Difference of β-carotene Bleaching of Lipoxygenase Extracts from Soybean with Various Seed Lipoxygenase Isoenzymes

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Abstract: β-carotene bleaching activity of Lox extracts from soybeans containing various Lox isoenzyme combinations was investigated under different extraction and reaction conditions. Results indicated that the optimum extracting solution for Lox1, Lox2, Lox3, Lox1,2, and Lox1,2,3 was Tris-HCl buffer(pH 6.8), while for Lox1,3 and Lox2,3, was sodium phosphate buffer(pH 6.0). The reaction time needed for extracts to bleach β-carotene completely was in the order of Lox1,2,3, Lox3 > Lox2,3, Lox1,3 > Lox1,Lox2 > Lox1,2. At their corresponding optimum reaction temperatures, the bleaching levels of extracts was in the order of Lox1,3 > Lox1,2,3, Lox2,3 > Lox3 > Lox1,2, Lox1 > Lox2, while at their corresponding optimum pH values in the order of Lox1,3, Lox2,3 > Lox1,2,3 > Lox1,2 > Lox2 > Lox3 > Lox1. With the laying time prolonged, the bleaching activity of extracts tended to decrease. Lox3 was indispensable for higher efficiency of β-carotene bleaching. The extracts of the isoenzyme combinations containing Lox3 (Lox1,3, Lox2,3, and Lox1,2,3) were found to have stronger bleaching activity than those without Lox3 or of single isoenzyme.

Key words: *Glycine max* (L.) Merrill; Lipoxygenase isoenzymes; Lox extract; β-carotene; Bleaching

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具不同脂肪氧合酶同工酶的大豆种子提取液漂白 β-胡萝卜素的能力差异

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摘 要:大豆种子中含有 3 种脂肪氧合酶同工酶(Lox1、Lox2 和 Lox3)。以含有不同脂肪氧合酶同工酶大豆品系种子为材料,揭示不同提取和反应条件下,不同品系 Lox 提取液漂白 β-胡萝卜素的特性差异。结果表明:Lox1,Lox2,Lox3,Lox1,2 和 Lox1,2,3 的最适提取液是 Tris-HCl 缓冲液(pH 6.8),而 Lox1,3 和 Lox2,3 的最适提取液是磷酸缓冲液(pH 6.0)。完全漂白 β-胡萝卜素所需反应时间长短顺序是 Lox1,2,3,Lox3 > Lox2,3,Lox1,3 > Lox1,Lox2 > Lox1,2;在最适反应温度条件下,Lox 提取液的漂白能力大小顺序是 Lox1,3 > Lox1,2,3,Lox2,3 > Lox3 > Lox1,2,Lox1 > Lox2,而在最适 pH 条件下,Lox 提取液的漂白能力大小顺序是 Lox1,3,Lox2,3 > Lox1,2,3 > Lox1,2 > Lox2 > Lox3 > Lox1。随着 Lox 提取液的放置时间延长,其漂白能力下降。含有 Lox3 的品系提取液(也就是 Lox1,3,Lox2,3 和 Lox1,2,3 的提取液)比不含 Lox3 或仅含单一同工酶品系的提取液具有更好的漂白能力,说明 Lox3 是获得高效漂白能力必不可少的同工酶。

关键词:大豆;脂肪氧合酶同工酶;Lox 提取液;β-胡萝卜素;漂白

Lipoxygenases (linoleate: oxygen 13-oxidoreductase, EC 1.13.11.12, Lox) are a family of non-heme, iron containing dioxygenases, which catalyze the hydroperoxidation of polyunsaturated fatty acids containing *cis*, *cis*-1, 4-pentadiene structure into hydroperoxides that are considered to be bitter and grassy flavor precursors[1-4]. Soybean seeds contain three distinct Lox isoenzymes i.e., Lox1, Lox2 and Lox3, that were differ in molecular weight and behaviors such as optimum pH activity, substrate specificity and thermostability etc[5-9].

In 1928, Bohn and Haas found that additions of small amounts of soybean flour to wheat flour dough decreased the normal yellow color of wheat[10]. Subsequently, lipoxygenases were found to be involved in this color loss[11]. Since then, many food scientists

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and soybean breeders have been interested in the bleaching activity of soybean seed lipoxygenases on β-carotene or in the detection of Lox isoenzymes in soybean seeds using β-carotene[2,12-16]. The mechanism of bleaching β-carotene by soybean lipoxygenases is reported that non-heme iron Fe(II) in lipoxygenase (inactive enzyme) is oxidized to Fe(III) (active enzyme) by e-transfer, and the lipoxygenase with Fe (III) catalyses linoleic acid (LH) to form linoleiyl radical copolymer [Lox-Fe(II)-L*]. And β-carotene reacts with the intermediate linoleiyl radical copolymer [Lox-Fe(II)-L*] by hydrogen transfer to form β-carotene*, and consequentially will lose color and absorbance at 460 nm[16-20].

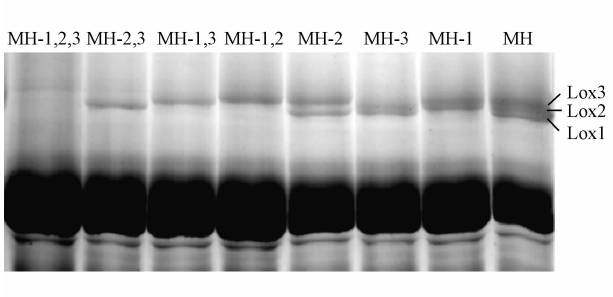
Differences of β-carotene bleaching activity have been found among the three soybean seed lipoxygenase isoenzymes. Lox2 and Lox3 were reported to be more effective at oxidation towards β-Carotene than Lox1[21]. Then Lox3 was farther found to have stronger activity towards the oxidation of both linoleic acid and β-carotene than do Lox1 or Lox2[14,22]. For the proper utilization of soybean Lox isoenzymes as a bleaching agent, further research is required to investigate the characteristics of β-carotene bleaching of three Lox isoenzymes and their combinations. Therefore, the objective of the present study was to evaluate the differences of β-carotene bleaching of the Lox extracts from soybean lines containing various seed Lox isoenzyme combinations under different extracting solutions, reaction times and temperatures, pH values of reaction buffer and laying time of extracting solutions.

1 Materials and methods

1.1 Materials and reagents

Eight soybean lines were used in this study. MH-1,2,3 was a line lacking all three types of seed Lox isoenzymes (Loxs null); MH-2,3, MH-1,3, and MH-1,2 were lines containing only one isoenzyme i.e., Lox1, Lox2, Lox3, respectively; MH-2, MH-3, and MH-1 were lines containing two isoenzymes i.e., Lox1 and Lox3 (Lox1,3), Lox1 and Lox2 (Lox1,2), Lox2 and Lox3 (Lox2,3), respectively. MH was a normal line containing Lox1, Lox2 and Lox3 (Lox1,2,3). All lines were planted in June, and harvested in November, 2007, in Jiangpu Agricultural Experiment Station, Nanjing Agricultural University, Nanjing, Jiangsu Province, P. R. China. Normal agronomic practices were followed. After harvested, all seeds of eight lines were stored at 4 ± 0.5℃. All lines were identified for the presence of seed Lox isoenzymes or null by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Liu et al.[23] with small modifications, and the electrophoretic pattern is shown in Fig. 1.

Linoleic acid (L-1376) and β-carotene (C-9750) were bought from Sigma Chemical Company (St. Louis, MO, America). All other chemicals were of the purest analytical grade, and purchased from Nanjing Chemical Industry (Nanjing, Jiangsu Province, PR China).



MH-1,2,3: containing none of Lox isoenzymes; MH-2,3, MH-1,3, MH-1,2: containing Lox1, Lox2, Lox3, respectively; MH-2, MH-3 and MH-1: containing Lox1 and Lox3 (Lox1,3), Lox1 and Lox2 (Lox1,2), Lox2 and Lox3 (Lox2,3), respectively; MH: containing Lox1, Lox2 and Lox3 (Lox1,2,3)

Fig. 1 Identification of the presence of Lox isoenzymes in soybean seeds by SDS-PAGE

1.2 Preparation of crude extracts of soybean seed Lox isoenzymes

The crude extracts of Lox isoenzymes from soybean seeds were prepared according to Kumar et al.[8] with small modifications. The seeds of soybean line MH, which contained Lox1,2,3, were ground in a Straub grinding mill (model 4E, Straub Company, Philadelphia, PA) to pass through a 100-mesh sieve. Ten mg of the soybean flour was extracted with 0.5 mL extracting solution by slowly stirring for 1 h at room temperature using a magnetic stirrer. The homogenized solution was centrifuged at 10 000 r · min^{-1} for 10 min at 4℃, and the supernatant so obtained was used as the crude extract of Lox1,2,3. The preparation procedure of the crude extracts of the other Lox isoenzymes was the same as that used in preparation of the crude extract of Lox1,2,3 with their corresponding lines.

1.3 Preparation of β-carotene saturated acetone solution

β-carotene saturated acetone solution was prepared according to Kikuchi and Kitamura[14]. 10 mg of β-carotene was dissolved in 10 mL of acetone. After being vigorously stirred several times and centrifuged, the orange-colored supernatant was used as the β-carotene saturated acetone solution. This solution should be prepared immediately prior to use.

1.4 Preparation of linoleic acid stock solution

The linoleic acid stock solution was prepared daily according to Serpen and Gökmen[16] with some modifications. Linoleic acid (310 μL) and Tween 20 (360 μL) were mixed in 10 mL of autoclaved distilled water, and was shaken vigorously for about 10 seconds. Then about 0.2 mL 1 mol · L^{-1} NaOH was added to increase the optical clarity. The final volume was adjusted to 100 mL with autoclaved distilled water to set the final concentration of linoleic acid stock solution to 10 mmol · L^{-1}, and then divided equably into vials. The oxygen was removed from the vials and replaced with nitrogen. The linoleic acid stock solutions were kept at -20°C until use.

1.5 β-carotene bleaching of Lox extracts under various extraction and reaction conditions

Five experiments were conducted to investigate the β-carotene bleaching activity of Lox extracts under various extraction and reaction conditions. Experiment I was designed to evaluate the effect of seven extracting solutions [distill water, Tris-HCl buffers (0.05 mol · L^{-1}, pH 8.0 and 6.8), sodium borate buffer (0.2 mol · L^{-1}, pH 9.0), sodium phosphate buffers (0.2 mol · L^{-1}, pH 6.8, 6.6, and 6.0)]; Experiment II investigated the reaction time of Lox extracts; Experiment III studied the effect of different reaction temperatures; Experiment IV studied the effect of different pH values of reaction buffer; Experiment V studied the effect of laying time of Lox extracts at room temperature.

Experiment I was conducted as follows: (i) 10 mg of the soybean flour was extracted with 0.5 mL extracting solution by slowly stirring for 1 h at room temperature using a magnetic stirrer, the homogenized solution was centrifuged at 10 000 r · min^{-1} for 10 min at 4℃, and the supernatant obtained was used as the Lox crude extract; (ii) 20 μL of Lox extract was added to 1.0 mL of 0.2 mol · L^{-1} sodium phosphate buffer (pH 6.8) containing 50 μL of 10 mmol · L^{-1} linoleic acid and 50 μL of carotene-saturated acetone solution; (iii) After incubation for 20 min at the room temperature, 1.0 mL of methanol was added to the reaction mixture to stop the bleaching reaction; (iv) β-carotene bleaching levels were measured in spectrophotometer at 452 nm.

Experiment II involved the same procedures as described in experiment I, except for the following modifications: (i) 10 mg of the soybean flour was extracted with 0.5 ml 0.05 mol · L^{-1} Tris-HCl buffers (6.8); after the procedure (ii), the absorbance of solution was immediately recorded at an interval of 40 seconds until 20 min.

Similarly, experiment III with the following modifications: (i) 10 mg of the soybean flour was extracted with 0.5 mL 0.05 mol · L^{-1} Tris-HCl buffers (6.8); (iii) the incubation was conducted at different temperatures (4, 15, 27, 50, and 80℃).

Experiment IV with the following modifications: (i) 10 mg of the soybean flour was extracted with 0.5 mL 0.05 mol · L^{-1} Tris-HCl buffers (6.8); (ii) 20 μL of Lox extract was added to 1.0 mL of 0.2 mol · L^{-1} buffer containing 50 μL of 10 mM linoleic acid and 50 μL of carotene-saturated acetone solution. The buffers used were sodium phosphate buffers (pH 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0 and 8.0) or sodium borate buffers (pH 8.8, 9.0 and 9.2).

Experiment V with the following modification: (i) 10 mg of the soybean flour was extracted with 0.5 mL 0.05 mol · L^{-1} Tris-HCl buffers (6.8); (ii) The Lox extracts were laid at room temperature for different times (0, 6, 12, 24, 48, 60, 72, 84, 96, 108 and 120 h), and then 20 μL of Lox extract was added to reaction buffer.

1.6 Determination of β-carotene bleaching of Lox extracts

To permit differentiation based on Lox isoenzyme type, rather than quantity, we evaluated the β-carotene bleaching of Lox extracts at the same protein concentration. The bleaching effect of Lox extracts on β-carotene was detected by spectrophotometric method as previously described[14] with small modifications. β-carotene bleaching levels were measured in a Spectramax Plus ELIASA (Molecular Devices Corporation, A-

merica) at 452 nm. The contrast wasn't added any Lox extract, therefore the absorbance was negative value in the present study. The bigger the negative value was, the stronger the bleaching activity of Lox extract was. All the reactions were conducted in the dark as β-carotene was unstable in the light.

1.7 Statistical analysis

Experiment dates, which were carried out in quintuplicate, were presented as mean ± standard deviations. Analysis of variance (ANOVA) and shortest significant range (SSR) test at the 5% level were used to evaluate differences among means (SAS 8.2, SAS Institute Inc., Cary, NC, America).

2 Results and discussion

2.1 Effect of extracting solutions on bleaching activity of Lox extracts

To extract Lox isoenzymes from the seeds of soybean lines, seven extracting solutions were used. The bleaching levels of the extracts were determined, and the results are shown in Table 1. The extracts prepared from MH-1,2,3 lacking all three Lox isoenzymes with seven extracting solutions showed no β-carotene bleaching activity. Moreover, the bleaching activity of the extracts prepared from soybeans with Lox1 and Lox3 with distilled water and Tris-HCl buffer (pH 8.0) were hardly detected. However, for each of soybean lines containing one, or two, or three Lox isoenzymes, significant differences ($P < 0.05$) in the β-carotene bleaching levels were found among the extracts prepared with different extracting solutions. The optimum extracting solution for Lox1, Lox2, Lox3, Lox1,2, and Lox1,2,3 was Tris-HCl buffer (pH 6.8), while for Lox1,3 and Lox2,3, was sodium phosphate buffer (pH 6.0). The second optimum extracting solution for Lox1,3 and Lox2,3 was Tris-HCl buffer (pH 6.8).

Table 1 Effect of different extracting solutions on β-carotene bleaching of soybean seed Lox isoenzymes (mean ± sd, $n = 5$)

	Lox null	Lox1	Lox2	Lox3	Lox1,2	Lox1,3	Lox2,3	Lox1,2,3
Distilled water	not detected	0 ± 0.0007^{a}	-0.0463 ± 0.0004^{b}	0 ± 0.0007^{a}	-0.0479 ± 0.0002^{b}	-0.0523 ± 0.0004^{a}	-0.0770 ± 0.0000^{a}	-0.0683 ± 0.0004^{b}
Tris-HCl buffer (pH 8.0)	not detected	0 ± 0.0021^{a}	-0.0269 ± 0.0023^{a}	0 ± 0.0011^{a}	-0.0340 ± 0.0010^{a}	-0.0854 ± 0.0002^{b}	-0.0995 ± 0.0035^{b}	-0.0638 ± 0.0011^{a}
Tris-HCl buffer (pH 6.8)	not detected	-0.0554 ± 0.0016^{e}	-0.1375 ± 0.0000^{g}	-0.0775 ± 0.0007^{e}	-0.1315 ± 0.0007^{g}	-0.1660 ± 0.0021^{f}	-0.1620 ± 0.0000^{d}	-0.1670 ± 0.0014^{g}
Sodium borate buffer (pH 9.0)	not detected	-0.0357 ± 0.0010^{c}	-0.0646 ± 0.0002^{c}	-0.0169 ± 0.0002^{b}	-0.1015 ± 0.0007^{f}	-0.1080 ± 0.0028^{c}	-0.1008 ± 0.0018^{b}	-0.1281 ± 0.0008^{e}
Sodium phosphate buffer (pH 6.8)	not detected	-0.0345 ± 0.0021^{c}	-0.0916 ± 0.0016^{e}	-0.0008 ± 0.0000^{a}	-0.0546 ± 0.0002^{c}	-0.1425 ± 0.0007^{d}	-0.1405 ± 0.0021^{c}	-0.0815 ± 0.0001^{c}
Sodium phosphate buffer (pH 6.6)	not detected	-0.0238 ± 0.0004^{b}	-0.0785 ± 0.0000^{d}	-0.0265 ± 0.0011^{c}	-0.0861 ± 0.0022^{d}	-0.1594 ± 0.0009^{e}	-0.1643 ± 0.0011^{d}	-0.1103 ± 0.0004^{d}
Sodium phosphate buffer (pH 6.0)	not detected	-0.0423 ± 0.0004^{d}	-0.1071 ± 0.0009^{f}	-0.0362 ± 0.0003^{d}	-0.0943 ± 0.0025^{e}	-0.1763 ± 0.0032^{g}	-0.1803 ± 0.0002^{e}	-0.1570 ± 0.0028^{f}

(a-g): The superscripts following each figure within the same column indicate significant differences at 0.05 level.

2.2 Reaction time of Lox extracts

Three soybean lipoxygenase isoenzymes are found to take different reaction time to catalyze linoleic acid[21,24]. Therefore, the effect of reaction time on β-carotene bleaching of Lox extracts were investigated, and the results are shown in Fig. 2. Similarly, no β-carotene bleaching activity was observed for the reaction mixture of the extracts prepared from MH-1,2,3 in a given reaction time (20 min). Whereas, seven extracts prepared from soybean lines with different Lox isoenzymes were found to take different reaction time to bleach β-carotene completely, and the order of the reaction time needed for them was in the order of Lox1,2,3, Lox3 > Lox2,3, Lox1,3 > Lox1, Lox2 > Lox1,2.

There was an interesting phenomenon that the extracts with Lox3 needed more reaction time to bleach β-carotene completely than those without Lox3. Some have reported that Lox3 takes more reaction time to bleaching β-carotene than do Lox2 and Lox1[22,23]. The reason for this may be that the action of Lox3 isoenzyme is different from those of Lox1 and Lox2 isoenzymes in the β-carotene bleaching mechanism, as suggested by Ramadoss et al[25].

2.3 Effect of reaction temperatures on bleaching activity of Lox extracts

Three soybean Lox isoenzymes may show diverse bleaching activities under different reaction temperatures, since their thermostabilities are different[5]. Therefore, in this study, β-carotene bleaching activity of Lox extracts under different reaction temperatures was investigated and the results are shown in Table 2.

No β-carotene bleaching activity was observed in the reaction mixture of the extracts prepared from MH-

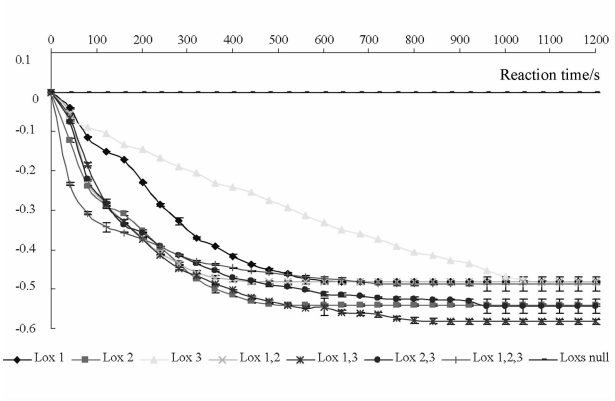


Fig. 2　The reaction time needed by Lox extracts to completely bleach β-carotene

1,2,3 under all the investigated temperatures. Whereas, significant differences ($P < 0.05$) in the β-carotene bleaching activity under different reaction temperatures were found among the extracts prepared from soybean lines with different Lox isoenzymes. The optimum reaction temperature for the extracts of both Lox1,3 and Lox1,2,3 were at 4℃, while, when the reaction temperature was raised, the bleaching activity of the extracts tended to decrease. A second optimum reaction temperature for the extracts of both Lox1,3 and Lox1,2,3 was observed at 27 ℃. The extracts of Lox2, Lox3 and Lox2,3 have the same optimum reaction temperature at 27℃, and when the reaction temperature was raised to more than 50℃, their bleaching activities decreased markedly, and even could not be detected. The optimum reaction temperatures for the extracts of Lox1,2 and Lox1 were 50 ℃ and 80 ℃, respectively. At their corresponding optimum reaction temperatures, the order of the bleaching levels of Lox extracts was in the order of Lox1,3 > Lox1,2,3, Lox2,3 > Lox3 > Lox1,2, Lox1 > Lox2. The extracts with Lox3 were found to have stronger bleaching activity than those without Lox3.

At higher reaction temperature (≥50℃), the extracts with Lox1 was observed being stronger to bleach β-carotene that those without Lox1. This should be related to that Lox1 isoenzyme is more thermostable than Lox2 and Lox3 isoenzymes[5].

Table 2　Effect of different reaction temperatures on β-carotene bleaching of soybean Lox extracts (mean ± sd, n = 5)

	4℃	15℃	27℃	50℃	80℃
Lox null	not detected	not detected	not detected	not detected	not detected
Lox1	-0.0452 ± 0.0005[a]	-0.0169 ± 0.0007[a]	-0.0900 ± 0.0010[a]	-0.0857 ± 0.0008[d]	-0.1055 ± 0.0018[d]
Lox2	-0.0550 ± 0.0006[b]	-0.0328 ± 0.0016[b]	-0.0921 ± 0.0010[a]	-0.0404 ± 0.0002[b]	not detected
Lox3	-0.1164 ± 0.0007[c]	-0.0690 ± 0.0027[c]	-0.1421 ± 0.0012[c]	-0.0185 ± 0.0011[a]	-0.0003 ± 0.0011[a]
Lox1,2	-0.0549 ± 0.0007[b]	-0.0306 ± 0.0004[b]	-0.0975 ± 0.0001[b]	-0.1061 ± 0.0009[e]	-0.0852 ± 0.0008[b]
Lox1,3	-0.2078 ± 0.0002[f]	-0.1145 ± 0.0012[f]	-0.1703 ± 0.0013[d]	-0.1141 ± 0.0002[g]	-0.0943 ± 0.0013[c]
Lox2,3	-0.1734 ± 0.0014[d]	-0.1106 ± 0.0004[d]	-0.1785 ± 0.0009[e]	-0.0587 ± 0.0003[c]	not detectable
Lox1,2,3	-0.1789 ± 0.0007[e]	-0.1310 ± 0.0012[e]	-0.1695 ± 0.0013[d]	-0.1119 ± 0.0009[f]	-0.1154 ± 0.0027[e]

(a-g): The superscripts following each figure within the same column indicate significant differences at 0.05 level.

2.4　Effect of pH value of reaction buffer on bleaching activity of Lox extracts

Three Lox isoenzymes from soybean seeds may show diverse bleaching activities on β-carotene in the reaction buffer of different pH values, since they have different optimum pH values[2,5]. Therefore, it is necessary to investigate the effect of pH values of reaction buffer on bleaching activity of Lox. Eleven reaction buffers with different pH values were chosen as reaction buffers in this study, and the results are shown in Fig. 3.

Under all the investigated pH values of reaction buffer, no β-carotene bleaching activity was observed in the reaction mixture of the extracts prepared from MH-1,2,3. The strongest β-carotene bleaching activity for extract of Lox1 was observed at pH 9.0, for extracts of Lox2 and Lox1,2,3 was at pH 6.8, and for the extracts of Lox3, Lox1,2, Lox1,3 and Lox2,3 was at pH 7.0. At their corresponding optimum pH values, the order of the bleaching levels of Lox extracts was in the order of Lox1,3, Lox2,3 > Lox1,2,3 >

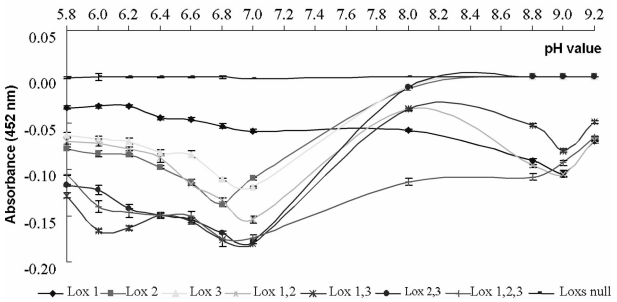


Fig. 3　Effect of pH of reaction buffer on β-carotene bleaching of Lox extracts

Lox1,2 > Lox2 > Lox3 > Lox1.

It is known that Lox1, Lox2 and Lox3 have the optimum isoenzymic activities at pH 9.0, 6.8, and 7.0, respectively[5]. The present result of Lox1, Lox2, and Lox3 is in agreement with those of Axelrod et al[5] and Robinson et al[2].

2.5 Effect of laying time of Lox extracts on bleaching activity

As we know, many enzymes are unstable and easily inactive. Therefore, it is necessary to study the stability of the bleaching activity of Lox extracts. The Lox extracts were laid at room temperature for different times (0, 6, 12, 24, 48, 60, 72, 84, 96, 108 and 120 h), and then the bleaching activity was determined. The results are shown in Fig. 4.

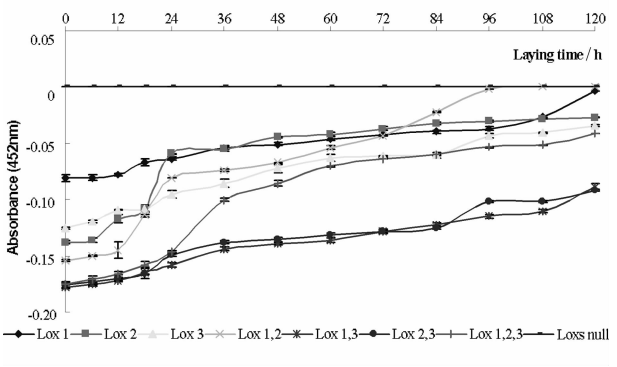


Fig. 4 Effect of laying time of Lox extracts on β-carotene bleaching

During the laying time of 120 h, the reaction mixture of extracts prepared from MH-1,2,3 showed no β-carotene bleaching activity at all. Whereas, with the laying time prolonged, the bleaching activity of seven Lox extracts prepared from soybeans with different Lox isoenzymes tended to decrease. For the extracts of Lox1,2, Lox2, and Lox1,2,3, a rapid decrease of bleaching activity was observed around 18 h or 24 h of laying time. The bleaching activity of the extracts of Lox1,2,3, Lox3, and Lox2 was lower than that of the extracts of Lox1,3 and Lox2,3 at 120 h, while the extracts of Lox1,2 and Lox1 showed no bleaching activity at 96 h and 120 h, respectively.

Ramadoss et al[25] has reported that the combination of either Lox1,3 or Lox 2,3 has a synergistic effect on β-carotene bleaching. In this study, we also found that at their corresponding optimum reaction temperatures or pH values of reaction buffer, the extracts of the isoenzyme combinations containing Lox3, namely, Lox1,3, Lox2,3 and Lox1,2,3, had stronger bleaching activity than those without Lox3 or of single isoenzyme, namely, Lox1,2, Lox3, Lox2, and Lox1. This indicated that Lox3 isoenzyme was indispensable for higher efficiency of β-carotene bleaching, suggesting that soybeans with Lox isoenzyme combinations containing Lox3 are maybe more useful as a bleaching agent in food processing.

3 Conclusions

The β-carotene bleaching activity of the Lox extracts prepared from soybean lines with different seed Lox isoenzymes was affected by type of extracting solution, reaction temperature and time, pH value of reaction buffer, and laying time of extract. Lox3 isoenzyme was indispensable for higher efficiency of β-carotene bleaching. Therefore, the extracts of the isoenzyme combinations containing Lox3 were found to have stronger bleaching activity than those without Lox3 or of single isoenzymes. This suggests that soybeans with Lox isoenzyme combinations containing Lox3 are maybe more useful as a bleaching agent in food processing.

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