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Lowering of Lipoxygenase Activity in Soy Milk Preparation by Propyl Gallate

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ABSTRACT

Lipoxygenase activity of soy milk was compared in the presence of different antioxidants. Various levels of antioxidant were used. Propyl gallate showed more inhibition than BHA, BHT and ascorbic acid. EDTA, citric acid and ascorbic acid combinations, added along with the propyl gallate. Addition of citric acid and ascorbic acid enhanced the inhibition of lipoxygenase by propyl gallate.

INTRODUCTION

Soy bean lipoxygenase has been studied for some time (Tappel *et al.*, 1952). Lipoxygenase catalyses the oxidation of linoleic and linolenic acids, esters and triglycerides (Eskin *et al.*, 1977) leading to the formation of several compounds like aldehydes, ketones and alcohols which are responsible for beany flavour in products like soy milk (Wilkens *et al.*, 1967; Mattick & Hand, 1969). The ability of phenolic antioxidants to inhibit lipoxygenase activity was studied in several model systems (Blain & Shearer, 1965; Rhee & Watts, 1966; Yasumoto *et al.*, 1970). Soy beans contain three lipoxygenase isozymes: L-1, L-2 and L-3, the first with optimum pH 6·8 and the others pH 9·0. Working with recessive mutant strains of soy beans which lacked L-1 activity Hildebrand & Kito (1984) showed that although all three are responsible for poor flavour of soy bean, L-1 was more active and difficult to

TABLE 1
Proximate Analysis of Golden Yellow Variety Soy beans

Fat	19·85%
Protein	42·1%
Ash	5·55%
Moisture	7·35%
Carbohydrate (By difference)	26·1%

inactivate by heat. Heat and homogenization were also studied for inactivation of L-1, L-2 and L-3 enzymes for preparing products like soy milk (Ediriweera *et al.*, 1987). The use of antioxidants in lipoxygenase inhibition in soy milk has not been extensively investigated. Some work has been done with NDGA (Blain & Shearer, 1965; Wilkens *et al.*, 1967). Lipoxygenase in broad beans has been shown to be inhibited by phenolic antioxidants and metal binding agents (Al-Obaidy & Siddiqui, 1981). The present work was done to explore the possibility that phenolic antioxidants, along with certain acidic compounds like EDTA, citric acid and ascorbic acid, might inhibit lipoxygenase in soy milk.

MATERIALS AND METHODS

Golden Yellow variety soy beans were used. The proximate analysis is shown in Table 1. Linoleic acid (99·0%) was obtained from Centron Research Laboratories, Bombay. Sodium phosphate, sodium borate, Tween 20, BHA, BHT, propyl gallate, citric acid, ascorbic acid and EDTA were all analytical reagent grade.

Soy milk was prepared by washing 50 g beans in water and then soaking them in 150 ml water for 18 h at room temperature (28 to 32°C). After draining and washing, the beans were ground with 400 ml water containing different antioxidants in a blender for 5 min. The slurry was filtered through a 4-fold muslin cloth to obtain soy milk. The control did not contain any antioxidants.

One millilitre of milk was diluted to 100 ml with water and this was used as the enzyme source. The reaction mixture contained 2 ml substrate and 0·1 ml enzyme source. Each sample was analysed in duplicate. The substrate was 1·23 mM linoleic acid prepared by emulsification with Tween 20 in sodium phosphate buffer (pH 6·8) and in sodium borate buffer (pH 9·0). This emulsion was prepared fresh before use and was stable for the duration of the test. After the reaction, the activity of lipoxygenase was measured at 234 nm using a B and L Spectronic 2000 spectrophotometer (Christopher *et al.*, 1972; Roza & Francke, 1973).

TABLE 2
Inhibition of Lipoxygenase L-1 (pH 9·0) Activity Using Different Antioxidants

Antioxidants	*Control*	*Absorbance at 234 nm*		
		Concentration (mg/litre)		
		320	*480*	*640*
BHT	0·019	0·022	0·034	0·026
		(116)	(179)	(137)
BHA	0·019	0·011	0·022	0·016
		(58)	(116)	(84)
Ascorbic acid	0·021	0·025	0·022	0·016
		(119)	(105)	(76)
Propyl gallate	0·027	0·019	0·016	0·010
		(70)	(59)	(37)

Figures in parentheses are values expressed as % of control.

RESULTS AND DISCUSSION

The effect of different antioxidants on lipoxygenase activity at pH 9·0 is shown in Table 2, and at pH 6·8 in Table 3. The activity at pH 9·0 is due to L-1 and that at pH 6·8 is due to L-2 and L-3 enzymes (Christopher *et al.*, 1970). Propyl gallate becomes increasingly effective as concentration is raised from 320 to 640 mg/litre. In the case of other antioxidants, increasing

TABLE 3
Inhibition of Lipoxygenases L-2 and L-3 Activity (pH 6·8) Using Different Antioxidants

Antioxidant	*Control*	*Absorbance at 234 nm*		
		Concentration (mg/litre)		
		320	*480*	*640*
BHT	0·025	0·044	0·058	0·074
		(176)	(232)	(296)
BHA	0·027	0·008	0·008	0·007
		(30)	(30)	(26)
Ascorbic acid	0·040	0·036	0·057	0·029
		(90)	(143)	(73)
Propyl gallate	0·038	0·037	0·021	0·008
		(97)	(55)	(21)

Figures in parentheses are values expressed as % of control.

TABLE 4
Inhibition of Lipoxygenase Activity Using Various Synergists with Propyl Gallate

	Absorbance at 234 nm			
	L-1 (pH 9·0)		*L-2 and L-3 (pH 6·8)*	
Control	0·019		0·045	
Propyl gallate (PG)	*a*	(37)	*b*	(21)
PG + EDTA	0·008	(42)	0·009	(20)
PG + EDTA + Ascorbic acid (AA)	0·007	(37)	0·009	(20)
PG + Citric acid (CA)	0·012	(63)	0·007	(15)
PG + CA + AA	0·004	(21)	0·010	(22)

PG = 640 mg/litre, EDTA = 160 mg/litre, AA = 320 mg/litre, CA = 160 mg/litre.
a = from Table 1; *b* = from Table 2.
Figures in parentheses are values expressed as % of control.

concentration does not improve inhibition. Of the antioxidants tested, propyl gallate shows the best inhibition.

Table 4 shows the effects of combinations of EDTA, ascorbic acid and citric acid together with propyl gallate. Although at pH 6·8 these compounds did not exhibit marked change in inhibition, the L-1 activity was markedly inhibited by ascorbic and citric acids together with propyl gallate. Even with L-2 and L-3 activity, inhibition was more when citric acid was added to propyl gallate.

Ascorbic and citric acids are known to have synergistic effects with phenolic antioxidants. They are common ingredients that may be added to flavoured soy milk. Propyl gallate, along with ascorbic and citric acids, can be used to control the activity of lipoxygenases in soy milk. When heat is used for inactivation of the enzyme the proteins undergo denaturation and the yield of soy milk is reduced. Hence, when cold extraction is applied, the flavour changes due to lipoxygenases can be minimized advantageously by phenolic antioxidants such as propyl gallate.

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