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Effect of β-Carotene on Enzymatic Browning of Chlorogenic Acid and Tyrosine

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베타-카로틴이 클로로젠산과 타이로신의 효소적 갈변화에 미치는 영향 연구

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Abstract

생감자주스와 감자주스에 당근주스나 물을 혼합했을 때 갈변화 반응의 속도를 실험한 결과 감자주스는 15분 이내에 빨리 갈변화하였고 30분 후에는 까맣게 되었다. 그러나 당근 주스를 첨가한 것은 서서히 갈변화가 시작되었고 45분이 지나서야 갈색화가 진하게 되었다.

첨가한 당근주스의 양이 증가함에 따라 갈변화 속도는 비례적으로 감소하였다. 당근 주스의 갈변화 억제효과가 당근주스의 베타-카로틴 인지를 알아보기 위해 모델시스템에서 클로로젠산과 타이로신의 갈변화 속도에 베타-카로틴이 어떤 영향을 미치는지 실험한 결과 클로로젠산의 초기 갈변화 속도는 베타-카로틴을 첨가한 클로로젠산의 갈변화 속도보다 빨랐다. 두 속도 사이의 차이는 시간이 경과함에 따라 증가하였다. 당근에서 추출한 카로티노이드를 클로로젠산에 가했을때도 비슷한 결과를 보였다. 타이로신 단독으로와 베타-카로틴을 첨가한 타이로신의 갈변화 경향도 클로로젠산의 경우와 같은 결과를 보였다.

키워드 : 베타-카로틴, 클로로젠산, 타이로신, 효소적 갈변화.

INTRODUCTION

Enzymatic oxidation of phenolic compounds is a great concern in maintaining the quality of fruit and vegetable products. The brown color development is caused by the endogenous polyphenoloxidase and the polyphenol compounds. Black spot of potatoes has been recognized as one of the most important tuber defects and it is mainly caused by phenolic compounds. Amoung polyphenol compounds found in potatoes the most prevalent phenolics are chlorogenic acid and tyrosine(Mapson et al 1963).

Recently it has been reported that many naturally occurring polyphenol compounds found in plant foods commonly have beneficial effects such as the ability to act as antioxidants, to scavenge active oxygen species and electrophiles, to block nitrosation, and to chelate metals(Huang & Ferraro 1992). Some phenolic compounds are also known to prevent carotenoids oxidation(Oszmianski & Lee 1990). There have been many recent reports on the beneficial effects of β-carotene and other carotenoids in cancer (Peto et al 1981, Terao 1989, Tee 1992)

An old Korean folk remedy of raw carrot and potato juice mixture has been used to cure stomach ulcers. There has been no published scientific proof that any specific compounds found in carrots and potatoes are directly related to the anticancer activity. However, the carrot-potato juice mixture has been used for a long time and it is our interest to investigate the chemical interactions between carotenoids in carrots and the major polyphenol compounds in potatoes.

The purpose of this study was to find the function of carotenoids in carrots in the enzymatic oxidation of phenolic compounds in potatoes using a model system of β-carotene, chlorogenic acid, tyrosine, and carotenoids extracted from carrots.

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MATERIALS AND METHODS

1. Reagent Solutions

Stock solutions(50 mM) of each tyrosine and chlorogenic acid(from Sigma Chemical Co.) were prepared in phosphate buffer at pH 6.5 and diluted solutions were made before use. Mushroom tyrosinase was dissolved in phosphate buffer (0.5 mg/mL). β-Carotene(1% CWS, Roche Chemical Division of Hoffmann-La Roche Inc.) stock solution was prepared by dissolving 0.1 mg in 50 mL phosphate buffer and 0.3 mL of the stock solution was diluted to 50 mL before use.

2. Measurement of potato juice browning in the presence of carrot juice

Potato juice and carrot juice were prepared using a ACME Supreme Juicerator. Potato juice (50 mL) was immediately transferred to a 100 mL circular glass sample cell and exposed to a Hunetr Colorimeter Optical Sensor. The degree of browning was measured in "L" values. The sample was transferred to a beaker and mixed on a magnetic stir plate at room temperature and the "L" values were measured various time intervals. The difference in "L" values between the initial time and at a given time was calculated as "$\delta L"=L_0 - L_T$ for the browning rate. To observe the effect of carrot juice on potato juice browning, various amounts of fresh carrot juice(a H_2O for reference)was added to fresh potato juice and the browning rate was measured as above.

3. Extraction of carotenoids from carrots

A 300 g of diced carrots were ground in Waring blender with an equal volume of acetone for 3 min. and filtered with Whatman No. 42 filter paper under vacuum. This process was repeated at least 5 times until the carrot sample was colorless. The combined filtrates were extracted with an equal volume of hexane several times until colorless. The hexane extract was evaporated with a rotary evaporator to dryness. The dry extract was dissolved in mixed solvent of 1 mL chloroform and 40 mL Tween 80 and brought to the final volume of 50 mL with water.

4. Measurement of enzymatic of tyrosine and chlorogenic acid in the presence of β-carotene

For the model system, 500 μL of the enzyme solution was added to the mixture of 20 mL of 2 mM tyrosine(or chlorogenic acid) and 5 mL of buffer solution to measure the rate of browning as a control. To measure effect of β-carotene on browning reaction, 5 mM of buffer solution was replaced with 5 mL of β -carotene solution. For the carrot carotene system, 500 μL of the enzyme solution was added to the mixture of 20 mL of 2 mM chlorogenic acid and 5 mL of carrot extract. Oxidation of phenolic compounds was measured by color differences in "L" values at various time intervals using a Hunter Colorimeter Optical Sensor(model D 26L). The difference in "L" values between initial time and at given time was calculated as "$\delta L"=L_0 - L_T$.

RESULTS AND DISCUSSION

Fig. 1 shows the browning rate of potato juice alone and potato juice with added carrot juice or water(1:1 by volume). Potato juice quickly browned in less than 15 minutes and became darker after 30 minutes. The browning rate increased steadily up to 50~60 minutes and then progressed slowly. The browning rate of the potato juice and carrot juice mixture(1:1) was very slow and took more than 45 minutes to show a dark brown color. The browning rate of water diluted potato juice followed almost the same pattern of potato juice alone. The browning rate of potato juice with different amounts of carrot juice is shown in Table 1. As the amount of carrot juice added was increased, the browning rate proportionally decreased: from 1.86 of potato juice alone to 0.36 for the potato juice and carrot juice mixture(1:1). This inhibitory effect of carrot juice on browning led us to assume that β-carotene, the major constituent of carrot carotenoids, may be involved in impeding the browning reaction of potato juice. Therefore, model system of enzymatic browning, consisting of chlorogenic acid/tyrosine and β-caro-

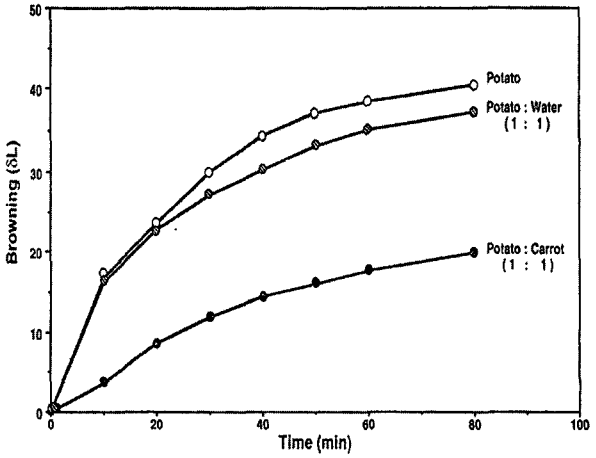

Fig. 1. Effect of carrot juice on enzymatic browning of potato juice.

Table 1. Initial browning reaction rate of potato juice added different amount of carrot juice

Potato juice+carrot juice	k=δL/min
Potato juice	1.86
Potato juice+6% carrot juice	1.00
Potato juice+15% carrot juice	0.78
Potato juice+25% carrot juice	0.70
Potato juice+35% carrot juice	0.43
Potato juice+50% carrot juice	0.36

tene/carrot carotenoids, were studied. Fig. 2 shows the browning rates of chlorogenic acid alone and chlorogenic acid with added β-carotene. The initial browning rate of chlorogenic lacid was faster(δL=1.1/min.) than that of chlorogenic acid with added β -carotene(δL=0.6/min.). It shows that the difference between two rates was increased as the time progressed. A similar result was obtained when the extracted carrot carotenoids was added to chlorogenic acid as shown in Fig. 3. The pattern of browning rate between tyrosine alone and tyrosine with added β-carotene was similar to that of chlorogenic acid(data not shown).

One of the proposed mechanisms of carotenoids' protection against oxidation in a biological system is the ability of carotenoids to deactivate reactive chemical species such as free oxygen(Krinsky 1989). Since molecular oxygen is one of the three components in the enzymatic browning reactions, any oxygen quenching activity would hinder the enzymatic browning reactions. It appears, therefore, that β-carotene in carrot juice acts just as an oxygen quencher and interferes the enzymatic browning of potato juice.

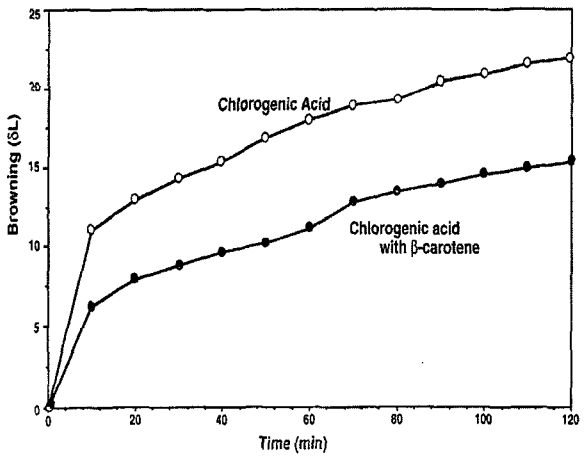

Fig. 2. Effect of β-carotene on enzymatic browning of chlorogenic acid.

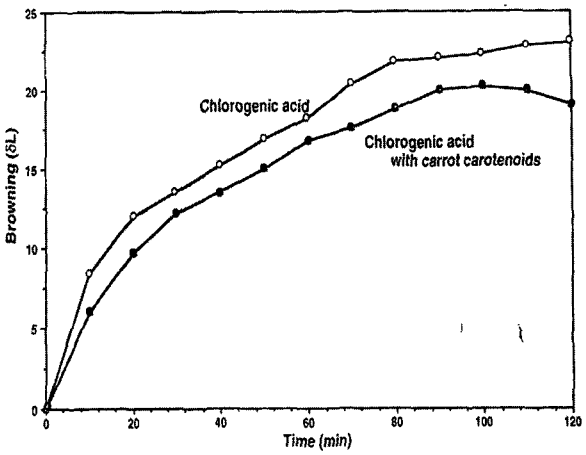

Fig. 3. Effect of carrot carotenoids on enzymatic browning of chlorogenic acid.

SUMMARY

The browning rate of potato juice alone and potato juice with added carrot juice or water(1:1 by volume) were studied. As the amount of carrot juice added was increased, the browning rate proportionally decreased: from 1.86 of potato juice alone to 0.36 for the potato juice and carrot juice mixture(1:1). This inhibitory effect of carrot juice on browning led us to assume that β -carotene, the major constituent of carrot carotenoids, may be involved in impeding the browning reaction of potato juice. Therefore, model system of enzymatic browning, consisting of chlorogenic acid/tyrosine and β-carotene/carrot carotenoids, were studied. The results shows the browning rates of chlorogenic acid alone and chlorogenic acid with added β-carotene. The initial browning rate of chlorogenic acid was faster(δL=1.1/ min.)than that of chlorogenic acid with added β-carotene(δ L=0.6/min.).

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