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BRIEF COMMUNICATION

Activities of antioxidant enzymes during strawberry fruit development and ripening

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Abstract

The analyses of some antioxidant enzyme activities were carried out in the course of strawberry fruits development and ripening. The catalase activity was maximum in small-sized green fruits, it decreased in middle-sized green fruits and increased again during the ripening stages. The highest superoxide dismutase and peroxidase activities were observed in white fruits.

Additional key words: catalase, peroxidase, reactive oxygen species, superoxide dismutase.

Reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide, and hydroxyl radical are produced as a result of many biochemical reactions and they are considered to be the main cause of oxidative damage (*e.g.* Jimenez *et al*. 2003). Susceptibility of plants to oxidative stress may depend on the balance between factors that increase ROS generation and those cellular components that exhibit an antioxidant capability (Foyer *et al*. 1994). Activities of various antioxidant enzymes are known to increase in defence responses (Anand *et al*. 2009) and in response to drought (Sairam and Saxena 2000, Sairam and Srivastava 2001), high temperature (Sairam *et al*. 2000) and salinity (Hernandez *et al*. 2000). Reactions involving ROS are an intrinsic feature of the senescence and fruit ripening (Jimenez *et al*. 2003). Since ROS levels and their harmful products are known to increase during senescence in many plants, it is possible that these changes are due to a decline in the activity of certain antioxidant enzymes (Procházková and Wilhelmová 2007). Increased levels of ROS have been reported during banana, pear, pepper and tomato ripening (Thompson *et al*. 1987, Rogiers *et al*. 1998) as well as during pea leaves senescence (Pastori *et al*. 1997). There must be a balance between the production of ROS and their removal by antioxidant systems. Fruit ripening is considered by some authors to be a specialized form of senescence (Seymour *et al*. 1993). The antioxidant system, which play an important role in both senescence and fruit ripening, includes enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and peroxidase (POX; EC 1.11.1.7).

Attractive characteristics of the strawberry (*Fragaria* × *ananassa*) fruit are not only aroma, taste, colour and texture, but also its chemical composition (contents of minerals, vitamins and antioxidants) (Aharoni *et al*. 2002). Strawberries contain many compounds with antioxidant activity such as phenolic acids, flavonoids and anthocyanins (Olsson *et al*. 2004). There are a lot of studies on antioxidant capacity and phenolic content in strawberry but there is little information about the activities of significant antioxidant enzymes in strawberry fruits at different maturation stages. So, the aim of the present research has been to study the activities of main antioxidant enzymes (SOD, POX and CAT) along the development and ripening of strawberry fruits.

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Abbreviations: CAT - catalase; NBT - nitroblue tetrazolium; OPD - *o*-phenylenediamine; POX - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
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Strawberry (*Fragaria* × *ananassa* Duch. cv. Chandler, an octaploid cultivar) plants were grown in controlled fields under natural conditions. Extreme caution was taken to avoid collecting fruits with signals of fungi or bacterial infestation. Fruits were harvested and immediately frozen in liquid nitrogen and kept at -80 ºC until use. According to the various stages of development and ripening the fruits were classified into six types: small-sized green fruits (G1, the fruit is in the first stages of growth), middle-sized green fruits (G2, the fruit continues growing and is more elongated), full-sized green fruits (G3, the fruit has reached the definitive size), white fruits (W), turning-stage fruits (T) and full-ripe red fruits (R).

Strawberry fruits (0.2 g) at different ripening stages were ground under liquid nitrogen and immediately, the fine powder was homogenized in a buffer containing 0.05 M potassium phosphate buffer (pH 7.0), 1 mM EDTA-Na_2 and 0.05 % (v/v) *Triton X-100*. The homogenate was centrifuged at 12 000 *g*. After that, the supernatant was filtered through a miracloth membrane. Catalase (CAT) activity was determined by following the absorbance change at 240 nm due to consumption of H_2O_2 in a reaction mixture containing 0.05 M phosphate buffer (pH 7.0) and 30 mM H_2O_2 (Aebi 1983). Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), according to the method of Beauchamp and Fridovich (1971). The reaction mixture contained 2 μM riboflavin, 75 μM NBT, 0.1 mM EDTA-Na_2, 0.05 M potassium phosphate buffer and homogenate from strawberry fruits at different stages of ripening. The reaction was started switching on a lamp and kept for 10 min. Subsequently, the absorbance change at 560 nm was measured. One unit of SOD activity was defined as the amount of enzyme required for 50 % inhibition of the rate of NBT reduction measured at 560 nm. Peroxidase (POX) activity was determined in a reaction medium containing 10 mM *o*-phenylenediamine (OPD), 30 mM H_2O_2 and homogenate from strawberry fruits. The increase in absorbance was monitored at 490 nm. One unit of CAT or POX activity was defined as the amount of enzyme that converts 1 μmol of substrate to product in 1 min. Measurements of all antioxidant activities were carried out using a *Beckman DU 7500* spectrophotometer at 25 °C and were done by triplicate. Protein was determined according to Bradford (1976) with bovine serum albumin as a standard. For evaluation of significance of differences the Student *t*-test was applied using the *SPSS* program for statistical analysis.

We have measured the activities of three significant antioxidant enzymes at different stages of the development and ripening of strawberry fruits. The maximum CAT activity, expressed on a dry mass basis, was found in G1 fruits, CAT activity decreased in G2 and increased during the ripening stages until reaching in red fruits similar values to G1 fruits. However, the maximum CAT activity expressed on a protein basis was observed in white and red fruits (Fig. 1*A*). So in agreement with Aharoni and O´Connell (2002), CAT is one of the antioxidant enzymes whose activity increased during ripening of strawberry fruits.

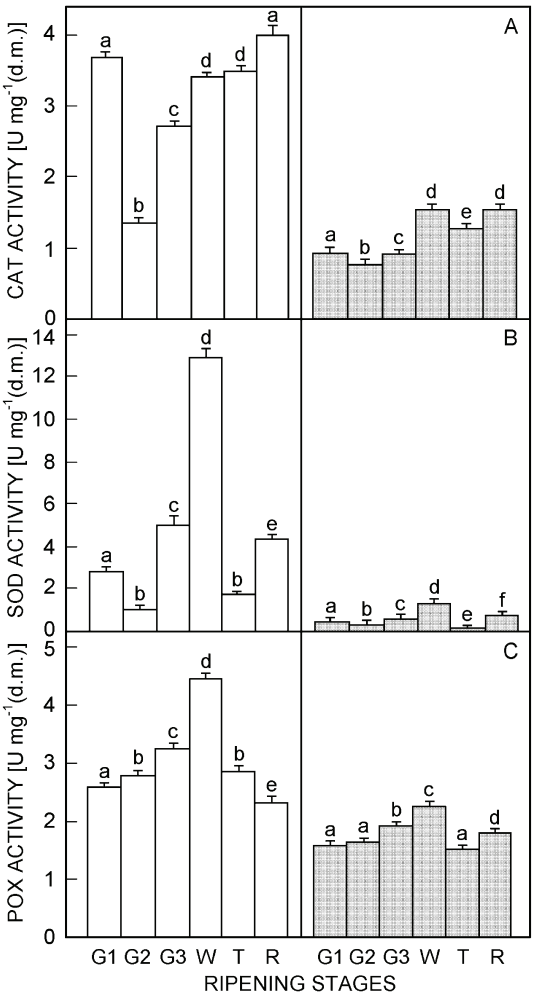

Fig. 1. CAT (*A*), SOD (*B*) and POX (*C*) activities in strawberry fruits at different stages of the development and ripening: small-sized green fruits (G1), middle-sized green fruits (G2), full-sized green fruits (G3), white fruits (W), turning-stage fruits (T), and full-ripe red fruits (R). Different letters indicate that the differences in activity were statistically significant ($P < 0.05$). *On the right* specific activities [U mg^{-1}(protein)] are presented.

The SOD activities (Fig. 1*B*), expressed on a d.m. and protein bases, increased during maturation stages up to the highest values in white fruits. However, in contrast to CAT activity, it decreased in turning stage and a slightly increased in red fruits. It has been demonstrated that growth and early fruit development of strawberry were stimulated by auxin (Nitsch 1950). Later during fruit development (W stages), auxin content declined and this invoked the ripening process (Given *et al*. 1998). We can

expect that the highest SOD activity occurred in white fruits because of dramatic changes in fruit pigmentation and texture (Manning 1994, 1998), which could be accompanied by oxidative stress.

POX activities expressed on a dry mass and protein bases (Fig. 1*C*), were similar to those obtained by Civello (1995). The white fruits had the highest activity and the minimum values were in red fruits (calculated on a d.m. basis) and in turning stage (calculated on a protein basis).

Stress may arise in the fruits during ripening as a result of changes in osmotic potential due to the accumulation and the storage of osmotically active compounds (*e.g*. hexoses), or from abiotic or biotic factors (Aharoni *et al*. 2002a). Another source of ROS production might be electron flow in mitochondria (Leprince *et al*. 2000). Further, it was proposed that the lignification of the vascular system is coupled to fruit maturation (Aharoni *et al*. 2002) and peroxidases are involved in the biosynthesis of lignine (Önnerud *et al*. 2002). Moreover, a basic peroxidase isozyme was located in the concentric array of the vascular bundles and in the vascular connections with the achenes in strawberry (Lopez-Serrano *et al*. 2001). Peroxidases have been suggested to be involved also in auxin catabolism (Normanly *et al*. 1995) and as was mentioned above, in this stage auxin content declined. These facts could explain why the highest POX activity was found in white strawberry fruits.

As shown by Aharoni *et al*. (2002a), there is an association between ripening-related gene expression and oxidative stress response in strawberry. Our results can support this idea and it is proposed that antioxidant enzymes such as CAT, POX and SOD could play an important role in the regulation of ripening processes.

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