KOREAN J. FOOD SCI. TECHNOL.
Vol. 17, No. 3 (1985)

Inhibiting Pattern of Dopachrome Formation as Influenced by Sodium Benzoate in Raw Potato Tubers

Young-Sook Moon and Mie-Soon Lee Kim

Department of Food and Nutrition, Duksung Women's College, Seoul

감자괴경에서 Sodium Benzoate에 의한 Dopachrome형성 억제 패턴

문영숙 · 이미순

덕성여자대학 식품영양학과

Abstract

The inhibiting properties of sodium benzoate on the dopachrome formation were investigated with crude enzyme preparations from homogenized tissues of raw potato tubers. The % inhibitin of dopachrome formation was increased with increasing concentrations of sodium benzoate and decreasing concentrations of substrate, 3,4-dihydroxyphenylalanine(DOPA). The inhibiting action was gradually reduced with increasing temperature. Dopachrome formation tended to be greatly inhibited in the range of pH 3-5, while it revealed a sharp increase above pH 6. The results suggested that sodium benzoate might compete with the substrate for the enzyme.

Introduction

Two types of enzymatic discoloration including "adventitious browning" and "functional browning" are encountered in plant tissues. The former follows Cellular damage and the latter occurs during normal development of plants.[1] Dark melanin compounds appearing in blackspot of potatoes, prepeeling blackening and pressure bruising are the results of adventitious browning. It is commercially undesirable in most cases because of the unsightly appearance and the concomitant development of off-flavor.

Polyphenol oxidase (o-diphenol : oxygen oxidoreductase, E.C.1.10.3.1.) is the enzyme responsible for the initiation of adventitious browning. Various inhibitors have been used to prevent this type of enzymatic browning.[2~7]

As the dopachrome is the first visible pigment concerned with the enzymatic browning, inhibiting pattern of dopachrome formation provides convenient system for enzymatic browning and its inhibition studies.

Inhibition pattern of dopachrome formation as influenced by sulfur dioxide has been previously investigated in this laboratory.[8,9]

Sodium benzoate, the sodium salt of benzoic acid, converts to benzoic acid which is an active form in the aqueous solution.[10] Sodium benzoate does not have deleterious or poisonous actions when applied to foods. Owing to the presence of detoxifying mechanism in the human body, there is no danger of accumulation of benzoate in the body.[4] As it has a good flavor, sodium benzoate has been used as an inhibitor in the enzymatic browning,[2~6,13~16] even though the mechanisms and the conditions of its inhibiting action are not fully known.

The present study was undertaken to investigate the inhibiting pattern of dopachrome formation as influenced by sodium benzoate in raw potato tubers.

Materials and Methods

'Irish Cobbler' potatoes donated from Alpine Experiment slation were provided as the source of

enzyme preparation. DOPA and sodium benzoate were the products of Sigma Chemical Company and Borjak Aromatics, respectively. All chemicals employed for enzyme assay were of reagent grade. Crude enzyme preparations were based on procedures described by Muneta.[11] Peeled potato 100*g* was ground with ice 85*g* and 12.5*ml* of 2.8%(W/V) ascorbic acid solution neutralized to pH 6.0 for 2 minutes at high speed in a waring blender. The homogenized tissues were filtered under suction through Toyo No. 2 filter paper. The filtered residue was washed 3 times with 75*ml* of distilled water. Then the washed residue was mixed well with 150*ml* of distilled water and refiltered. This final filtrate was used as crude enzyme preparation.

The reaction mixture contained 2.5-15 *nm* DOPA, 0.25-2.0 *nm* sodium benzoate, and 0.5*ml* enzyme preparation. Its volume was made up to 8*ml* with 0.2M phosphate buffer(pH 6.0) except for specified cases. All reactions were carried out at 20±1℃.

Various pHs of the reaction mixture were adjusted with 0.1M citrate buffer between pH 3-5 or 0.2M phosphate buffer between pH 6-8. Temperature experiment was performed by maintaining specified temperatures±1℃ in the water bath. Enzyme preparation was added to the incubation mixture after it reached thermoequilibrium state. Dopachrome formation was proceeded at 10, 20, 30, 40 or 50℃.

The pattern of dopachrome formation was also investigated by varing the incubation time of assay mixture in the presence of sodium benzoate. The reaction mixture was incubated for 0, 1/2, and 1 hr, respectively. DOPA was placed immediately after the termination of each incubation time. To investigate the effect of adding time, sodium benzoate was added after 20 minute reaction. In the above two experiments, absorbance (at 480 *nm*) was measured at regular time interval.

Results and Discussion

Fig. 1 shows that enzymatic browning developed with 2 mM DOPA within 10 minutes under the present experimental conditions. However, sodium benzoate, ranging from 0.5 *nm* to 2.0 *nm*, greatly retarded the

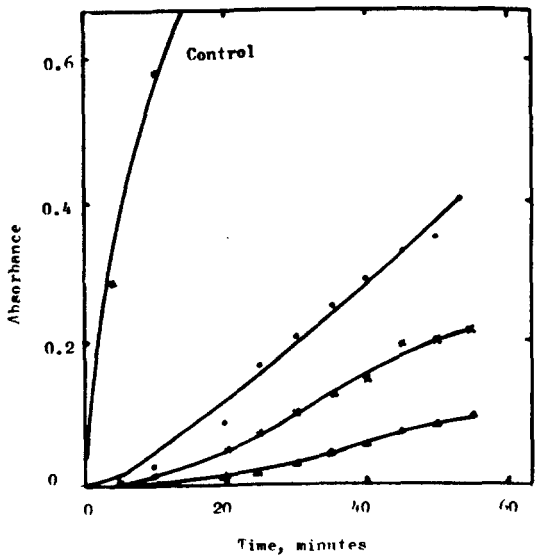

Fig.1 The pattern of dopachrome formation as influenced by various concentrations of sodium benzoate added at the initiation of reaction. Sodium benzoate; 0 *m*M(■), 0.5 *m*M(●), 1.0 *m*M(×), 2.0 *m*M(▲). Concentration of DOPA was 2.0 *m*M.

dopachrome formation, showing greater inhibition with increased concentration.

Increasing DOPA concentration up to 15 *nm*, various concentrations of sodium benzoate resulted in similar patterns of inhibition for dopachrome formation. There was no interaction between DOPA and sodium benzoate concentrations. At the same level of DOPA, the degree of inhibition for dopachrome formation gradually increased as the concentration of sodium benzoate became higher. Sodium benzoate tended to inhibit dopachrome formation less effectively as the concentration of DOPA increased(Table 1).

The above results clearly indicated that the % inhibition of dopachrome formation was great independent of DOPA levels at the high concentrations of sodium benzoate. On the other hand, it varied depending on DOPA levels at the low concentrations of sodium benzoate. This fact suggests that the % inhibition of dopachrome formation is more greatly influenced by the levels of enzyme substrate(DOPA) rather than enzyme inhibitor(sodium benzoate) when low concentration of inhibitor is applied. To reach the same extent of inhibition, much higher concentra-

Table 1. The pattern and the % inhibition of dopachrome formation as influenced by various concentrations of sodium benzoate and DOPA.

Concn of sodium benzoate (*m*M)	Concen of DOPA(*m*M)					
	2.5	5.0	7.5	10.0	12.5	15.0
	—Absorbance—					
0.00	0.174 (0.0)	0.339 (0.0)	0.379 (0.0)	0.525 (0.0)	0.523 (0.0)	0.603 (0.0)
0.25	0.026 (85.0)	0.125 (63.1)	0.200 (46.8)	0.314 (40.1)	0.391 (26.2)	0.430 (28.1)
0.50	0.035 (79.8)	0.088 (74.0)	0.155 (58.7)	0.267 (49.1)	0.309 (41.7)	0.343 (43.1)
1.00	0.013 (92.5)	0.065 (80.8)	0.079 (79.0)	0.174 (66.8)	0.197 (62.8)	0.238 (60.5)
2.00	0.016 (90.8)	0.022 (93.5)	0.047 (87.5)	0.091 (82.6)	0.127 (76.0)	0.147 (75.6)

*( ) indicates % inhibition.

tions of sodium benzoate are required in the presence of larger amounts of DOPA.

As shown in Fig. 2, pH greatly affects the inhibiting action of sodium benzoate. Maximum development of browning occured at pH 7.0 without sodium benzoate. It is apparent that the relative % of dopachrome formation decreased as the pH became lower in case maximal value of absorbance at pH 7.0 was designated as 100%.

According to Heymamn *et al.*,[10] optimum pH of catechol oxidase is 5-7 and Robb et al.[17] suspected that the enzyme probably had tertiary structure at neutral pH. Benzoic acid might act as competitive inhibitor for polyphenol oxidase.[18] Otherwise, in low pH, protonation of free carboxyl group might lead to the neutralization of negative charge in the enzyme molecule itself and cause the electrostatic repulsion between positively charged amino groups. It is presumable that this electrostatic repulsion destroys the parts of tertiary stucture of the enzyme.

In the present study, it is seen that sodium benzoate largely prevented the dopachrome formation at pH 3-6 range, while the inhibiting action was abruptly decreased above pH 6.As suggested by Ben-shalom *et al.*,[5] the inhibiting action of sodium benzoate might depend on its undissociated acid. Furia[19] also reported that the action of benzoate as antimicrobial agent was possible only in undissociated state.

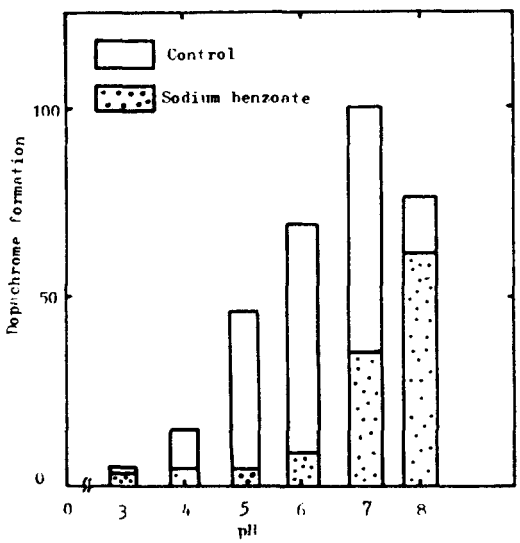

Fig.2 The effect of pH on the pattern of dopachrome formation in the presence of 1.0 *m*M sodium benzoate.

As a result of considering all the above facts, pH could influence both on the enzyme and on sodium benzoate. Conquently, it is probable to inhibit enzymatic discoloration only by lowering the pH, however, the extremely low pH brings about softening of raw potato tubers. Therefore, it would be more efficient to treat sodium benzoate under the condition of pH 3-5.

Table 2 shows the effect of 1.0*mM* sodium benzoate on dopachrome formation at various tempera-

Table 2. The effect of temperature on dopachrome formation in the presence of sodium benzoate.

Temperature (℃)	Concn of sodium benzoate	
	0 *m*M	1.0 *m*M
	—Absorbance—	
10	0.295	0.004
20	0.626	0.018
30	0.547	0.091
40	0.379	0.220
50	0.254	0.218

tures. Browning developed maximally at 20℃, with 2 times higher value than the one at 10℃, and then gradually decreased with increasing temperatures. It appears that browning is well controlled by 1.0*mM* sodium benzoate at 20℃ near room temperature. However, the degree of inhibition for dopachrome formation tended to reduce at higher temperatures above 20℃.

To find out whether the particular site of enzyme is affected by sodium benzoate, the enzyme mixture without substrate was preincubated for 0, 1/2 and 1 hr in the presence of sodium benzoate and provided with DOPA. Then the extent of dopachrome formation was measured at regular time interval. Sodium benzoate of 1.0*mM* greatly inhibited dopachrome formation compared with the control containing no benzoate. However, preincubation of 1/2 or 1 hr resulted in little difference of dopachrome formation; the shift of time axis allows 3 curve to overlap(Fig. 3). This result means that sodium benzoate does not directly affect enzyme molecule.

Fig. 4 depicts the pattern of dopachrome formation when 0.5*mM* or 2.0 *mM* of sodium benzoate was added after 20 minutes of reaction time elapsed. Sodium benzoate completely prevented further dopachrome formation at 2.0*mM*, even though it produced slightly higher amount of dopachrome than that already formed at 5.0*mM*. As the degree of competitive inhibition for the enzyme is largely determined by relative concentrations of substrate and inhibitor, this result supports a possibility that sodium benzoate might act as a competitive inhibitor. It is feasible that sodium benzoate combines with the enzyme at its

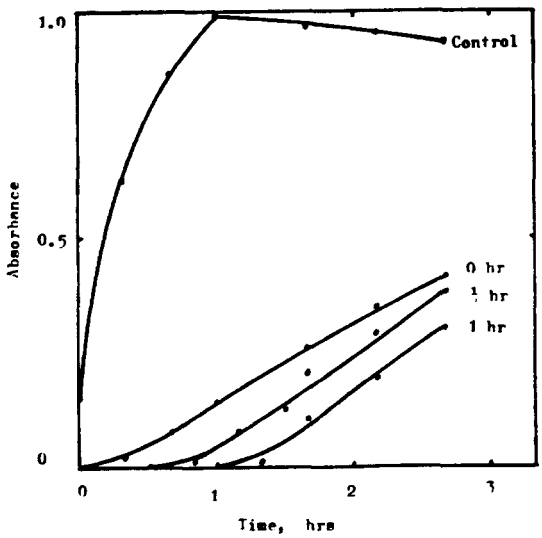

Fig.3 The pattern of dopachrome formation as influenced by preincubation. The reaction mixture was incubated respectively for 0, 1/2 and 1 hr in the presence of sodium benzoate and than provided with DOPA.

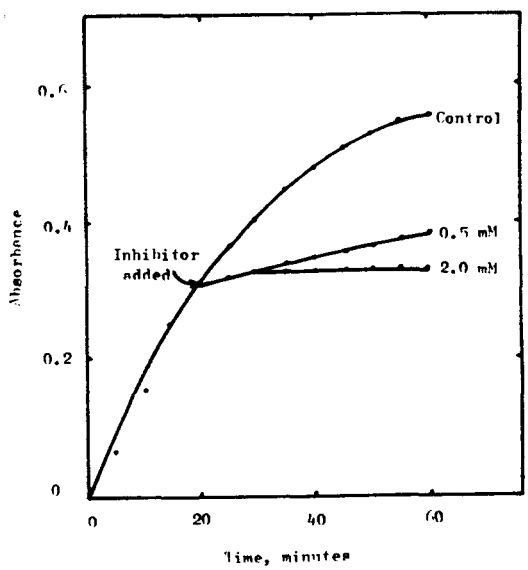

Fig.4 The pattern of dopachrome formation as influenced by 0.5 and 2.0*m*M sodium benzoate added after 20 minute reaction time. Concentration of DOPA was 0.5*m*M.

active site and thereby compete with DOPA for polyphenoloxidase. In practical consideration, it would be undesirable to treat sodium benzoate after the dopachrome formation poceeds.

Based on the results of this study, it is assumed that benzoic acid exerts great inhibiting action in

undissociated state and competes with DOPA for the enzyme because of its structural similarity with DOPA. The extent of inhibition might be varied since the chemical state of sodium benzoate is affected by various conditions studied in the present investigation.

요 약

Dopachrome형성에 미치는 sodium benzoate의 억제작용을 감자괴경조직을 마쇄하여 얻은 crude enzyme preparation을 사용하여 조사하였다. Dopachrome 형성의 억제정도는 sodium benzoate 농도를 증가시키고 기질(DOPA)의 농도를 감소시킴에 따라 증가하였다. pH 3-6 사이에서 억제정도는 매우 컸고 반면 pH 6 이상에서 dopachrome 형성은 급속히 증가하였다. 억제작용은 온도가 증가함에 따라 서서히 감소하였다. 본 실험결과에서 sodium benzoate는 기질인 DOPA와 효소에 대하여 경쟁적으로 작용한다고 시사되었다.

Reference

1. Reyes, P. and Luh, B.S.: *Food Technol.*, **14**, 570(1960)
2. Mayer, A.M. and Harel, E.: *Phytochemistry*, 18, 193(1979)
3. Mathew, A.G. and Parpia, H.A.B.: *Adv. in Food Research*, **19**, 75(1971)
4. Ikediobi, C.O. and Obasuyi, H.N.: *Phytochemistry*, **21**, 2815(1982)
5. Ben-Shalom, N., Kahn, V., Harel, E. and Mayer, A.M.: *Phytochemistry*, **16**, 1153(1977)
6. Eskin, N.A.M. and Henderson, H.M.: *Biochemistry of Foods*, Academic Press, New York and London, p.70(1971)
7. Bedrosian, K., Steinberg, M.P. and Nelson, A.I.: *Food Technol.*, **14**, 480(1960)
8. Kang, K.J., Cho, Y.O. and Kim, M.S.L.: *Am. Potato J.*, **60**, 451(1983)
9. Cho, Y.O. and Kim, M.S.L.: *Korean J. Nutr.*, **17**, 60(1984)
10. Igoe, R.S.: *Dictionary of Food Ingrdients*, Van Nostrand Reinhold Company Inc. p.119(1983)
11. Muneta, P.: *Am. Potato J.*, **54**, 387(1977)
12. Muneta, P. and Wang, H.: *Am. Potato J.*, **54**, 73(1977)
13. Pifferi, P.G., Baldassari, L. and Cultrera, R.: *J. Sci. Food Agric.*, **25**, 263(1974)
14. Heymann, H., Rogach, Z. and Mayer, R.L.: *J. Am. Chem. Soc.*, **76**, 6330(1954)
15. Duckworth, H.W. and Coleman, J.E.: *J. Biol Chem.*, **245**, 1613(1970)
16. De Jesus Rivas, N. and Whitaker, J.R.: *Plant Physiol.*, **52**, 501(1973)
17. Robb, D.A., Swain, T. and Mapson, L.W.: *Phytochemistry*, **5**, 665(1966)
18. Talburt, W.F. and Smith, O.: *Potato Processing*. Avi. Publishing Company Inc., Westport Connecticut, p.29(1967)
19. Furia, T.E.: *Handbook of Food Additives*, CRC Press, Ohio, p.120(1977)