BROCCOLI PROCESSING

Accumulation of Alcohol in Underscalded Frozen Broccoli

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In continuation of investigations on the nature of the changes in color and flavor of frozen vegetables, the effect of scalding on the accumulation of acetaldehyde, alcohol, and related compounds in broccoli during freezing storage was determined. In broccoli tissues scalded in steam or boiling water for various periods of time and held frozen up to 1 year, the content of volatile aldehydes was found to remain fairly constant at a level of 2 to 15 p.p.m. The alcohol content, however, continuously increased during freezing storage from 0 to 370 p.p.m. The volatile alcohol was determined to be largely ethyl alcohol. Recently, the formation of acetoin, acetylmethylcarbinol, as well as ethyl alcohol, was observed when sterile broccoli tissues were held anaerobically. These results indicate that unlike asparagus and peas, ethyl alcohol rather than acetaldehyde content may serve as an objective criterion of quality in frozen broccoli.

A DISTURBANCE IN THE NORMAL glycolytic reactions rather than cleavage of large storage or structural components such as fats and proteins (*12*) is believed to be involved in the production of off-flavors in the tissues of underscalded vegetables during freezing storage (*1*, *9–11*). As part of this series of investigations, data on the accumulation of alcohol in frozen broccoli are presented here. Additional details will be found in the thesis of Buck (*7*).

Materials and Methods

Italian green-sprouting broccoli, *Brassica oleracea*, var. italica, Plenck (*3*), was purchased as commercially packed with ice for the fresh market in tied bunches 7 to 8 inches in length.

Samples of small, medium, and large heads were selected for the study of heat penetration during scalding. The spikes were trimmed to a length of 4.5 inches, and copper-constantan thermocouples were inserted 1 inch from the top of the flower buds and 1 inch from the butt. Temperature measurements were recorded by the use of a Leeds and Northrup potentiometer. The samples were scalded in water at 100° C., then cooled rapidly by immersion in cold running tap water. Peroxidase and some catalase tests were used to evaluate the effectiveness of scalding.

A portion of the same broccoli was trimmed to remove defects, then successively cut into 1-inch lengths using stainless steel knives. Thus, segments of the spikes characterized by positions of 0 to 1, 1 to 2 inches, etc., along the stem were obtained from the butt end. The leaves and the terminal flower buds were also collected. These samples were placed in hermetically sealed cans, frozen, and stored at −17° C. for periods of 6 months and 1 year.

Another portion of the broccoli was trimmed to remove defects and large leaves, cut to 4.5 inches in length, and scalded in steam and water for varying periods of time. The scalded samples were sealed in friction lid No. 2½ tin containers, frozen, and stored as above.

After storage, the samples for the enzyme and chemical analysis were opened and the broccoli was quickly ground at an air temperature of 1° C. in a food chopper using the fine blade. The ground tissue material was well mixed and fractions were taken for the different analyses. Whenever an analysis was not performed immediately, the sample was rapidly transferred to clean, dry, glass-stoppered bottles and stored at −17° C.

Commercial samples were used for comparative studies, and two samples were found to have objectionable haylike flavor. Quality was judged by reference to a point system, and the samples were graded A, B, or C.

Organoleptic Analysis After storage for 1 year, one can of each treatment in the series of scalded, frozen broccoli was opened and divided into equal portions. One half of the contents of each can was allowed to thaw in air at room temperature, and the other half was cooked by dropping the frozen broccoli into just sufficient boiling water so that rapid cooking occurred. The prepared samples were judged by a four-membered tasting panel. All other samples were tasted by the authors at the time of analysis.

Catalase and Peroxidase Activity The distribution of the enzymes, catalase, peroxidase, and phenolase was determined in the successive portions of the stem, leaves, and flower buds by the qualitative method of Joslyn (*10*). In addition, partially purified extracts were tested for the same enzymes by several chromogenic reagents (*9*). The catalase activity was determined quantitatively by the modified Balls and Hale procedure (*4*). Peroxidase activity was determined by the colorimetric guaiacol procedure (*9*).

Table I. Identification of Alcohol by Duclaux Procedure

Distillate, Ml.	0.02 N NaOH, Ml.		Constants (Average), %	Duclaux Constants, %	
				For apparatus	For acetic acid
20	0.28	0.31	18.5	15.7	15.7
40	0.20	0.23	32.1	33.4	32.7
60	0.30	0.33	51.8	51.4	51.4
80	0.30	0.34	72.1	73.2	72.6
100	0.43	0.46	100.0	100.0	100.0

Volatile Aldehydes And Alcohols

Acetaldehyde and alcohol were determined as in previous investigations (*1*, *11*). The alcohol was identified by the Duclaux procedure as modified by Van der Lek. The data shown in Table I indicate that ethyl alcohol is the only alcohol formed.

Results and Discussion

The data plotted in Figure 1 show that the ethyl alcohol content of frozen broccoli increased from 11 mg. per 100 grams of fresh tissue in the unscalded sample to about 40 in the lightly scalded sample, then decreased as the time of scalding increased. The volatile aldehyde content in the same sample was much lower and decreased slowly with increase in time of scalding. All tissues of broccoli, green stems, leaves, and flowers behaved similarly. Berger and Avery (*6*) reported in 1943 that alcohol dehydrogenase of *Avena coleoptile* increased in activity in plants treated with auxin. Because broccoli is growing vigorously prior to harvesting, a series of analyses was made to determine the effect of the distance of the tissue from the terminal meristem on the accumulation of volatile aldehydes and alcohols. No effect of distance was observed either on flavor or on alcohol and aldehyde content under the conditions of the investigation. Unscalded sections of the three tissues developed off-flavor rated as poor, bitter flavor, and showed accumulation of ethyl alcohol, but no sequence due to position was observed even after storage for 1 year at −17° C. In all samples the volatile aldehyde content remained fairly constant during freezing storage, as it did in spinach (*7*), but ethyl alcohol accumulated.

The thermal inactivation of the enzymes involved in the formation of off-flavors paralleled that of peroxidase. Table II shows the correlation among the extent of scalding, the activity of peroxidase and catalase, the accumulation of volatile aldehydes and ethyl alcohol, and the changes in flavor. Scalding for 1 and 2 minutes was insufficient to prevent the loss of green color and development of off-flavor. The 1-minute water-scalded samples were markedly poorer in quality and flavor than the control receiving no scald. The off-flavor was described by members of the taste panel as haylike in flavor but acid or sharp in aroma. Fixation of color occurred between 2 and 3 minutes. The data summarized in Figure 2 show that broccoli catalase is much more thermolabile than the peroxidase. For retention of flavor in frozen broccoli the peroxidase activity must be reduced to less than 100 units and the alcohol accumulation checked completely.

The heat penetration into the tissues of broccoli was studied by inserting thermocouples in various segments of the plant. The rate of penetration was dependent in part upon the thickness of the tissue, even in the branched flower head. Only the thick butts had a measurable temperature lag. The flower head and butt measurements of spikes used to plot the rate of heat penetration and cooling are given in the legends of Figures 3 and 4. As curves for steam scald and for water scald were nearly identical, only a typical determination has been plotted. When the data of Figure 3 are plotted using semilogarithmic paper by the regular conventions for heat penetration, the plots are linear. The f_h, which is the slope of the heating curve, is 8.5 for the typical thick spike and 3.4 for a typical thin-stemmed spike. Because the position of the thermocouple affected the readings, in each determination the thermocouples were checked to ensure

Figure 1. Volatile aldehyde and ethanol content of fresh scalded broccoli

Milligrams per 100 grams of fresh broccoli, scalded for various times in water at 100° C. and stored at −17° C. for 1 year before analysis
1. Ethanol content
2. Volatile aldehyde content

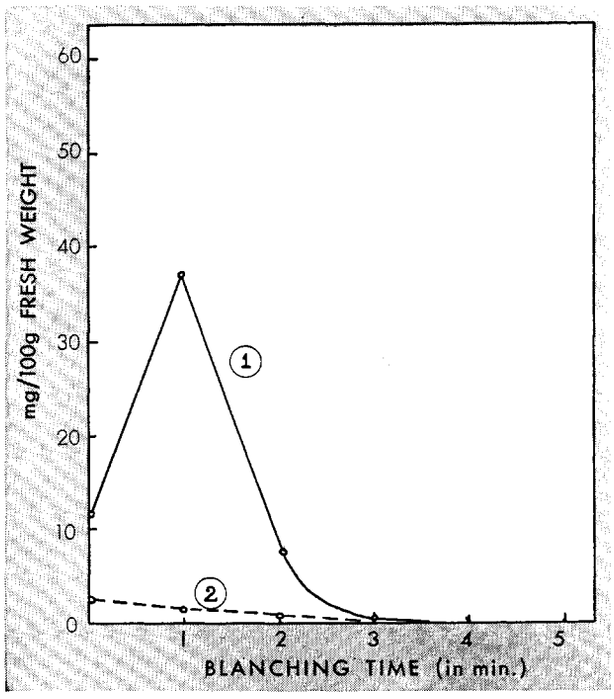


Figure 2. Peroxidase and catalase activity per gram of fresh broccoli tissue

Broccoli scalded for various times in water at 100° C. and stored at −17° C. for 1 year before analysis.
1. Peroxidase activity. Readings using Klett-Summerson photoelectric colorimeter, logarithms of transmission, T, were plotted against time.
2. Catalase activity, calculated from formula $K = 1/t \log a/a-x$ using titration method, and multiplied by 10^3 for convenience in plotting

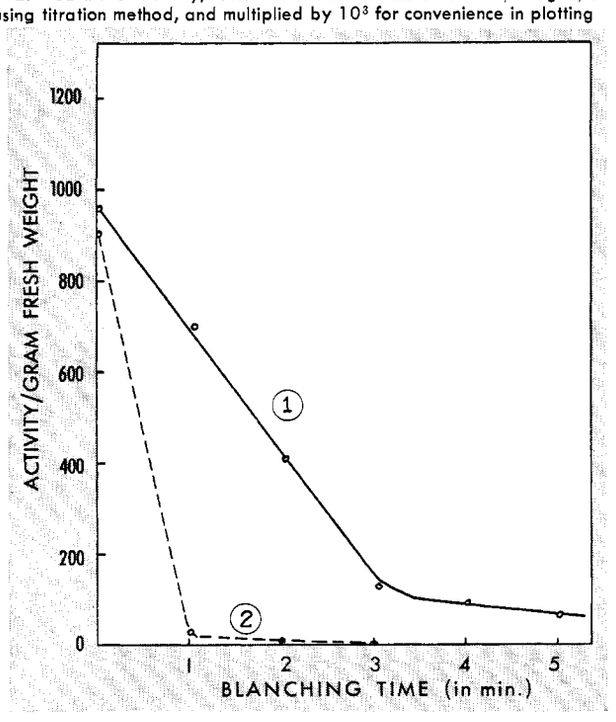


Table II. Organoleptic Tests and Peroxidase and Catalase Activity in Scalded Broccoli

(Compared to volatile aldehyde and ethyl alcohol content after 1-year storage at —17° C.)

Scalding Medium	Time of Scalding, Min.	Color	Odor	Taste	Quality	Peroxidase Activity per G. Fresh Tissue	Catalase Activity per G.	Mg./100 Groms Fresh Tissue	
								Aldehyde	Ethyl alcohol
Steam	0	Olive green	Off-odor	Bitter	C	960	0.949	3.01	10.8
	1	Light green	Off-odor	Bitter	B	710	0.021	0.21	33.8
	2	Green	Trace	Bitter	B	400	0.009	0	2.1
	3	Green	Normal	Trace	A	70	0.004	0	0
	4	Green	Normal	Normal	A	13	0	0	0
	5	Green	Normal	Normal	A	0	0	0	0
Water	0	Olive green	Off-odor	Bitter	C	...	...	...	...
	1	Olive green	Off-odor	Bitter	C	450	0.017	1.46	37.0
	2	Light green	Trace	Trace	B	440	0.011	0.01	8.6
	3	Green	Normal	Trace	A	130	0.007	0	0
	4	Green	Normal	Normal	A	100	0.008	0	0
	5	Green	Normal	Normal	A	80	0	0	0
Commercial sample selected		Light green	Trace	Bitter	B	15	0.011	5.3	...
for noticeable off-flavor		Light green	Trace	Bitter	B	0	0.001	3.5	...

that the junction of the leads were centered in all segments. The temperature lag, by convention termed J, of flower heads of broccoli was negligible, which means that no dead air spaces retarded the heat inactivation of the enzymes in these experiments. The temperature lag at the butt was measurable but small; the value was 1.13 for the typical thick spike and 1.06 for the thin spike. The slight change of slope noted in many of the curves around a temperature of 70° C. is due to a collapse of the vegetable tissue during scalding.

The heat inactivation of catalase and peroxidase in broccoli tissues is shown in Table II. The inactivation in situ depended mainly upon the rate of heat penetration, although a slight residual peroxidaselike activity remained even after 8 and 15 minutes of steam scalding at 100° C. The activity of the peroxidase in broccoli was sufficiently reduced by scalding for 3 minutes at this temperature to permit storage for more than a year without deleterious change.

The formation of acetylmethylcarbinol, acetoin, was observed to occur in sterile broccoli subjected to anaerobiosis. Increasing the holding time from 3 to 7 days at room temperature increased the amount of acetoin. Fresh broccoli did not give a test for acetoin even after 14 days' storage. These findings of increasing amounts of acetoin are in agreement with those of David on peas subjected to anaerobiosis (*8*).

Isolation of α-carboxylase and of alcohol dehydrogenase from broccoli is being attempted. The authors hope to investigate their role under established conditions in the formation of off-flavors. They suspect that pyruvic acid formed by glycolysis of sugars is decarboxylated to acetaldehyde under freezing storage, just as it is under anaerobic conditions. Then α-carboxylase converts the acetaldehyde into acetoin (*13*) and subsequently to off-flavors. Alcohol de-

Figure 3. Heating and cooling curves for selected broccoli spikes during steam scalding

Water-scalding curves were nearly identical and have been omitted
1. Blossom end of typical spikes
2. Butt end of spikes measuring 0.75 to 1 inch in diameter
3. Butt end of spikes measuring 1.25 to 1.5 inches in diameter.

Arrow indicates time when cooling was begun.

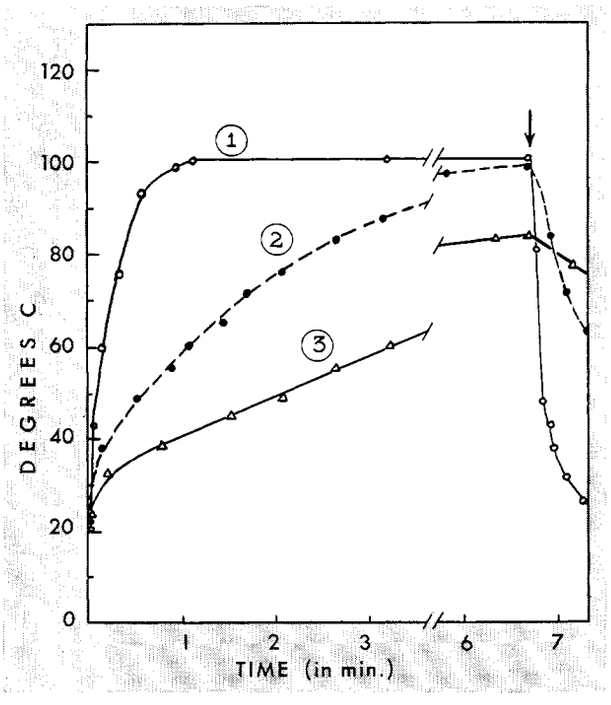
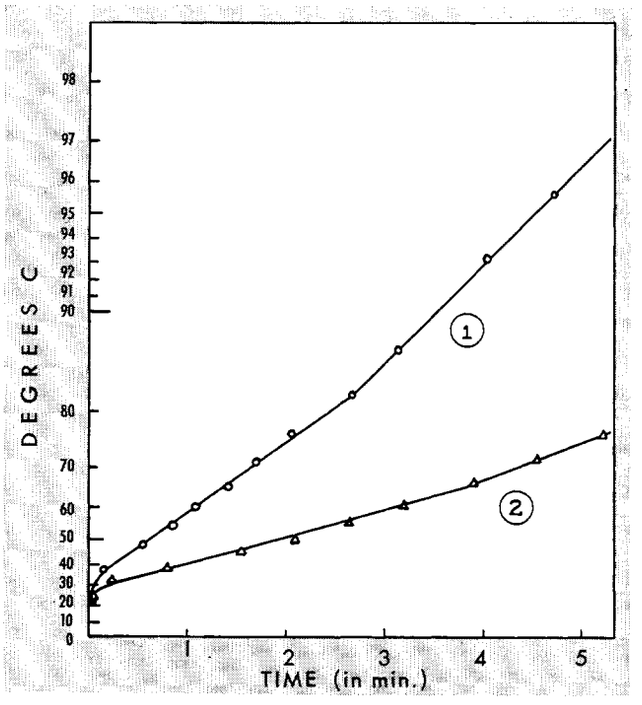


Figure 4. Logarithmic plot of temperature at center of broccoli tissues against time of heating during water scalding at 100° C.

1. Butt end of spike measuring 0.75 to 1 inch in diameter
2. Butt end of spike measuring 1.25 to 1.5 inches in diameter



hydrogenase converts part of the acetaldehyde into ethyl alcohol, but possibly its role may be indirect by maintaining the reduced diphosphopyridine nucleotide in an oxidized form, permitting further glycolysis.

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VANILLA-LIKE SYNTHETICS

Solubility and Volatility of Propenyl Guaethol, Bourbonal, Vanillin, and Coumarin

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Flavor studies on the vanilla-like synthetics, propenyl guaethol, bourbonal, vanillin, and coumarin, required an extension of the literature information on their solubilities and volatilities. The solubility of propenyl guaethol, in per cent by weight at 24° C., was found to be 0.002 in water, 0.54 in 50% ethanol by weight, 16.7 in 100% ethanol, 0.11 in 50% propylene glycol by weight, and 4.00 in 100% propylene glycol. In water, the solubility of bourbonal in per cent by weight at 25° C. was found to be 0.35, of vanillin 1.24, of coumarin 0.222, and of propenyl guaethol 0.0022. The relative volatilities over the range 24° to 105° C. are, in increasing order, bourbonal, vanillin, propenyl guaethol, and coumarin. However, the differences are not considered significant for flavoring purposes. The data on solubility and volatility of the synthetic vanilla-type flavoring materials are useful in preparing solutions of these compounds for use as flavor concentrates and in estimating their probable loss through volatilization during cooking.

RECENT FLAVOR STUDIES (*1*) of the high-flavor-strength, vanilla-like synthetic, 5-propenyl guaethol, in comparison with bourbonal (ethylvanillin), vanillin, and coumarin, required knowledge of solubilities in water, ethanol, propylene glycol, and mixtures thereof for each of these compounds, and of their relative volatilities.

Nothing on volatility, and not enough on the desired solubilities, was found in the literature. Some information, mostly qualitative, on the solubility of vanillin and coumarin appeared in the usual handbooks. One trade bulletin (*4*) gave solubility tables for bourbonal, vanillin, and coumarin, while another (*5*) gave some solubility data for propenyl guaethol. Mange and Ehler (*3*) reported solubilities of vanillin in alcohol-water and glycerol-water solutions, describing in detail their methods of determination, and noting especially the danger of error due to supercooling.

A simplified adaptation of the methods of Mange and Ehler was used to obtain additional solubility data for immediate use. Comparative volatilities of the four compounds were simply determined at several temperatures. It is hoped that the methods and data here reported will prove useful, and especially that they will stimulate further work in this neglected field.

Samples and Solvents

All synthetic flavoring materials used were commercial samples as currently offered for use in foods.

Propenyl guaethol, 1-ethoxy-2-hydroxy-4-propenylbenzene or 5-propenyl guaethol, was purchased from Shulton, Inc., under the trade name Vanitrope.

Bourbonal, 3-ethoxy-4-hydroxybenzaldehyde, also known as ethylvanillin and as vanillal, was purchased from Monsanto Chemical Co. under the trade name Ethavan.

Vanillin, 3-methoxy-4-hydroxybenzaldehyde, was from Monsanto.

Coumarin, *o*-hydroxycinnamic acid lactone, was from Monsanto.

Distilled water and U.S.P. XIV grades of absolute alcohol and propylene glycol were used as solvents.

Experimental Methods

The method used for solubility determinations, adapted from the work of Mange and Ehler (*3*), requires only simple laboratory equipment, guards against supercooling errors, and appears to be convenient and efficient for general application.

Each solubility curve was first roughly determined by a few trials and then verified at a number of points by more accurate determination. As an illustration, the determination of the approximate solubility curve for propenyl guaethol in ethanol-water solutions at 24° C. is described.

To approximately 0.05 gram of propenyl guaethol, accurately weighed in a 2-ounce glass bottle with an aluminum foil–lined screw cap, was added slightly less than the required quantity of ethanol. After weighing, the solution was warmed with shaking until clear, then cooled to 24° C., resulting in crystal