POLYPHENOLASE ACTIVITY AS A PRIMARY CAUSE IN DARKENING OF BOILED POTATOES[1]

IT has recently been reported[2] that a compound (or compounds) reacting like catechol (orthodihydroxy phenol) occurs in potatoes during winter storage in proportions which show general correlation with the degree of blackening after boiling. Following that work we have investigated the activity of the polyphenolase system in tubers covering a wide range of discoloration after cooking. The difference in this function, as between normal and seriously discoloring potatoes, is much more definite than that found for the catechol reaction. As the polyphenolase system is much more active in the oxidation of catechol than of tyrosine, it appears that compounds of the latter type may not accumulate in proportion to the capacity of the tuber for discoloration. Our results thus indicate that departure from the normal respiratory relations after harvesting is a primary cause in darkening of boiled potatoes. A more extensive account of this work will be published soon.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A COLORIMETRIC TEST FOR VITAMIN K_1

DAM *et al.*[1] while working on the isolation of vitamin K discovered that the isolated purified material gave, in the presence of sodium alcoholate, a violet blue color, changing to red and eventually to brown. Subsequently, both vitamins, K_1 and K_2, were found to give this reaction.[2, 3, 4] As far as it can be ascertained there has been no further report on the development of this test.

Sullivan and Irreverre[5] have shown that 1,4-naphthoquinone-2 potassium sulfonate is a highly specific reagent for creatinine. Since vitamins K_1 and K_2 are derivatives of α-naphthoquinone it was thought that perhaps there might be a compound in the creatinine group that would give a color reaction with vitamin K, particularly the naturally occurring K_1 and K_2. No such reaction could be obtained by any of the derivatives of creatinine at our disposal. However, on experimenting with vitamin K_1 and compounds belonging to the carbamic acid series, it was found that vitamin K_1 and 2,3-dimethyl-1,4-naphthoquinone gave a cobalt blue color in the presence of sodium diethyl dithiocarbamate and alkali, or better alcoholic alkali.

The test is performed as follows: To 2 cc of a 95 per cent. alcohol solution of the material to be tested, add 2 cc of 5 per cent. sodium diethyl dithiocarbamate[6] in 95 per cent. alcohol and 1 cc of alcoholic alkali (made by dissolving 2 gm of sodium in 100 cc of 95 per cent. ethyl alcohol). Under this condition vitamin K_1 (0.5 mg per 2 cc) gives a deep cobalt blue color attaining its highest intensity in 5 minutes and fading slowly after 8 minutes. At the end of 30 minutes the color is faintly reddish orange. This reaction was tried on a number of quinones: 2-methyl-1,4-naphthoquinone[7]; 2-chlor-1,4-naphthoquinone; 2-amino-1,4-naphthoquinone; 2-hydroxy-1,4-naphthoquinone; 2,5-dimethyl-1,-4-naphthoquinone[8]; 2,6-dimethyl-1,4-naphthoquinone[8]; 2,7-dimethyl-1,4-naphthoquinone[8]; 2,8-dimethyl-1,4-naphthoquinone[8]; 2,6-dimethyl-3-hydroxy-1,4-naphthoquinone[8]; 1,4-naphthoquinone; 2,3-dichlor-1,4-naphthoquinone; 2-methyl-3-hydroxy-1,4-naphthoquinone[7]; 1,2-naphthoquinone; 3-methyl-2,3-oxido-1,4-naphthoquinone[7]; 1,4-naphthoquinone-2,3-oxide. All the compounds enumerated gave a color: pink, red, green, brown or violet. The cobalt blue color, however, was exhibited only by vitamin K_1 and 2,3-dimethyl-1,4-naphthoquinone.[7] It is believed that the cobalt blue color is characteristic of 2,3-dialkyl substituted α-naphthoquinones in the α-naphthoquinone series, since the 2,3-dichlor-1,4-naphthoquinone gave a brownish violet color. Vitamin K_2 was not on hand. All the hydroxy derivatives gave red colors. The Dam *et al.*[1] test was also tried on all the substances mentioned, as follows[9]: To 4 cc of a 95 per cent. alcohol solution of the material to be tested, add 1 cc of alcoholic alkali (made by dissolving 2 gm of sodium in 100 cc of 95 per cent. ethyl alcohol). In our hands all the substances tested gave a color: yellow, orange,

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[2] C. O. Clagett and W. E. Tottingham, *Jour. Agr. Research*, 62: 349, 1941.

[1] H. Dam, A. Geiger, J. Glavind, P. Karrer, W. Karrer, E. Rothschild and H. Salomon, *Helv. Chim. Acta*, 22: 310, 1939.

[2] P. Karrer, *Helv. Chim. Acta*, 22: 1146, 1939.

[3] L. F. Fieser, *Jour. Am. Chem. Soc.*, 61: 2559, 3467, 1939.

[4] A. A. Klose and H. J. Almquist, *Jour. Biol. Chem.*, 132: 469, 1940.

[5] M. X. Sullivan and F. Irreverre, *Jour. Biol. Chem.*, 128: ci, 1939.

[6] The sodium diethyl dithiocarbamate can be obtained from Eastman Kodak Company. If the substance is not pure it should be recrystallized from warm 95% alcohol with decolorization by carboraffin. A 5% solution of the colorless purified material in 95% alcohol should be practically colorless. This reagent must be freshly made and will keep for only a day.

[7] These compounds were furnished through the courtesy of Merck and Co., Inc.

[8] These naphthoquinones were kindly supplied by Dr. Louis F. Fieser.

[9] Dam *et al.* do not give any details for running their test.