

Effects of Salts and Edible Oils on Calcium and Water Contents in Ripening Banana Fruits

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Abstract: Bananas were treated by dipping them for 24 h separately in CaCl₂ and MgSO₄ solutions at different concentrations. The treated fruits were either further anointed or not with palm kernel oil or coconut oil. Untreated fruits were used as controls. Results showed that Ca²⁺ content in the peel increased significantly after treatments with CaCl₂ or MgSO₄ solutions, the concentrations of 150 and 200 mg L⁻¹ being more effective. No similar trends were observed in the pulp. The water content decreased and increased, respectively in the peel and pulp during ripening of both control and treated bananas. However, individual or simultaneous treatments with salt solutions and oils delayed these alterations in the water content. Simultaneous treatments were more effective in delaying the decrease and increase in water content in the peel and pulp, respectively. These results indicated that treatments with CaCl₂ or MgSO₄ solutions which induce an increase in the level of Ca²⁺ in the peel, when used simultaneously with palm kernel or coconut coating have synergistic delaying effects on some processes that usually accompany ripening.

Key words: Banana fruits, calcium ion, cell wall firmness, delay of ripening, lipids

INTRODUCTION

Fruits fulfil essential requirements for human nutrition due to their contents in minerals, vitamins and carotenoids (Bramley, 2002; Nakasone and Paul, 1998). The ripening of many fruits can be assessed visually through changes in the color or texture. However, the detailed biochemical processes involved in these changes and the nature of their coordination during fruit ripening are not completely elucidated. Knowledge about the implication of phytohormones in the ripening have led to the classification of fruits into climacteric (e.g., banana, mango and tomato) and non-climacteric (e.g., pineapple and orange). In climacteric fruits the massive production of ethylene commences at the onset of respiratory climacteric period and exogenously applied ethylene induces ripening and endogenous ethylene production (John and Marchal, 1995; Nakasone and Paul, 1998). In non-climacteric fruits such as citrus ethylene is also necessary for color change even if it may not be the primary inducer (Purvis and Barmore, 1981; Alonso *et al.*, 1995). It has long been speculated that the gene system I is responsible for ethylene production in both climacteric and non-climacteric fruits as well in vegetative tissues whereas system II operates during ripening of climacteric fruits (Lelièvre *et al.*, 1977; Wills *et al.*, 1998). However, Katz *et al.* (2004) reported that in non-climacteric citrus fruits there was a transition from system II-like activity to system I activity in the course of maturation. Furthermore, during the ripening of strawberry, another non-climacteric fruit, even little ethylene produced might be sufficient to trigger ripening-related physiological processes (Trainotti *et al.*, 2005). Obviously, the mechanisms of ethylene action in triggering fruit ripening process are far from being completely understood. Phytohormone signal pathways may involve the binding of hormones to their membrane

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receptors, a change in the expression of specific genes and/or allosteric alterations in the activities of existing enzymes (Luettge *et al.*, 2002; Oepik and Rolfe, 2005). Transmembrane ethylene receptors have been identified in *Arabidopsis thaliana* and related proteins have been isolated from other species such as tomato (Trewavas, 2000; Oepik and Rolfe, 2005). Alterations in receptors concentration and/or in physical nature of their environment can modify the sensitivity of cells to signals (Luettge, 2002; Trewavas, 2000). Yet, it is well known that lipids are constituents of plant and animal cell membranes; their chemical nature may determine the physical properties of membranes and modulate many physiological processes (Aghofack-Nguemezi and Tatchago, 2006; Aghofack-Nguemezi, 2001; Thompson, 1986; Raison, 1980). In flower senescence and ripening of banana fruits, short-chain-length fatty acids may increase the sensitivity of the tissue to ethylene as a result of an increase in ethylene binding (Whitehead and Vasiljevic, 1993; Whitehead and Bossé, 1991). Treatments with coconut oil and palm kernel oil induced a delay in several changes that usually accompany ripening, thereby prolonging the shelf-life of banana fruits (Aghofack-Nguemezi *et al.*, 2006).

Calcium ion may be required for a variety of ethylene-dependent processes (Raz and Flur, 1992). It acts as second messenger in coupling many stimulus-response systems and thus occupies a pivotal position in plant signal transduction pathways, some of which are associated with hormones (Trewavas, 2000; Navarro-Avino and Bennett, 2005). The divalent cations Ca^{2+} and Mg^{2+} play other physiological roles in plant growth and development. There is evidence that calcium inhibits the activities of cell wall degrading enzymes in ripening fruits (Brady, 1987). The effects of treatments with calcium chloride solutions on the ripening of fruits depend on the concentration and the mode of application (Aghofack-Nguemezi and Yambou, 2005; Perera and Karunaratne, 2002). Calcium ions delay the senescence of plant tissues by stabilizing cell membrane and increasing the rigidification of monolayers. The Ca^{2+} -mediated cross-linking may occur as bridge between phospholipids, between phospholipids and carboxy tails of embedded membrane proteins and between phospholipids and cytoskeleton. Magnesium ions affect the electrostatic cross-linking between membrane components to a lesser extent than calcium ions (Leshem, 1991; Hepler and Wayne, 1985). While intracellular Ca^{2+} concentration is submicromolar, the concentration of closely related divalent cation Mg^{2+} is millimolar. Despite the concentration difference that favors Mg^{2+} , cellular processes often display an enormous selectivity for Ca^{2+} (Hepler and Wayne, 1985). It is not known to which extent exogenous supply of Ca^{2+} and Mg^{2+} could affect their endogenous content.

The present research was undertaken to examine the effect of simultaneous treatments with coconut oil or palm kernel oil and calcium chloride or magnesium sulphate on the water content in the peel and pulp of banana fruits during ripening. Furthermore, the influence of treatments with calcium chloride and magnesium sulphate solutions on the endogenous Ca^{2+} and Mg^{2+} contents was also determined.

MATERIALS AND METHODS

Plant Materials and Treatments

Musa accuminata Colla fruits were donated by the High Penja Plantation Company (PHP) in Njombe, Cameroon. These fruits were from the second hand of bunches. The oils were obtained from the endosperm of palm kernel and coconut after grinding and squeezing. Green, unripe bananas were either not treated and used as control or treated with oils or salt solutions singly and simultaneously. One hundred microliter of palm kernel oil or coconut oil were homogeneously applied on each banana fruit. Treatments of banana fruits with salt solutions were done by dipping them in 50, 100, 150 or 200 mg L^{-1} calcium chloride or magnesium sulphate solutions for 24 h. Control and treated bananas were packed in transparent polythene bags. The seven ripening stages were visually assessed according to Dadzie and Ochard (1997). Bananas at stage 1 (green, unripe), 3 (greener than yellow),

5 (yellow with green tip) and 7 (yellow, slightly flecked) of ripening of control fruits were used for the determination of water content. The endogenous Ca^{2+} and Mg^{2+} contents were estimated using unripe bananas treated with calcium chloride or magnesium sulphate solutions.

Determination of Water Content

Fresh peel and pulp were weighed and dried in an oven at 105°C for 24 h. They were then weighed for the determination of dry matter weight. The water content was calculated using fresh and dry matter weight according to Chapman (1976).

Determination of Calcium and Magnesium Contents

Fresh banana peel and pulp were dried in an oven at 105°C for 24 h. One gram of the dried samples was then calcined at 405°C for 24 h. Ten milliliter of 1 N nitric acid solution were added to the ash and the mixture was heated till the evaporation of half of the volume. The residue was completed to 50 mL with distilled water. This solution was further threefold diluted before use. Ca^{2+} and Mg^{2+} contents were determined by the complexometric method as described by Pauwels *et al.* (1992).

Statistical Analysis

Group comparisons were made using One-way Analysis of Variance (ANOVA) to see if variations among the means were significantly greater than expected by chance. The Student-Newman-Keuls Test was used to compare means differences, whereby a p-value of <0.05 was considered as statistically significant.

RESULTS

Table 1 shows the influence of treatments with salt solutions at different concentrations on the endogenous level of Ca^{2+} and Mg^{2+} in the peel and pulp of banana fruits. Treatments of bananas by dipping in CaCl_2 (CC) or MgSO_4 (MS) solutions for 24 h induced significant increases in the Ca^{2+} content in the peel. The Ca^{2+} content in the peel of bananas treated with 100, 150 or 200 mg L^{-1} CaCl_2 solutions (CC100, CC150 or CC200) and 50, 100, 150 or 200 mg L^{-1} MgSO_4 solutions (MS50, MS100, MS150 or MS200) was significantly ($p<0.001$) higher than the level of Ca^{2+} in the peel of control fruits. The level of calcium ion in the peel of banana fruits treated with CC50 was also significantly ($p<0.01$) higher than that of control fruits. There was no linear increase in the Ca^{2+} content in the peel of treated bananas with increasing concentrations of CC or MS solutions. However, treatments with 150 and 200 mg L^{-1} CC or MS solutions were more efficient in inducing increases in

Table 1: Effects of treatments with calcium chloride (CC) and magnesium sulphate (MS) solutions at different concentrations (50, 100, 150 and 200 mg L^{-1}) on calcium (C) and magnesium (M) ion content in the peel (PE) and pulp (PU) of banana fruits. Values are means \pm SD (n = 4)

Types of treatments	Ion content (meq kg^{-1})			
	CPE	MPE	CPU	MPU
Control	5.23 \pm 1.75	36.1 \pm 0.5	15.07 \pm 1.00	24.9 \pm 7.9
CC50	10.3 \pm 3.3 ^{2a}	28.1 \pm 4.0 ^a	17.9 \pm 3.7 ^a	32.7 \pm 9.4 ^a
CC100	12.3 \pm 1.4 ^{23a}	30.9 \pm 1.9 ^b	13.8 \pm 2.8 ^a	21.2 \pm 3.3 ^a
CC150	15.1 \pm 0.4 ^{23b}	29.3 \pm 1.4 ^a	16.8 \pm 2.2 ^a	26.2 \pm 2.6 ^a
CC200	16.0 \pm 1.0 ^{23b}	36.1 \pm 6.5 ^a	10.5 \pm 1.5 ^b	31.7 \pm 6.3 ^a
MS50	13.4 \pm 0.2 ^{23a}	35.1 \pm 0.3 ^a	16.1 \pm 0.1 ^a	28.6 \pm 2.9 ^a
MS100	15.2 \pm 0.3 ^{23b}	37.8 \pm 5.8 ^a	21.3 \pm 3.1 ^{11b}	29.6 \pm 8.0 ^a
MS150	18.1 \pm 1.5 ^{23c}	30.5 \pm 0.5 ^b	12.5 \pm 0.5 ^c	31.6 \pm 0.1 ^a
MS200	17.2 \pm 2.3 ^{23c}	26.0 \pm 0.5 ^{1c}	14.3 \pm 3.8 ^c	33.6 \pm 0.5 ^a

^{a, b, c}: Values from each fruit part and for each salt bearing different superscript within a column differ significantly, ^{1, 2}, ²³: Significantly different from the control in the same column, respectively at $p<0.05$, $p<0.01$ and $p<0.001$

the endogenous Ca²⁺ content in the peel than treatments with 50 or 100 mg L⁻¹ solutions of both salts. Treatments with MS200 rather induced significant (p<0.05) decrease in the level of Mg²⁺ in the peel. Treatments with other concentrations of CC and MS solutions did not affect significantly the level of Mg²⁺ in the peel of treated fruits in comparison to the control. Nevertheless, there were differential effects of individual salt concentrations on the Mg²⁺ content, the peel of fruits treated with different solutions of CC having almost the same Mg²⁺ content and the peel of fruits treated with MS200 having lower level of Mg²⁺ than that of fruits treated with MS solutions at lower concentrations. Apart from the effect of treatment with MS100, no significant (p>0.05) effect of divalent cation salts on Ca²⁺ or Mg²⁺ content in the pulp of treated banana fruits could be observed as compared to control fruits. The level of Ca²⁺ in the pulp of fruits treated with CC50 was significantly (p<0.05) higher than that in the pulp of fruits treated with CC200. The Ca²⁺ content in the pulp of bananas treated with MS100 was significantly (p<0.05) higher than that in the pulp of fruits treated with other concentrations of this salt. There were no significant (p>0.05) differences between the levels of magnesium ion in the pulp of fruits treated with solutions of CC or MS at different concentrations.

The water content in the peel of control banana fruits decreased significantly with increasing stages of ripening. A similar trend was observed in the peel of treated fruits (Table 2). However, the rate of decrease in the water content was generally lower in the peel of treated than in that of control fruits. Thus, at stage 3 of ripening, the water contents in the peel of bananas treated with CC50, MS 100 or MS150 and MS200 + coconut oil (CNO) were significantly higher than that in the peel of

Table 2: Changes in water content in the peel of banana fruits during ripening as related to treatments with coconut oil (CNO), palm kernel oil (PKO) and/or calcium chloride (CC) and magnesium sulphate (MS) solutions at different concentrations (50, 100, 150 and 200 mg L⁻¹). Values are means±SD (n = 4)

Types of treatments	Water content (% fresh weight)			
	Ripening stages of control banana fruits			
	1	3	5	7
Control	96.3±2.0	90.3±1.4 ²	90.5±0.4	87.2±3.5
CNO	96.3±2.0	91.5±0.2 ²	91.5±2.4	87.4±0.5 ¹
PKO	96.3±2.0	92.9±0.3 ²	91.3±0.8	92.0±0.9
CC50	96.3±2.0	96.3±0.9 ²	91.2± 0.3 ²	91.8±1.9
CC100	96.3±2.0	92.4±0.7 ²	91.3±1.3	89.4±1.4
CC150	96.3±2.0	90.5±0.3 ³	90.5±0.2	92.5±0.4 ¹ ¹
CC200	96.3±2.0	93.0±0.1 ²	94.7±2.6	92.5±0.8 ¹
MS50	96.3±2.0	92.4±1.2 ¹	92.3± 0.5	92.3±0.1 ¹ ¹
MS100	96.3±2.0	95.6±1.5 ²	90.0±4.4	89.6±1.9
MS150	96.3±2.0	94.4±1.6 ¹	92.2±0.4	92.8±0.5 ¹
MS200	96.3±2.0	88.9±0.3 ³	90.6±0.8	89.8±0.5
CC50+CNO	96.3±2.0	90.7±1.5 ²	92.5±0.1	92.8±0.2 ¹
CC100+CNO	96.3±2.0	90.8±3.6	92.4±0.3	92.0±0.8 ¹
CC150+CNO	96.3±2.0	91.6±2.5 ²	92.0±0.8	91.4±0.3
CC200+CNO	96.3±2.0	89.9±0.2 ³	92.2±3.1	91.1±1.3
CC50+PKO	96.3±2.0	90.3±0.1 ³	91.2±0.4	85.4±2.0 ³
CC100+PKO	96.3±2.0	91.5±1.0 ²	93.5±1.3	91.1±1.3
CC150+PKO	96.3±2.0	89.8±0.2 ³	93.4± 0.9 ²	91.4±2.5
CC200+PKO	96.3±2.0	90.4±0.5 ²	91.2±0.2	89.2±4.4
MS50+CNO	96.3±2.0	91.3±0.6 ²	90.7±0.2	80.3±16.6
MS100+CNO	96.3±2.0	90.2±0.5 ³	90.3±1.1	93.2±0.3 ¹ ¹
MS150+CNO	96.3±2.0	91.0±0.6 ²	92.1±0.0	91.9±0.6
MS200+CNO	96.3±2.0	94.9±1.3 ¹	90.8±0.6 ²	91.7±0.5
MS50+PKO	96.3±2.0	89.6±2.0 ³	90.7±0.7	89.5±1.3
MS100+PKO	96.3±2.0	91.1±0.5 ²	92.4±0.6	89.9±3.4
MS150+PKO	96.3±2.0	92.5±2.7	92.5±1.7	92.1±1.3
MS200+PKO	96.3±2.0	87.9±1.7 ³	91.8±0.2 ²	90.4±1.5

¹, ², ³: Significantly different from the preceding stage, respectively at p<0.05, p<0.01 and p<0.001, ¹, ²: Significantly different from the control in the same column, respectively at p<0.05 and p<0.01

control fruits. No significant difference could be observed between the water content in the peel of control and treated bananas at stage 5 of ripening. At ripening stage 7, the water content in the peel of bananas treated with CC150, CC200, CC50 + CNO, CC100 + CNO or MS100 + CNO was significantly higher than that observed in the peel of control fruits. Obviously, simultaneous treatments with CC or MS solutions and oils were more effective in inducing a delay of water loss from the peel of banana fruits during ripening than singly treatments with oils.

Contrarily to the peel, the water content in the pulp of both control and treated banana fruits increased significantly with increasing stages of ripening, this increase being more pronounced from ripening stages 1 to 3 in the pulp of most of the treated fruits (Table 3). Almost 8%-increase in the water content was observed in the pulp of control banana fruit from ripening stages 1 to 7 with only 2%-increase between the stages 1 and 3 of ripening. In the pulp of treated fruits however, an increase of 6 to 9% in the water content could be observed from ripening stages 1 to 7 with up to 6%-increase recorded in the pulp of some treated fruits only between the two first ripening stages studied. Thus, at ripening stage 3 the water content in the pulp of most of the treated fruit was significantly higher than that observed in the pulp of control fruits. At this stage, the water content in the pulp of fruits which have been treated with MS50, CC50 + PKO, CC150 + PKO or MS200 + PKO was significantly different from that measured in the pulp of control fruits. No significant difference could be observed between the water content in the pulp of control and treated fruits at stage 5 of ripening.

Table 3: Changes in water content in the pulp of banana fruits during ripening as related to treatments with coconut oil (CNO), palm kernel oil (PKO) and/or calcium chloride (CC) and magnesium sulphate (MS) solutions at different concentrations (50, 100, 150 and 200 mg L⁻¹). Values are means±SD (n = 4)

Types of treatments	Water content (% fresh weight)			
	Ripening stages of control banana fruits			
	1	3	5	7
Control	70.9±0.1	72.3±0.0 ¹	75.3±2.5	78.8±0.2 ¹
CNO	70.9±0.1	76.1±0.2 ^{3r3}	77.1±1.1	82.7±4.2
PKO	70.9±0.1	76.9±0.1 ^{3r3}	77.0±0.3	79.7±0.6 ²
CC50	70.9±0.1	76.3±0.2 ^{3r3}	75.5±0.4	78.9±1.1 ²
CC100	70.9±0.1	75.9±0.3 ^{3r3}	72.5±1.3 ²	75.6±1.7 ^{1r2}
CC150	70.9±0.1	75.0±0.7 ^{3r3}	74.9±0.2	76.7± 0.8 ¹
CC200	70.9±0.1	75.3±0.2 ^{3r3}	75.2±0.1	77.0±0.6 ¹
MS50	70.9±0.1	72.6±0.8 ¹	74.2±0.0 ¹	77.5±1.3 ¹
MS100	70.9±0.1	75.2±0.1 ^{3r3}	75.4±0.1	77.4±0.3 ²
MS150	70.9±0.1	74.6±0.5 ^{2r2}	74.7±0.1	79.1±1.9 ³
MS200	70.9±0.1	73.4±0.7 ^{1r2}	73.9±0.0	75.9±4.2
CC50+CNO	70.9±0.1	74.0±0.7 ^{2r2}	76.3±0.7 ²	78.2±0.0 ¹
CC100+CNO	70.9±0.1	74.1±0.1 ^{2r2}	75.5±0.2	77.2±1.6
CC150+CNO	70.9±0.1	74.0±0.4 ^{1r2}	75.1±0.2 ¹	78.1±0.7 ²
CC200+CNO	70.9±0.1	73.7±0.2 ^{1r2}	75.1±0.8 ¹	77.4±0.2 ²
CC50+PKO	70.9±0.1	72.7±0.2 ¹	75.1±0.1 ^{1r2}	79.5±0.6 ³
CC100+PKO	70.9±0.1	75.4±0.1 ^{3r3}	77.4±2.8	79.0±0.3
CC150+PKO	70.9±0.1	72.2±0.7 ¹	74.5±0.2 ²	75.8±0.0 ^{2r1}
CC200+PKO	70.9±0.1	74.3±0.4 ^{3r2}	74.8±0.7	77.9±0.5 ²
MS50+CNO	70.9±0.1	75.1±0.0 ^{3r3}	74.4±0.7	77.6±0.5 ²
MS100+CNO	70.9±0.1	73.9±0.2 ^{1r2}	76.2±0.3 ²	76.4±0.5 ¹
MS150+CNO	70.9±0.1	74.0±0.1 ^{1r2}	78.6±0.6 ^{1r3}	77.4±0.6 ¹
MS200+CNO	70.9±0.1	74.7±0.7 ^{2r2}	74.2±0.2	76.4±0.2 ²
MS50+PKO	70.9±0.1	73.8±0.7 ^{1r2}	75.5±0.1 ¹	74.8±0.2 ²
MS100+PKO	70.9±0.1	73.4±0.4 ^{1r2}	75.5±3.7	78.3±0.2
MS150+PKO	70.9±0.1	75.4±0.2 ^{3r3}	77.8±2.2	77.2±0.3
MS200+PKO	70.9±0.1	72.7±0.0 ¹	75.2±3.1	76.1±3.2

¹, ², ³: Significantly different from the preceding stage, respectively at p<0.05, p<0.01 and p<0.001, ^{r1}, ^{r2}, ^{r3}: Significantly different from the control in the same column, respectively at p<0.05, p<0.01 and p<0.001

At ripening stage 7, the water content in the pulp of bananas treated with CC50, CC100, CC150 + PKO, MS50+ PKO or MS100 + CNO was significantly lower than that observed in the pulp of control fruits. The treatment with CC150 + PKO was more effective in delaying the increase of water content in the pulp of bananas. There was no significant difference between the water content in the pulp of bananas that received other treatments and the water content in the pulp of control fruits.

DISCUSSION

Treatments of banana fruits with CaCl_2 or MgSO_4 solutions induced significant increases in the Ca^{2+} contents in the peel. Simultaneous treatments with these solutions and palm kernel oil or coconut oil were more effective in inducing an inhibition of the decrease in water content in the peel than treatment with each oil alone (Table 1 and 2). If the increased level of calcium ions in the peel of fruits treated with CaCl_2 solutions may be attributed to its uptake from the external medium during the treatments, the mechanisms whereby treatments with MgSO_4 solutions induced increases in the endogenous Ca^{2+} content are not known. Ca^{2+} contributes to the membrane stability in plant cells by its association with membrane phospholipids and it is necessary for the maintenance of the normal permeability of the plasmalemma. It also contributes to cell wall firmness as calcium pectate, a major component of the middle lamella which cements cell wall together (Oepik and Rolfe, 2005). Thus, an increase in Ca^{2+} content in the peel may have led to a hindrance of water loss by the peel either through transpiration or diffusion into the pulp by osmosis. This effect of calcium ion could have been reinforced by the protecting effect of oils on water loss by the peel. Indeed, it is well established that oil represents an excellent barrier against water (Badwin, 2001). Exogenously applied palm kernel and coconut oils may have also modified the lipid nature of the cell membranes and hence affected the binding sites of hormones such as ethylene that are implicated in fruit ripening processes. In this context, Gil-Villarino *et al.* (1999) reported that in animal, coconut oil feeding induced very rapid changes in hepatic mitochondrial lipid. Furthermore, palm kernel oil rich diet influences the metabolism of cholesterol (Abdel-Fattah *et al.*, 1998; Ebesunum *et al.*, 2003). Plant tissues also contain different types of sterols including cholesterol (Eichenberger, 1977; Duperon *et al.*, 1984). Certain free sterols are precursors of steroid hormones (Fujioka and Yokota, 2003; Mori, 2004) that have been implicated in many processes including the regulation of gene expression (He *et al.*, 2003; Schaller, 2003). Although it remains unclear whether chlorophyll degradation triggers the water loss in the peel of ripening fruits and other ripening related changes or *vice-versa*, it has already been shown that ethylene is necessary for chlorophyll breakdown (Apelbaum *et al.*, 1976).

The water content in the pulp increased with increasing stages of ripening of control as well as treated fruits. Individual or simultaneous treatments with salt solutions and oils differently delayed this increase in water content (Table 3). During ripening of fleshy fruits the water content usually increases in the pulp due to transformation of starch into reducing sugars and consequently to an increase of osmotic potential that induces the movement of water from the peel to the pulp; the water-producing reactions like respiration also contribute to the increase in the water content in the pulp (Ngalani *et al.*, 1999; Cordenunsi and Lajolo, 1995). The mechanisms whereby treatments with oils and/or CaCl_2 solutions inhibit these increases in water contents in the pulp of banana fruits are not exactly known. Alterations in water content in the pulp as a result of treatments of fruits with oils may be an indirect effect of changes in metabolic activities of cells in the anointed peel.

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