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Inhibitory effects of propyl gallate on membrane lipids metabolism and its relation to increasing storability of harvested longan fruit

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ABSTRACT

Effects of propyl gallate on membrane lipids metabolism and its relation to storability of harvested longan fruits were studied. The results showed that the propyl gallate-treated longans maintained lower activities of pericarp phospholipase D (PLD), lipase and lipoxygenase (LOX) than those in control fruits. Such treatments could maintain higher levels of pericarp unsaturated fatty acids (USFAs), higher pericarp indices of unsaturated fatty acids (IUFA), and higher pericarp ratio of unsaturated fatty acids to saturated fatty acids (U/S) than those in control fruits. Furthermore, propyl gallate also delayed color changes of pericarp in the harvested longans. Therefore, the postharvest treatments of longan fruits with propyl gallate for increasing storability of longan fruits might be explained by a decrease in activities of PLD, lipase and LOX, and an the increased unsaturation of fatty acids, which could delay membrane lipids metabolism and maintain cell membrane characteristics.

1. Introduction

Longan (*Dimocarpus longan* Lour.) fruit is consumed globally and has a high commercial value. China is the primary producer of longan fruit (Apai, 2010; Jiang & Li, 2001; Jiang, Zhang, Joyce, & Ketsa, 2002; Lin, Chen, & Lin, 2010; Lin et al., 2014; Su et al., 2005). However, longan fruit has a short shelf-life with aril breakdown and pericarp browning, which can be attributed to the fruit maturing in summer with high temperatures and high humidity (Lin, Lin, Chen, Chen, & Lin, 2013; Lin, Hu et al., 2013; Lin, Lin, Chen et al., 2016). Pericarp browning is the major factor limiting for storage and marketing of harvested longan fruit (Duan et al., 2007; Duan, Zhang et al., 2011; Holcroft, Lin, & Ketsa, 2005; Lin et al., 2015).

Recently, more attention has been given to changes in cell membrane properties in this respect (Rui et al., 2010; Saquet, Streif, & Bangerth, 2003). Cell membrane degradation is highly correlated with membrane lipids metabolism (Lin et al., 2016), and the process includes the hydrolysis of membrane phospholipids to free fatty acid, peroxidation of fatty acid in cell membrane, and generation of hydroperoxide or other reactive oxygen species (ROS) (Liu et al., 2011). Excessive ROS generation also damages the cell membrane, which promotes browning (Lin et al., 2016). Previously, it has been reported that internal browning of loquats involved the loss of membrane integrity, increased in activities of phospholipase D (PLD) and lipoxygenase (LOX), higher levels of palmitic and stearic acids, belong to saturated fatty acids (SFAs), lower levels of inoleic and linolenic acids, belong to unsaturated fatty acids (USFAs), and a lower USFAs to SFAs (U:S) ratio. However, the heat-treatment of loquat fruit was associated with less internal browning, lower activities of PLD and LOX, and reduced SFAs content as well as higher concentrations of USFAs and a higher U:S ratio (Rui et al., 2010). Moreover, Lin et al. (2014) suggested that hydrogen peroxide, as an exogenous reactive oxygen, could promote the browning development of longan pericarp by reducing endogenous scavenging capacity. In addition, hydrogen peroxide could enhance LOX activity, reduce the levels of USFAs, U:S and IUFA (Lin et al., 2016). In contrast, propyl gallate has been reported to be associated with increased endogenous antioxidant activities and increased ROS scavenging capacity. These functions reduce the impact of ROS production and peroxidation of fatty acid in cell membranes and, accordingly, inhibited pericarp browning retaining the commercial value of the crop (Lin, Lin et al., 2013; Lin et al., 2015).

Currently, no studies have been published on the effect of propyl gallate on membrane lipids metabolism in longan fruit in relation to retardation of pericarp browning and maintenance of fruit

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commercial value. Therefore, to advance knowledge of this mechanism, this study investigated changes in pericarp color, activities of PLD, lipase and LOX, and fatty acid composition in cell membrane of harvested longan fruit.

2. Materials and methods

2.1. Plant material and treatments

Mature longan (*Dimocarpus longan* Lour. cv. Fuyan) fruit were harvested and treated as described in our previous studies (Lin, Hu et al., 2013; Lin et al., 2015).

2.2. Pericarp color measurement

The method described by Zhang et al. (2015) was used to measure chromaticity L^*, a^*, b^* values of longan pericarp color.

2.3. Assay of PLD, lipase and LOX activities

The methods of Liu et al. (2011) and Lin et al. (2016) were used to determine PLD, lipase and LOX activities. Protein content was determined according to the method of Bradford (1976).

2.4. Determination of membrane fatty acid composition

The method described by Lin et al. (2016) was used for extraction and determination of lipid as well as the analysis of composition and relative content of membrane fatty acids (FAs) by gas chromatograph (Model 7890A, Agilent Technologies Co. Ltd., USA). IUFA and U/S were calculated according to Lin et al. (2016)

2.5. Statistical analyses

All experiments were repeated in triplicate. Each value in figures are presented as the mean ± standard error (n = 3). Analytic variance was tested by SPSS version 17.0.

3. Results and discussion

3.1. Propyl gallate - delayed changes in chromaticity values

The loss of pericarp color, characterized by increasing pericarp browning, is the key factor accounting for reduced apparent quality and commercial value of longans (Chen et al., 2015). The objective measurement of color using chromaticity L^*, a^* and b^* system was used to represent the change in apparent color. L^* represents lightness, and positive a^* and b^* indicate red and yellow, respectively (Venkatachalam & Meenune, 2012; Zhang et al., 2015). Previously, fruit color has been measured at 13, 14, 15 and 16 weeks after anthesis and the result showed increased browning, as evidenced by lower values for L^* and b^* (Venkatachalam & Meenune, 2012). The loss of red color in litchi fruit coincided with decreased L^*, a^* and b^* (Zhang et al., 2015). Apple polyphenols could postpone the loss of red color and pericarp browning by maintaining higher values of L^*, a^* and b^* (Zhang et al., 2015).

In the present study, L^*, a^* and b^* in control fruit gradually decreased during storage (Fig. 1), indicating the gradually declined lightness of longans. The results were accordance with our previous works, in which pericarp browning index increased and commercial value declined during storage (Lin, Lin et al., 2013; Lin et al., 2015). Pericarp browning index was inversely associated with L^*, a^*, b^* values (correlation coefficient $r = -0.9809$, -0.9879, -0.9813, respectively, $p < 0.05$), while commercial value was strongly positive correlated with chromaticity L^*, a^*, b^* values ($r = 0.9906$, 0.9460, 0.9686, respectively, $p < 0.05$).

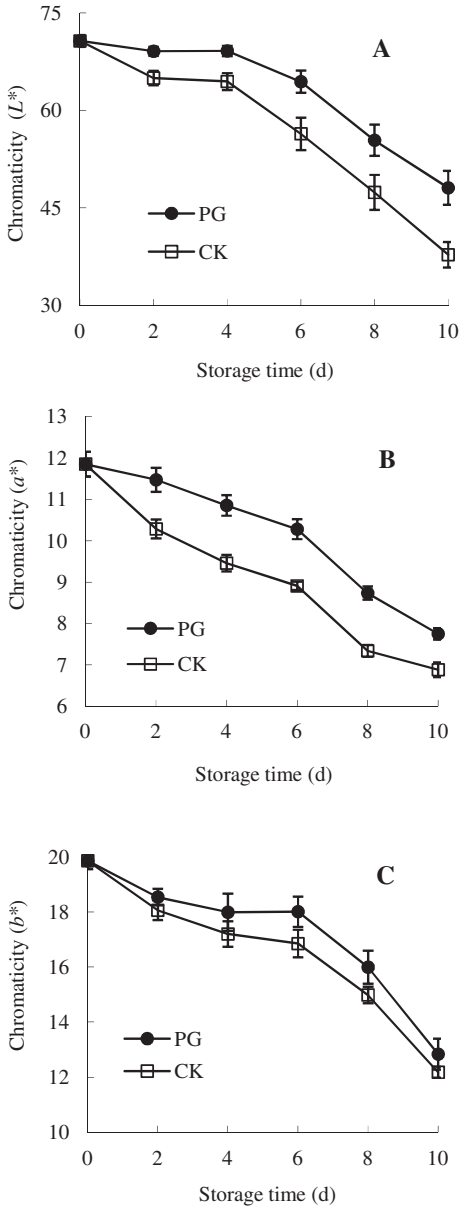

Fig. 1. Changes in chromaticity values of L^* (A), a^* (B) and b^* (C) in pericarp of harvested longan fruit.

Values for L^*, a^* and b^* reduced slowly in the propyl gallate-treated fruits. They were significantly ($p < 0.05$) higher than corresponding values in control fruits. These results are consistent with suppressed of pericarp browning and higher commercial value of longans treated by the propyl gallate (Lin et al., 2013). These results above demonstrated that propyl gallate exerted a strong effect on the lightness of apparent color suggestion inhibition of pericarp browning, and, thus, a higher rate of commercially acceptable fruit. Inhibition of browning through the application of propyl gallate on cold-stored banana fruits has also been reported (Jiang, Chen, Lin, & Chen, 1991).

3.2. Propyl gallate – inhibited changes in activities of PLD, lipase and LOX

Browning occurrence or decreased storability are the basic characteristics of senescence in harvested fruits such as peaches (Jin, Zhu, Wang, Shan, & Zheng, 2014), loquats (Cao, Yang, Cai, &

Zheng, 2011), longans (Apai, 2010; Chen et al., 2014; Lin et al., 2014, 2015; Su et al., 2005) and lychees (Duan et al., 2004; Duan, Liu et al., 2011; Yang et al., 2009). There are many reports of senescence of plant tissue being closely related to the deterioration of cell membrane (Shewfelt & del Rosario, 2000; Lin et al., 2016). Cell membranes are mainly composed of lipid, protein and sugar, the contents of which is about 50%, 40% and 2–10%, respectively (Meyer & Terry, 2010). Moreover, phospholipids like phosphoglyceride, are form the basis of lipids in cell membrane. PLD, lipase and LOX play key roles in membrane lipid metabolism (Liu et al., 2011; Lin et al., 2016). PLD can hydrolyze the ester bond of phosphoglyceride and is thought to be the first one to initiate the phospholipid catabolism. To a certain extent, the degree of phospholipids catabolism is determined by PLD activity, because lipase and LOX cannot break down phospholipids directly (Yi et al., 2009). Lipase can esterify diacylglycerol to free fatty acids (Liu et al., 2011). USFAs can be degraded by LOX. Furthermore, these processes can lead to the peroxidation of membrane lipid fatty acid and the generation of ROS, which in turn contribute to membrane ion leakage and further damage to the membrane (Cao et al., 2011; Meyer & Terry, 2010; Sun, Liang, Xie, Lei, & Mo, 2010; Yi et al., 2010; Zhang & Tian, 2010).

A previous study reported litchi pericarp browning as well as increased activities of PLD, lipase and LOX at ambient temperature (25 °C) with time (Liu et al., 2011). Furthermore, hydrogen peroxide can aggravate pericarp browning of 'Fuyan' longans via enhanced superoxide anion (O_2^-) production rate, LOX activity, and membrane ion leakage (Lin et al., 2014, 2016). In contrast, application of chlorine dioxide lowed ROS levels and LOX activity as well as membrane ion leakage, and alleviate pericarp browning of harvested 'Daw' longan fruit (Chomkitichai, Chumyam, Rachtanapun, Uthaibutra, & Saengnil, 2014).

Activities of PLD, lipase and LOX exhibited different tendencies during storage (Fig. 2). PLD activity increased rapidly during the first two days, changed only slightly from the second day to sixth day, and then increased sharply during the last four days of storage (Fig. 2A). Lipase activity increased during the first four days and then decreased (Fig. 2B). LOX activity increased slightly during the first six days but increased sharply from the 6th to 8th day (Fig. 2C). These results indicate that, in the process of cell membrane lipid deterioration, before the first two days, PLD is the main enzyme to degrade phospholipids to phosphatidyl choline and diphosphatidyl glycerol during storage. In addition, lipase has a leading role during the first four days of storage, which could degrade diacylglycerol, the degradation product of phospholipids hydrolyzed by PLD. Moreover, days six to eight, LOX had a key role in degrading USFAs, a partial product of diacylglycerol degradation. However, the specific phospholipid components of cell membrane and their degradation composition, in the process of membrane lipid metabolism need further elaboration.

Our previous work showed that propyl gallate could reduce cell membrane permeability and browning index of longans (Lin et al., 2013, 2015). We also determined that propyl gallate could decrease ROS levels, reduce peroxidation of cell membrane lipid, and maintain cell membrane integrity and, consequently, postpone the browning (2013a, 2015). In the present work, significantly lower activities of PLD, lipase and LOX were found in the propyl gallate-treated longan fruit compared with control fruit (Fig. 2), suggesting inhibition of pericarp browning in propyl gallate-treated longans might be due to reduced activities of these enzymes (Fig. 2) and maintenance of membrane lipids. These results are also in accordance with previous reports claiming lower LOX activity, and reduced ROS accumulation and membrane damage were associated with less pericarp browning in longans (Duan et al., 2007; Duan, Liu et al., 2011), lychees (Duan et al., 2004; Sun et al., 2010; Yang et al., 2009) and loquats (Cai et al., 2006).

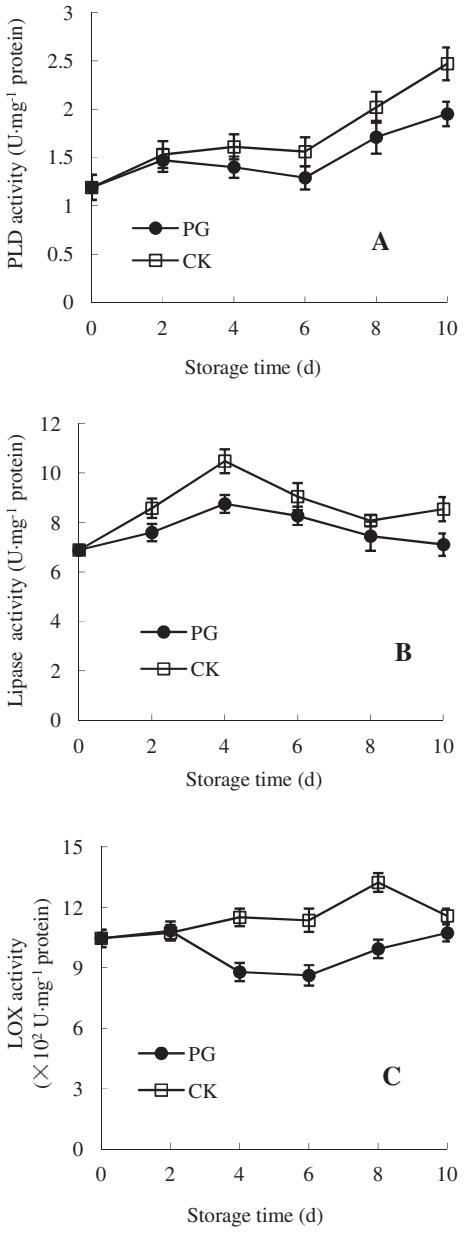

Fig. 2. Changes in activities of PLD (A), lipase (B) and LOX (C) in pericarp of harvested longan fruit.

3.3. Propyl gallate – inhibited change in compositions and alterations of fatty acid in cell membrane

A growing number of studies have proved that the decompartmentalization of cell membrane can contribute to browning development (Apai, 2010; Jiang & Li, 2001; Lin et al., 2014). In addition, cell membrane decompartmentalization is related to membrane lipid metabolism (Lin et al., 2016). Clearly, membrane lipid metabolism depends on membrane lipid composition and the composition of fatty acid in membrane lipid (Liu et al., 2011; Yi et al., 2009). The latter, in particular, is of great significance, because it influences the fluidity and stability of cell membrane. These are two basic requirements for membrane structure functionality (Saquet et al., 2003; Shewfelt & del Rosario, 2000) because normal function is regulated by fluidity, which depends on FAs chain length and FA unsaturation (Campos, Quartin, Ramalho, & Nunes, 2003; Jin et al., 2014; Lin et al., 2016). Shorter chains and higher FA unsaturation increase the fluidity of membranes (Campos et al., 2003).

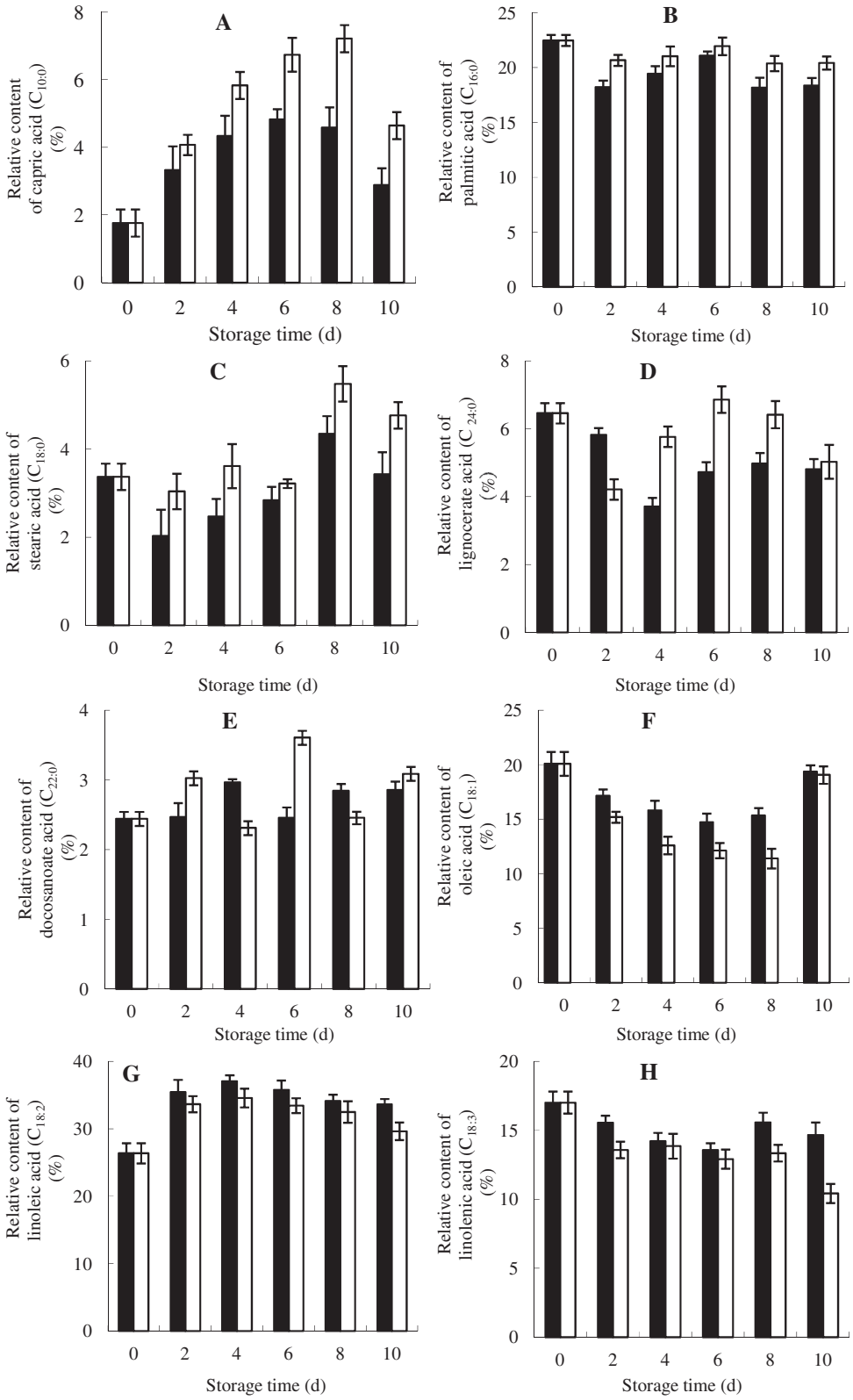

Fig. 3. Changes in fatty acid compositions of membrane lipids in pericarp of harvested longan fruit. □, control, ■, propyl gallate.

FAs, saturated and unsaturated, are essential in plant cell membrane. IUFA and U:S are two factors in the degree of unsaturation of cell membrane and membrane dysfunction. Any change in compositions of FAs, IUFA and U:S affects the fluidity, stability and other properties of cell membranes (Lin et al., 2016). These factors can trigger the decompartmentalization of cell membranes and lead to the development of browning (Zhang & Tian, 2010). Higher levels of USFAs were associated with inhibition of browning in pears during delayed controlled atmosphere storage (Saquet et al., 2003). Moreover, Cao et al.

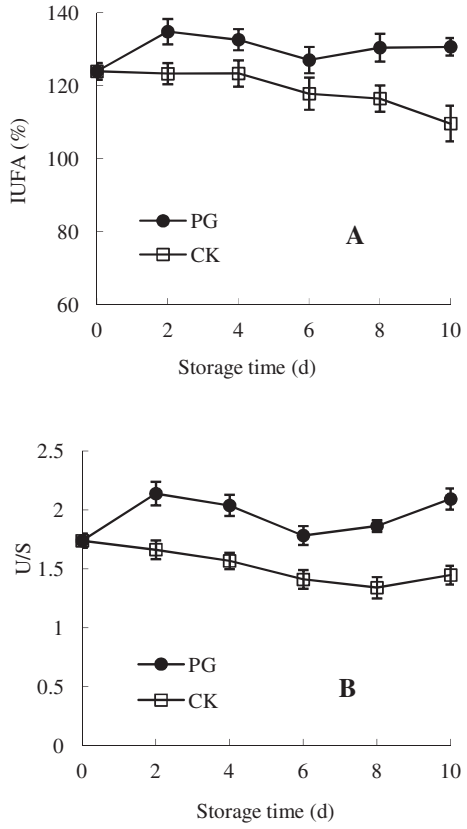

Fig. 4. Changes in IUFA (A) and U/S (B) in pericarp of harvested longan fruit.

(2011) reported that 'Qingzhong' loquat fruits were less susceptible to core browning than 'Fuyang' loquat fruits, because 'Qingzhong' loquat fruits maintained lower SFAs levels, and higher levels of USFAs and U:S. It has also been reported that a higher U:S is associated with greater tolerance to stress and less browning in peaches (Jin et al., 2014).

In the present study, as shown in Fig. 3, SFAs of harvested longan accounted for 36.5% the membrane content, with palmitic acid (C16:0) being predominant. While, USFAs accounted for 63.5%, with linoleic (C18:2) being predominant. The results are in agreement with the study on peaches by Jin et al. (2014).

When compared with control fruits, propyl gallate altered the FAs levels in cell membrane (Fig. 3), increasing the relative contents of USFAs (Fig. 3F–H). It was also associated with a decrease in the relative contents of SFAs, like palmitic acid (C16:0) and stearic acid (C18:0) (Fig. 3A–E). Moreover, propyl gallate enhanced the levels of IUFA and increased U:S (Fig. 4). In general, higher USFAs would enhance the fluidity, flexibility and integrity of cell membrane. Decreased IUFA and U:S were associated with the changes in relative contents of FAs. These results suggest that propyl gallate could delay the senescence and deterioration of the cell membrane, retarding pericarp browning and increase the shelf-life of longan fruits.

4. Conclusion

Propyl gallate reduced activities of PLD, lipase and LOX as well as higher levels of USFAs, IUFA and U:S. Moreover, it postponed changes in pericarp color. This increased storability of longans could be attributed to improved cell membrane integrity and delayed senescence because of the decreased enzyme activities and higher degree of unsaturation in cell membranes.

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