0.5% Sodium Benzoate
	Bact.per gram; fish quality	Bact.per gram; fish quality	Bact.per gram; fish quality	Bact.per gram; fish quality
0	1,062,000 Slight fishy odor.	428,000 Good condition.	440,000 Good condition.	250,000 Good condition.
2	660,000 Distinct fishy odor.	643,000 Good condition.	1,160,000 Definite fishy odor.	520,000 Good condition.
4	60,000 Definite coagulation. Decided odor. Dried appearance.	948,000 Flesh softening. Slight odor.	1,225,000 Signs of coagulation. Dried appearance.	650,000 Good condition.
7	180,000 Tissue hard. Coagulated. Fishy odor. Dried appearance.	1,445,000 Flesh softening. Good condition. Strong odor.	3,125,000 Strong fishy odor. Definite coagulation.	875,000 Fishy odor. Tissue softening.
9	12,100 Flesh stiff. Discolored. Heavy fish odor. Dried appearance.	2,275,000 Good condition. Odor no stronger. Flesh firm.	1,250,000 Flesh stiff. Brown discoloration. Heavy odor.	2,125,000 Flesh soft. Odor no stronger.
11	5,830 Flesh stiff. Discolored. Strong odor. Dried appearance.	13,650,000 Flesh firm. Odor not prevalent.	500,000 Flesh stiff. Brown discoloration.	3,650,000 Fair condition. Odor no stronger.
Percent benzoate retained.	0.65	0.15	0.55	0.07
pH of the ice.	3.8	7.6	4.3	6.6

Table IV. Bacterial Counts per Gram and Organoleptic Tests of Fillets Stored in Benzoate Ice at 40°F.

Days in storage	0.5% Ammonium Benzoate	0.5% Magnesium Benzoate	Control I	Control II
	Bact. per gram; fish quality	Bact. per gram; fish quality	Bact per gram; fish quality	Bact. per gram; fish quality
0	850,000 Good condition.	432,000 Good condition.	647,000 Good condition.	1,700,000 Stale.
2	1,115,000 Sweet odor. Tissue in good condition.	794,000 Good condition. Little odor.	765,000 Good condition.	2,900,000 Tissue soft. Fishy odor.
4	6,258,000. Sweet odor. Flesh in good condition.	1,500,000 Sweet odor. Tissue in good condition.	1,350,000 Sweet odor. Tissue softer.	17,8000,000 Tissue soft. Strong odor.
7	22,350,000 Sweet odor. Flesh in good condition.	1,744,000 Tainted odor. Flesh firm.	3,850,000 Definite softening of flesh.	59,400,000 Sour odor. Tissue soft. Almost putrid
9	4,300,000 Flesh in good condition. Slight fishy odor.	2,750,000 Tainted odor. Flesh firm.	60,000,000 Sour odor. Almost putrid.	117,360,000 Putrid. Very soft.
11	2,225,000 Flesh in good condition. Slight fishy odor.	7,748,000 Flesh very firm. Tainted odor.	139,000,000 Putrid. Very soft.	300,000,000 Putrid. Mushy.
Percent benzoate retained	0.11	0.07	---	---
pH of the ice.	6.8	7.5	6.3	6.5

Table V. Bacterial Counts per Gram and Organoleptic Tests of Fillets Stored in Benzoate Ice at 40°F.

Days in storage	1.0% Ammonium Benzoate	1.0% Calcium Benzoate	1.0% Sodium Benzoate	1.0% Magnesium Benzoate
	Bact. per gram; fish quality	Bact. per gram; fish quality	Bact. per gram; fish quality	Bact. per gram; fish quality
0	180,000 Excellent.	117,000 Excellent.	3,490,000 Sweet odor.	224,000 Excellent.
2	1,000,000 Flesh firm. Sweet odor.	212,000 Good condition. Sweet odor.	977,000 Sweet odor. Flesh good.	1,100,000 Sweet odor.
5	1,200,000 Flesh firm. Sweet odor.	144,000 Slight odor. Flesh a bit stiff.	938,000 Sweet odor. Flesh good.	33,000 Flesh good. Sweet odor.
7	950,000 Flesh firm. Sweet odor.	212,000 Little odor. Flesh a bit stiff.	3,066,000 Good condition. Faint odor.	1,018,000 Good condition. Slight odor. Flesh a bit stiff.
9	481,000 Flesh firm. Good odor.	2,240,000 Little odor Flesh a bit stiff and dry appearing.	3,977,000 Good condition. Faint odor. Flesh a bit stiff.	3,700,000 Good condition. A bit stiff. Little odor.
11	112,000 Definite fishy odor. A bit stiff.	2,846,000 Flesh stiff. Little odor. Dry appearing.	5,133,000 Flesh stiff to a slight degree. Good condition in tissue.	2,877,000 Flesh a bit stiff at one end and very stiff at the other. Very little odor.
Percent benzoate retained	0.31	0.24	0.18	0.20
pH of the ice	6.7	7.6	7.0	7.5

unfit for practical use as a fish preservative. The low temperature of the melting ice probably increased the efficiency of the benzoic acid by lowering its dissociation constant. The high benzoate retention in the tissue upholds Held's (1915) evidence on the protein binding power of benzoic acid. The melting ice, however, kept a fresh supply bathing the fish and prevented the loss in disinfection activity that accompanies the protein binding of this compound.

Antimol ice produced a similar coagulation of the fish flesh. On continued exposure to the ice, the flesh assumed a brown discoloration.

Ammonium benzoate ice seemed to stimulate rapid bacterial growth to a point where the flesh would normally have started to decompose. However, the flesh remained relatively unchanged and a sharp decrease in the bacterial count followed. This is more pronounced in the ice at the lesser benzoate concentration. The high retention of the benzoate radical by the fish may be of significance in explaining the rapid decrease. Antimol presented a similar problem.

Sodium benzoate appeared to be the most efficient benzoate salt ice. A marked preservative action was noted during the first seven days of storage.

The 0.5 percent calcium and magnesium benzoate ice

exerted an efficient preservative action on haddock fillets for about four days. The 1.0 percent ices of these salts extended this time to seven days. Calcium benzoate in this case was slightly more active. The magnesium and calcium ions exerted a definite firming effect on the flesh, even in the case of high bacterial counts.

However, as previously mentioned, the benzoates are selective in their action on microorganisms. Some cells are easily inhibited or destroyed while others are not influenced to a great degree by these compounds. Many different species of bacteria are known to cause fish spoilage. The species present on a fillet depends on the source of contamination. Therefore, it would be difficult to definitely state how effective benzoate ice would be in preserving fish under all conditions of contamination and storage. The temperature of storage affects the efficiency of benzoate ice, for higher temperatures cause more rapid melting, making available a greater quantity of the preservative.

The taste threshold of the benzoates was determined by tasting fried fillets that had previously been soaked in 1.0 and 0.5 percent solutions of sodium benzoate for five days at 40° F. The characteristic benzoate taste was so pronounced that the fish was declared inedible. It seems likely that this fish contained less benzoate than

that retained by the fillet stored eleven days under 0.5 percent sodium benzoate ice. Therefore, we can judge the taste threshold to be less than 0.07 percent benzoate. Since all the fillets preserved under benzoate ice retained more than that amount, they can probably all be declared inedible regardless of condition.

Therefore, although benzoate ice exercises a mild preservative action at low concentrations of benzoate, the benzoate retained by the fish may affect the flavor. Furthermore, the preserving effect of benzoate ice cakes containing less than 0.5 percent benzoate is insufficient to greatly enhance keeping quality.

Summary

1. A critical literature survey of benzoic acid and its salts as bactericidal compounds and food preservatives was made. The factors influencing their efficiency were pointed out.

2. Solutions containing various concentrations of benzoic acid and sodium benzoate were frozen and the distribution of the solute throughout the ice was traced and found to vary, but not sufficiently to prevent the use of the ice when flaked. Probable factors influencing the variations were noted.

3. Ice containing 0.2 percent benzoic acid, 0.5 and 1.0 percent sodium magnesium, calcium and ammonium benzoates and 0.5 percent Antimol were examined for their efficiency as preservatives for fresh fish. The relative values of these substances were discussed.

Conclusions

1. A literature survey indicates that the bactericidal action of benzoic acid and its salts is due to the presence of undissociated benzoic acid.

2. The bactericidal efficiency of benzoic acid is greater than that of its salts.

3. The efficiency of benzoic acid and its salts depend on the pH and composition of the medium. Acid media are necessary for efficient action.

4. The toxicity of benzoic acid and its salts to micro-organisms varies with the species.

5. Solutions may be frozen under certain conditions, yielding ice with a distribution of solute allowing practical use when flaked.

6. Benzoic acid and Antimol ice are not practical for fish preservation because the low pH coagulates.the protein. Benzoic acid ice is a strong bactericide.

7. Ice containing benzoate salts in concentrations of 0.2, 0.5 and 1.0 percent inhibit to some extent bacterial growth on haddock fillets.

8. Calcium and magnesium ions have a firming effect on either fresh or stale fish tissue.

9. The use of benzoate ice is not practical in the preservation of haddock fillets due to the benzoate taste imparted to the flesh.

10. The taste threshold for sodium benzoate (calculated on the basis of the benzoate radical) in fried haddock fillets is less than 0.07 percent.

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