THE USE OF SPICES AS PRESERVATIVES.

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During recent years there has been a good deal of discussion and legislation on food preservatives. Sodium benzoate has been of particular interest because some experts maintain that it is not harmful to the human system when used in quantities that will prevent the growth of microörganisms in food, whereas others ascribe injurious properties to it, and strongly condemn its use. However, much of it has been used, especially where an inferior grade of material is utilized and as yet there is no law preventing its use. As a result of this controversy over sodium benzoate, the public has become suspicious of it, and most people would prefer not to eat food containing it. At this time, therefore, it is interesting to consider the preserving properties of various articles which we commonly use in our food.

It is a matter of common knowledge that fruits or vegetables prepared with the addition of spices and vinegar keep longer without being carefully sealed than do those same fruits or vegetables put up alone or with only ordinary amounts of sugar. Unsealed pickles and ketchups will keep for a long time without the growth of microörganisms. Cakes containing spices are also known to keep longer than other cakes. Fruit cake, which always contains spices, is often made several weeks before using, since its quality is said to improve with age. Although it is known in a general way that spices have something to do with the keeping of food to which they have been added, yet they have never been used with the idea of exerting a preservative effect. The spices are added on account of the flavor which they impart, and it is only a lucky coincidence that they also help to keep the food from spoiling.

When spices are used in food, several kinds are almost always added together, and usually the cooking is done in a liquid which contains more or less vinegar. It is common to add to pickles and ketchups, especially, as many as six or eight kinds of spices. Just which of them have any value in preserving the food is not generally known.

All condiments are irritating to the mucous membrane and are injurious when used in excessive amounts. Their use, then, must be limited. Hence every housewife should know just which of the condiments she adds in her cooking will help to preserve the food, and which of them are of no value except for their flavor, so that she can regulate her use of them accordingly. As will be seen in the tables below, some of the most irritating spices have no value whatever as preservatives.

Vinegar in sufficiently high concentration prevents the growth of microörganisms, and must be considered an important preservative. A single test was made with vinegar: 100 cc. of vinegar were added to 100 grams of apple sauce (about 1 part water to 8 parts of apples by volume), the mixture was boiled over a flame until the volume was again reduced to 100 cc., it was exposed to the air for a few days and then stoppered to prevent evaporation. The sauce has not spoiled after $4^1/_2$ months. Although a single experiment was performed, it seems to indicate that the vinegar exerts a preservative action. In much less concentration the vinegar would no doubt retard growth. The vinegar employed in this operation contained about 25% acetic acid.

A few experiments were carried out under conditions that would obtain in any kitchen to compare the preserving properties of various spices, and to determine the amounts of them which will prevent all growth of microörganisms. Apple sauce to which a small amount of sugar had been added was used in all of the tests except those on ginger, black pepper, and cayenne pepper recorded in Table I, where cabbage was used. In every case the given amount of spice was mixed with 100 grams of the cooked material in small Erlenmeyer flasks. After adding the spice the flasks were placed in the steam sterilizer and steamed for 30 minutes. When removed from the sterilizer they were left open in the laboratory for a few days to receive contamination from the air and then closed with cork stoppers to prevent evaporation. The figures in the tables show the number of days after being put up when a change was first observed in the flasks. It was assumed that the growth of yeasts, molds, or bacteria would change the appearance or odor of the sauce, and no further tests for growths were made. In every case it was the molds which first made their appearance. The flasks in Table I were not examined between the 60th and 100th days, so that the exact time of the spoilage of the apple sauce containing allspice in 3 of the flasks is not known. The tests recorded in Table I were preliminary, and the results served to suggest the amounts of spices to be used in later experiments.

Table I.—Time of Spoilage of Food Containing Varying Amounts of Ground Spices.

Amount of spice. Grams.	Control.	Nutmeg.	Cinnamon.	Allspice.	Cloves.	Ginger.	Black pepper.	Cayenne pepper.	Mustard.
0	4 days	....	....	....	....	...	...	...	....
2.5	...	5 days	Not spoiled in 14 months	16 days	Not spoiled in 14 months	5 days	4 days	4 days	Not spoiled in 14 months
5.05	...	6 days				5 days	4 days	4 days	
7.5	...	7 days		Between 60 and 100 days		5 days	4 days	4 days	
10.0	...	16 days				5 days	4 days	4 days	
15.0	...	27 days		195 days		5 days	4 days	4 days	

From Table I it appears that ginger, black pepper and cayenne pepper do not prevent the growth of microörganisms, but that cinnamon, cloves, and mustard are valuable preservatives and nutmeg and allspice delay growth. Further experiments were carried out

to determine more accurately the amounts that will prevent growth.

Flasks were again put up with ginger, black pepper, and cayenne pepper in large amounts. The results are given in Table II. Even where the large amounts of 30 grams of spice to 100 grams of apple sauce were used growth took place only two or three days later than in the control flask. Bitting[1] reports that molds will grow in a few days on a paste made of these ground spices mixed with water. It is obvious, then, that ginger, black pepper, and cayenne pepper can be of no value as preservatives in the small proportions they are used in cooking.

TABLE II.—TIME OF SPOILAGE OF APPLE SAUCE CONTAINING VARYING AMOUNTS OF SPICE.

Amount of spice. Grams.	Control.	Ginger.	Black pepper.	Cayenne pepper.
0	4 days	...	...	...
15	...	6 days	...	7 days
20	...	8 days	6 days	7 days
30	...	7 days	6 days	6 days

The effect of cinnamon, cloves, mustard, allspice, and nutmeg in small amounts was tested in another experiment. The results are given in Table III.

TABLE III.—TIME OF SPOILAGE OF APPLE SAUCE PRESERVED WITH SPICES.

Amount of spice. Grams.	Control.	Cinnamon.	Cloves.	Mustard.	Allspice.	Nutmeg.
0	9 days	....	....	....	....	....
0.25		9 days	15 days	115 days	9 days	
0.50		30 days	15 days	60 days	9 days	
1.00		84 days	74 days			9 days
1.50		84 days	Not spoiled in 8 months			
2.00		Not spoiled in 8 months		Not spoiled in 8 months	9 days 16 days	
2.50					9 days	9 days
5.00						9 days
7.50						9 days
10.00						10 days

Allspice in the proportion of 5 grams of spice to 100 grams of sauce did not have even a retarding effect, although the flask containing 2.5 grams kept 16 days, whereas the control flask kept 9 days. Nutmeg in the proportion of 10 grams to 100 grams of sauce delayed growth only 1 day. Hence these two spices cannot be considered of practical value as antiseptics.

Cinnamon, cloves, and mustard are about equal in their efficiency. The flask containing 0.5 gram of mustard kept for almost four months; the flask containing 1 gram kept two months; and the flasks containing 2, 2.5, and 5 grams are still keeping, eight months after having been put up. The flask containing 0.5 gram of cinnamon kept one month; the flasks containing 1 and 1.5 grams kept almost three months; and the flask containing 2 grams is still keeping. Cloves in the proportion of 0.25 gram to 100 grams of sauce delayed growth for several days; the flask containing 1 gram kept for $2^1/_2$ months; and the flasks containing 1.5 and 2 grams are still keeping.

The three spices, cinnamon, mustard and cloves, must be considered important preservatives. Cinnamon and mustard are particularly valuable, for they are palatable even when used in proportions that prevent all growth. Cloves in the proportion which prevented growth (1.5 grams to 100 grams of sauce) had too much of a burning taste to be palatable. However, it retards growth in much smaller amounts.

The active antiseptic constituents of mustard, cinnamon, and cloves are their aromatic or *essential* oils, as they are termed. Clove buds yield about 15 per cent of essential oils, of which the greatest part is eugenol, or oil of cloves. Cinnamon bark yields from 0.5–1 per cent of essential oils. About 70 per cent of this is cinnamic aldehyde, which gives to cinnamon its characteristic odor. The essential oil of cinnamon also contains from 4–8 per cent of eugenol and some other aromatic compounds in smaller amounts.

The much smaller yield of the essential oil from cinnamon bark as compared with that from clove buds is compensated by its much greater antiseptic properties, which are brought out in Tables IV and V. These tests also were made with apple sauce.

Cinnamic aldehyde even in the smallest proportion used—a little over $^1/_{100}$ of a gram to 100 grams of sauce—delayed growth 60 days, and in double this amount prevented growth entirely in the second set of flasks. As in the case of the cinnamon from which it is derived, cinnamic aldehyde is palatable in proportions which will preserve food.

TABLE IV.—TIME OF SPOILAGE OF APPLE SAUCE PRESERVED WITH ESSENTIAL OILS.

Amount of oil. Gram.	Control.	Cinnamic aldehyde.	Eugenol.
0	3 days	...	...
0.026	...	80 days	6 days
0.052	...	120 days	12 days
0.105	...	Not spoiled in 14 months	Not spoiled in 14 months
0.157	...		
0.21	...		

TABLE V.—TIME OF SPOILAGE OF APPLE SAUCE PRESERVED WITH ESSENTIAL OILS.

Amount of oil. Gram.	Control.	Cinnamic aldehyde.	Eugenol.
0	9 days	...	...
0.013	...	60 days	9 days
0.026	...	Not spoiled in 8 months	13 days
0.052	...		9 days
0.105	...		Not spoiled in 8 months

It required considerably higher proportions of eugenol for complete preservation. In both tests, 0.105 gram of eugenol to 100 grams of sauce prevented all growth. This amount gives too much of a

[1] "Experiments on the Spoilage of Tomato Ketchup," by A. W. Bitting, Bureau of Chemistry, *Bull.* **119**, U. S. Dept. of Agriculture.

burning taste to be palatable, which is in agreement with the results obtained with cloves. One-half of this amount delayed growth somewhat, and did not give too strong a flavor. The essential oils of both cinnamon and cloves, however, lack much of the good flavor of the spices.

In continuation of the above work, it was thought advisable, in view of the present controversy relative to the manufacture of ketchups and the use of preservatives therein, to examine various ketchups as to the types of organisms present. Spices are extensively used in the manufacture of ketchup and in many commercial brands sodium benzoate is employed as a preservative. The use of sugar also has been adopted as aiding the keeping qualities of ketchups.

Accordingly any organisms which could grow in such a medium would be more or less of a resistant type and upon such a critical and crucial test of the preservative action of various substances could be made. In previous work these substances had been added to the medium and the latter then exposed to natural contamination. The work reported below was performed on pure cultures of organisms isolated from ketchups which were undergoing spoilage, the method adopted for this work being analogous to that employed in testing the strengths of disinfectants.

On examining various ketchups, the predominant type of organism usually found proved to be yeasts, presumably several of the wild species. These undoubtedly find a favorable medium in ketchup, particularly that containing sugar which they ferment with evolution of CO_2 resulting in the characteristic gassy fermentation of many of the spoiled ketchups. Of the bottles examined several were so charged with CO_2 that on opening, the contents were blown out of the bottle in all directions with explosive force. Besides yeasts several organisms of the bacillar type were isolated, and in several bottles of ketchup which had been bottled cold, an organism identical in most respects to the ordinary lactic acid bacterium was found. A large spore-forming bacillus was isolated from one of the heated ketchups. The predominant flora throughout, however, proved to be yeasts.

After preliminary isolation of these various organisms on an acidified tomato-broth agar, replating and reisolation were performed to insure purity of the cultures. No attempt was made to identify these various organisms by detailed study. They were employed directly to test out the preservative action of cinnamic aldehyde, eugenol, and benzoic acid, respectively. Numerous difficulties were here encountered before a suitable medium and a satisfactory method were devised. The medium finally adopted was a tomato broth bouillon containing 1 per cent sugar and adjusted to 1.5 per cent normal acid. But even with this medium, which gave good growth, it was difficult to test out the action of the various substances above mentioned, owing to the fact that their addition even in minute quantities occasioned a more or less pronounced turbidity which was with difficulty differentiated from that produced by bacterial growth. In several cases it was necessary to make microscopical examinations in order to ascertain whether or not growth had occurred.

Suspensions of the respective organisms were prepared by inoculating tubes of the tomato bouillon and incubating until turbidity had occurred which usually required forty-eight hours. After thorough shaking one loopful of these cultures was transferred to tubes containing 5 cc. of sterile broth. To these were then added varying amounts of cinnamic aldehyde, eugenol, and benzoic acid, respectively, equivalent to 1, 2, 5 and 10 parts per 10,000 of inoculated bouillon. Two tubes of each organism with each of the varying amounts of the different preservatives were prepared. All tubes were incubated at 37° C. and examined daily for three days. The results secured are recorded in Tables VI and VII.

TABLE VI.—INFLUENCE OF PRESERVATIVES UPON GROWTH OF CERTAIN ORGANISMS.

		Cinnamic aldehyde.				Eugenol.				Benzoic acid.			
Organism employed.	Day of examination.	1 pt. per 10,000.	2 pts. per 10,000.	5 pts. per 10,000.	10 pts. per 10,000.	1 pt. per 10,000.	2 pts. per 10,000.	5 pts. per 10,000.	10 pts. per 10,000.	1 pt. per 10,000.	2 pts. per 10,000.	5 pts. per 10,000.	10 pts. per 10,000.
23*d* (Spore-former)..	1st day	+	—	—	—	+	±	—	—	+	+	+	—
	2d day	+	—	—	—	+	+	—	—	+	+	+	±
	3d day	+	±	—	—	+	+	±	—	+	+	+	+
5*x* (Yeast).....	1st day	—	—	—	—	—	—	—	—	+	—	—	—
	2d day	+	—	—	—	—	—	—	—	+	—	—	—
	3d day	—	—	—	—	—	—	—	—	+	—	—	—

TABLE VII.—INFLUENCE OF PRESERVATIVES UPON THE GROWTH OF VARIOUS ORGANISMS.

	Cinnamic aldehyde.				Eugenol.			Benzoic acid.		
Organism	5 pts. per 100,000.	1 pt. per 10,000.	2.5 pts. per 10,000.	5 pts. per 10,000.	5 pts. per 100,000.	1 pt. per 10,000.	2 pts. per 10,000.	5 pts. per 10,000.	10 pts. per 10,000.	20 pts. per 10,000.
5*x* Yeast.....	+	±	—	±	+	+	+	—	—	—
23*y* Bacillus (spore-former).....	+	+	—	—	+	+	+	+	+	+
23*d* Bacillus (spore-former).....	+	+	±	±	+	+	+	—	+	±
23*e* Blunt bacillus.....	+	+	—	—	+	+	+	+	+	+
5*n* Yeast.....	+	±	—	—	+	+	+	—	+	+
27*a* Yeast.....	+	+	+	+	+	+	+	±	+	+
5*b* Yeast.....	+	±	—	—	+	+	+	—	+	±
5*d* Yeast.....	+	±	—	—	+	+	+	+	+	±
23*g* Bacillus in chains.....	+	+	+	+	+	+	+	+	+	+

From the data submitted it is at once apparent that cinnamic aldehyde possesses a more marked antiseptic action than either eugenol or benzoic acid. Of the two latter, benzoic acid appears to be by far

the least effective as a preservative. In case of cinnamic aldehyde a concentration of 2 parts per 10,000 was sufficient to inhibit the growth of most organisms, only two out of the nine employed showing growth at this concentration. With eugenol 5 parts per 10,000 proved insufficient to prevent the growth of all the organisms tried, four out of nine having grown. Benzoic acid on the other hand revealed a much weaker antiseptic action than either of the above, eight forms out of nine growing in a concentration equivalent to 10 parts per 10,000, and five out of nine in a concentration of 20 parts per 10,000, while three others showed doubtful evidence of growth.

In view of the fact that this work on pure cultures was performed by one of the authors, whereas the work on the apple sauce was performed independently by the other, it is interesting to note that the results in both cases coincide remarkably with one another. For direct contamination of the specially prepared apple sauce, it was found that 0.01 gram of cinnamic aldehyde per 100 grams of sauce (equivalent to 1 part per 10,000) was sufficient to delay growth at least 60 days and that 2 parts per 10,000 prevented all growth, results with which the data in Tables VI and VII correspond very closely. With eugenol the same remarkable coincidence maintains, the results of both observers showing that 10 parts per 10,000 were sufficient to inhibit growth. Thus, both methods, the more practical household method of direct exposure to contamination, as well as the laboratory pure-culture method, yielded identical results in all respects.

In view of the above, it appears that cinnamic aldehyde and eugenol as such possess considerable preservative action and aid materially in preserving substances to which they are added. Both are contained in such spices as cinnamon and cloves. No doubt the marked preservative action of these spices, as shown in the above experiments, must be attributed to their content of these essential oils. As this preservative action takes place, even when the spices are used in the small quantities necessary for flavoring, their use can be recommended in contrast to such spices as pepper and ginger which have been shown to possess but little, if any, preservative action. The more liberal use of cinnamon and cloves in the preparation of ketchup may perhaps remove the necessity of adding sodium benzoate for preserving it, a practice to which there is so much objection.

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THE DETERMINATION OF GLIADIN OR ALCOHOL-SOLUBLE PROTEIN IN WHEAT FLOUR.

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Osborne[1] in his monograph, "The Proteins of the Wheat Kernel," states that the following proteins are present in the wheat kernel: "Gliadin insoluble in neutral aqueous solutions, but distinguished from all others by its ready solubility in 70 per cent. alcohol; glutinin, a protein having a similar elementary composition to gliadin, soluble in dilute acid and alkaline solutions, and yielding a wholly different proportion of decomposition products when boiled with strong acids; leucosin, an albumin-like protein, freely soluble in pure water, and coagulated by heating its solution to 50–60° C.; a globulin, similar in composition and properties to many globulins found in other seeds, and one or more proteoses which are present in very small quantity."

Osborne states that globulin, albumin and proteose are the proteins found in the embryo of the wheat kernel, while the endosperm consists nearly entirely of gliadin and glutinin. The latter part of this statement of Osborne's is hardly correct, since wheat flour contains considerable proteid material—globulin, albumin and proteose, soluble in dilute salt solution —usually amounting to 10–15 per cent. of the total protein.[1] However, the proteins of the wheat endosperm do consist very largely of gliadin and glutinin.

The quantitative separation of the proteins of wheat flour is very difficult and not possible of absolute accuracy, since no one protein is entirely insoluble in the solvent used to extract another protein. Thus while a 10 per cent. sodium chloride solution will extract albumin, globulin and proteoses, yet it will also extract some gliadin. Likewise, 70 per cent. alcohol, while presumably extracting only gliadin, also extracts some albumin, globulin, etc. Chamberlain[2] has done a large amount of work in the quantitative separation of proteins of wheat flour. In his latest report, *Journal of the American Chemical Society*, 1906, he makes, among others, the following conclusions: "As recommended by the author in the Association of Official Agricultural Chemists, the separation of the proteins of wheat into more than two groups, *viz.*, first, alcohol-soluble, second, alcohol-insoluble, seems unwarranted, both because of the difficulty of making a further quantitative separation, and because of the indefinite value of such separation.

In the light of all data available to the writer, this statement of Chamberlain's seems wholly justified. It is to be hoped, however, that methods may be devised by which an accurate quantitative separation of the wheat proteins may be accomplished.

A large amount of chemical investigation has been conducted during the past ten years with wheat flour, and one of the chief objects in this work has been to determine some relation between the composition of the flour and its strength, *i. e.*, ability to produce a large, well piled loaf. An immense number of gliadin determinations have been made in this work, to ascertain if any relation existed between the ration of gliadin to the total protein, and the strength of the flour. The writer proposes to discuss this matter in detail in a later paper.

The determination of gliadin, or to be scientifically

[1] *Proteins of the Wheat Kernel*, pub. by Carnegie Inst., 1907.

[1] See Chamberlain, *Bull.* **90**, p. 124, Bureau of Chem., U. S. Dept. of Agr.

[2] See Chamberlain, *Bulls.* **81** and **90**, Bureau of Chem., U. S. Dept. of Agr.; also Vol. **28**, No. 11, *J. Am. Chem. Soc.*