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Catalase and Superoxide Dismutase Enzymes Activities in Flaxseed (*Linum Usitatissimum*) Extract

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In this study, catalase (E.C.1.11.1.6) and superoxide dismutase (EC 1.15.1.1) activities were determined in the extract of flaxseed (*Linum usitatissimum*) with high antioxidant effect. Activities were determined after homogenization, ultracentrifugation and PD-10 (Sephadex G-25M) column chromotography steps. After the ultracentrifugation step, catalase and superoxide dismutase specific activities were found as 15.94 U/mg prot. and 2.05 U/mg prot., respectively. After desalting with PD-10 column chromotography, catalase and superoxide dismutase specific activities were found as 17.56 U/mg prot. and 4.90 U/mg prot., respectively.

Key Words: Flaxseed, Catalase, Superoxide Dismutase, Free radicals.

INTRODUCTION

Developing scientific technology has increased the awareness of the relationship between diet and disease and there has been a growing interest in functional foods and their role in the maintenance and improvement of health and vigour[1,2,3].

The human body is constantly exposed to free radicals and oxygen derived species from endogenous and exogenous sources. Exogenous sources include air pollutants, oxides of nitrogen, tobacco smoke, car exhausts, irradiatinon and the diet. Complex antioxidant defense mechanisms against the damage that free radicals cause have evolved, but these defenses are not completely efficient and the presence of free radicals can result in damage to DNA molecules, proteins and lipids[4,5]. Oxidative stress in the human body is proposed to be involved in the pathophysiology of many chronic human diseases[6]. The consumption of foodstuffs rich in antioxidants provides protection against cancer and cardio- and cerebrovascular diseases. This protection can be explained by the capacity of these active compounds to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids[7]. On the other hand, the oxidation of lipids has long been classified as the major deterioration process affecting both the sensory and the nutritional quality of foods. The principle route of deterioration and possible economic loss of vegetable oils is through rancidity resulting from oxidation which takes place at the double bond sites in the triacylglycerol molecules. The free radical chain process proceeds *via* initiation, propagation and termination steps. Antioxidant compounds are gaining

importance because of their dual role in the food industry as lipid stabilizers and in preventive medicine as suppressors of excessive oxidation that causes cancer and ageing. In addition there is evidence that these substances may exert their antioxidant effects within the human body[8,9]. The safety of synthetic antioxidants has been questioned and a trend toward the use of natural additives in foods has been apparent for quite some time as a result of consumer demand[10]. Recently, there has been a global trend toward using naturally present phytochemicals, such as those in fruits, vegetables, oilseeds and herbs, as antioxidants and functional foods. Recent researches have focused on isolation and characterization of effective phytochemicals and natural antioxidants from vegetable oils[11-15].

Plants possess enzymatic systems that protect them against H_2O_2 and other harmful active oxygen species. These include superoxide dismutase (SOD) and catalase (CAT). Superoxide dismutase converts superoxide radicals to hydrogen peroxide and CAT converts H_2O_2 to water and oxygen[16,17].

Flaxseed is obtained from *Linum usitatissimum* belonging to family linaceae, commonly known as linseed. Flaxseed is an important oilseed crop grown around the world for its oil and fiber. Flaxseed has been cunsumed as an ingredient in various food formulations and currently has a high demand in food industries[18-20]. Few studies have been made on flaxseed CAT and SOD although flaxseed has long been regard as a cure all. In the present study, the aim is to investigate activities of antioxidant enzymes (SOD, CAT) in flaxseed extract.

EXPERIMENTAL

All reagents were of the highest purity available and were obtained from BDH, Merck or Sigma Chemical Company. Flaxseed (*Linum usitatissimum*) were obtained from Cukurova University, Agricultural Faculty.

Antioxidant enzymes extraction and assay: The extraction method has been described earlier by Malgorzata[17]. Flaxseeds were homogenized with 0.2 g hydrated PVP (insoluble polyvinylpyrrolidone) in 10 mL of 0.1 M phosphate buffer (pH 7.8) suplemented with 2 mM dithiothreitol, 0.1 mM EDTA and 1.25 mM PEG-4000 and centrifuged (16000 g for 15 min). The resulting supernatant was filtered through Miracloth, desalted on a PD10 column and used for enzymes assays. All steps of the extraction procedure were carried out at 1-4 °C.

Assay of superoxide dismutase activity: Superoxide dismutase activity was measured according to the method of Sun *et al.*[21]. Superoxide dismutase activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT.

Assay of catalase activity: Catalase activity was measured according to the Lartillot *et al.*[22] which is a modification of the method described by Aebi[23]. CAT activity was measured spectrophotometrically at 240 nm using a specific absorption coefficient of 0.0392 cm^2 μmol $H_2O_2^{-1}$.

The protein content in enzymatic extracts was determined by the method of Lowry *et al*.[24] by using bovine serum albumin as protein standard. All tests were repeated at least three times and all samples were analyzed 4 times. The results presented are the mean ± SD.

RESULTS AND DISCUSSION

Results are expressed in Tables 1 and 2. Homogenate was centrifuged and the supernatant was filtred through miracloth. Protein content, CAT activity and SOD activity in the supernatant were found 4.30 mg/mL, 15.94-2.05 U/mg prot., respectively. The supernatant was desalted on a PD10 column. Fractionated samples were collected. Protein content and enzyme activities for each fraction were determined and the enzyme containing fractions were pooled. Protein content, CAT and SOD activities in the pooled fraction were found 1.36 mg/mL, 17.56-4.90 U/mg prot., respectively (Tables 1 and 2).

TABLE-1
EXTRACTION OF FLAXSEED CATALASE

	V_T (mL)	$C_{protein}$ (mg/mL)	Total Protein (mg)	Activity (U/mL)	A_T (U) (V_Tx U/mL)	Specific activity (U/mg)
Ultracentrifuge	9, 40	4, 30	40, 42	68, 55	644, 37	15, 94
Pooled fractions	2, 75	1, 36	3, 74	23, 88	65, 67	17, 56

TABLE-2
EXTRACTION OF FLAXSEED SUPEROXIDE DISMUTASE

	V_T (mL)	$C_{protein}$ (mg/mL)	Total Protein (mg)	Activity (U/mL)	A_T (U) (V_Tx U/mL)	Specific activity (U/mg)
Ultracentrifuge	9, 40	4, 30	40, 42	8, 80	82, 72	2, 05
Pooled fractions	2, 75	1, 36	3, 74	6, 67	18, 34	4, 90

Environmental consciousness and cost factors have led to extraction of natural antioxidants from easily renewable sources, including plant waste materials. Extracts from plants, including waste materials such as old tea leaves[25] have shown antioxidant activity. Certain seeds have also shown to possess antioxidant activity: *e.g*., cowpea seeds[26] and rice seeds[27]. Other examples are peanut hulls, which showed considerable antioxidant activity in soybean and peanut oils[28].

There is currently a great deal of interest in promoting increased intakes of flaxseed (*Linum usitatissimum*) in the diet for its potential health benefits, specifically anticarcinogenic[29] and antiatherogenic effects[30] linked to its lignan and n-3 fatty acid contents. Flaxseed represents an excellent source of dietary fibre and α-linolenic acid (C18:3, n-3; approx. 55 % of total fatty acids). Previous reports have demonstrated that dietary flaxseed was protective against chemically induced mammary carcinogenesis[29] and early markers of colon carcinogenesis[31,32]. The high levels of C18:3, n-3 in flaxseed and its oil are thought to play a role in reducing low density

lipoprotein (LDL) cholesterol as a risk factor of cardiovascular disease[33]. One of the hypothesized mechanisms underlying these chronic diseases is the role of oxidized species and *in vivo* protection from enzymatic and non-enzymatic antioxidant processes.

Stajner *et al*.[34] determined activities of antioxidant enzymes (SOD and CAT) of bulb, leaf and stalk of *Allium ursinum*. They reported that the extracts from all plant organs exhibited antioxidant activity, the highest having been observed the leaves. Malgarzato *et al*.[17] investigated changes of activity antioxidant enzymes (CAT and SOD) in the roots and hypocotyls of soybean seedlings, submitted to cold. They reported that an increase in CAT and SOD activity in hypocotyls was observed both at 1 °C and after transfer of plants to 25 °C. In roots, CAT activity increased after 4 days of chilling, while SOD activity only after rewarming. Ning *et al*.[35] purificated SOD from garlic. Their study provided the most basic evidence for the structure and properties of garlic SOD.

This is the first study in the literature, on the antioxidant enzyme activities (SOD and CAT) in extract of flaxseed. The results indicated that flaxseed has antioxidant enzyme activities (CAT and SOD). This study provided the basic evidence for the existing of SOD and CAT in flaxseed. Their physiological function will be most probably revealed only if the protein is biochemically analyzed further in detail.

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