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Loss of tolerance to desiccation in germinated Norway maple (*Acer platanoides* L.) seeds. Changes in carbohydrate content

Abstract: Carbohydrates were analyzed in Norway maple embryo axes and cotyledons after imbibition, in the middle of cold stratification, before germination and during radicle protrusion to 8–10 mm and 20–25 mm. Simultaneously desiccation tolerance of seeds was determined by tetrazolium (TTC) test, after desiccation of seeds to 10–20% of water content. The cotyledons were tolerant to desiccation throughout all stratification and germination period. Embryo axes became sensitive to desiccation when hypocotyls-radicle protrusion reached 20–25 mm length. In this period the significant increase of monosaccharides: glucose, fructose and galactose in embryo axes occurred. This was not observed in cotyledons. During the germination period significant decrease of sucrose and raffinose content was noted in embryo axes and cotyledons. Relatively less changes appeared in stachyose content in embryo axes while in cotyledons it decreased evidently. The mass ratio of sucrose to oligosaccharides was higher in cotyledons of germinated seeds. The marked decrease of mass ratio of oligo to monosaccharides was observed in embryo axes in the last period of germination. The role of carbohydrates in loosing tolerance to desiccation in germinated Norway maple seeds is discussed.

Additional key words: dormancy, fructose, galactose, glucose, raffinose, stachyose, stratification, sucrose, viability

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Introduction

Seeds of Norway maple belong to the orthodox category. After shedding they can be dried to low level of water (6–10%) without any loss of viability (Hong and Ellis, 1990, Pukacka and Czubak 1998). Our observations during the years showed that these seeds acquire tolerance to desiccation in developmental period, after reaching the maximum of reserve deposition i.e. at 18th WAF (weeks after flowering) (Pukacka and Pukacki 1997, Pukacka 1998, Pukacka 1999). The dependence of desiccation tolerance of seeds of many species upon soluble sugars concentration was indicated in many reports (Koster and Leopold 1988, Chen and Burris 1990, Bernal-Lugo and Leopold 1992, Leprince et al. 1993, Pukacka and Pukacki 1997). Oligosaccharides have been considered as the factors responsible to glass formation in cells and prevention of macromolecular structures against deterioration during desiccation (Caffrey et al. 1988, Horbowicz and Obendorf 1994, Sun et al. 1996, Crowe et al. 1998). However, the recent reports suggest that, besides sugars other molecules play a crucial role in intracellular glass formation, probably proteins (Sun and Leopold 1997, Wolkers et al. 1998, Buiting et al. 2000). During seed germination their tolerance to desiccation disappears, but it happen at different time depending on species. Correlative studies showed that in germinating seeds the loss of oligosaccharides was always associated with the disappearance of tolerance to des-

iccation at the radicle (Koster and Leopold 1988, Leprince et al. 1992, Górecki et al. 1997). Seed germination is distinctly different from seed development, as germinating seedlings is going through a number of significant transitions toward of autotrophy. The oligosaccharides in germinating seedlings play a role as an energy sources for active metabolism leading to building new structures and organs. The purpose of presented study was to establish the changes in sugar composition in Norway maple seeds from their imbibition through cold stratification to germination including radicle emergence leading to the loss of tolerance to desiccation.

Material and methods

The seeds were collected from single Norway maple tree growing in the Kórnik Arboretum (Poland) after shedding in October. After their desiccation at ambient temperature to 8–10% of water content the seeds were stored in tightly sealed foil bags in stable temperature of –3°C.

The seedlot was imbibed in distilled water through 48h at 20°C and then transferred in closed box to +3°C, where their dormancy delayed. After 8 weeks of cold stratification the seeds started to germinate. Seed samples to analyses were collected after imbibition, after 5 weeks of cold stratification, after 8 weeks of stratification and after radicle protrusion to 8–10 mm and 20–25 mm.

Water content in embryo axes and cotyledons was determined by weighing three samples of 30 embryo axes and 20 cotyledons before and after drying at 110°C through 24h.

Desiccation tolerance test

Seeds were deprived of samaras and seed coats were removed. Embryo axes were excised from cotyledons. Four samples of 25 embryo axes and cotyledons were dried in the stream of cold air at ambient temperature and water loss was monitored by weighing them. The samples were drying to 10–20% of water content calculating on dry weight basis. After drying the samples were carefully rehydrated in moist paper towel and then tetrazolium test was performed according to ISTA (1976). Not stained parts of seeds were considered as dead.

Soluble sugar determination

The axes and cotyledons were separated and three samples of 50 embryo axes and 20 cotyledons were weighed and frozen in liquid nitrogen and stored at –80°C until use. Sugars were determined by HPLC (Waters) analysis according to Pukacka and Pukacki (1997). The Sugar Pak 1 (Waters Associates Milford, MA) column was used, with water as the mobile phase. Individuals were identified and quantified with those of the individual sugar standard.

Results and discussion

Norway maple seeds after shedding are in the state of deep dormancy and require to germination several weeks of cold stratification at +3°C and moisture content above 30% (Tomaszewska 1976, Tylkowski 1996). The dormancy breaking in Norway maple seeds is accompanied by the changes in the level of growth regulators (Tomaszewska 1976, Pinfield et al. 1990), protein synthesis and composition (Szczotka and Tomaszewska 1979, Pawłowski and Szczotka 1997), respiration (Szczotka and Żymańczyk 1994). During cold stratification Norway maple seeds remained tolerant to desiccation (Tab. 1) and in each time they could be dried to low level of water and stored in dry state without loss of viability (Tylkowski 1995). The decline of tolerance to desiccation appeared during germination, in radicles, when the hypocotyls-radicle axes reached the length of 20–25 mm (Tab.1). In geminating seeds the water content in cotyledons maintained constant, whereas in embryo axes it dramatically increased (Tab. 1). Loss of tolerance to desiccation was accompanied by marked increase of monosaccharides: glucose, fructose and galactose (Fig. 1) and decrease of mass ratio of oligosaccharide to monosaccharide (Fig. 5B) in embryo axes comparing to those in cotyledons and embryo axes in earlier stages of germination and stratification. Sucrose content diminished significantly in embryo axes where the desiccation tolerance was lost, whereas, raffinose concentration decreased earlier, in

Table 1. The water content and tolerance to desiccation of Norway maple (*Acer platanoides*) seeds during cold stratification and germination

	Water content (%)		Desiccation (%)		Viability (%)	
	E.axes	Cotyled.	E.axes	Cotyled.	E.axes	Cotyled.
Imbibed seeds	53.1	52.7	20.4	11	100	100
½ Stratification	54.8	53.6	17.2	12.1	100	100
End of stratif.	58.2	54.5	18.6	15.3	100	100
Radicle 8–10mm	70.0	56.3	20.0	10.8	100	100
Radicle 20–25mm	77.0	56.8	19.8	17.0	20	100

the end of stratification (Fig. 2). The lesser changes were observed in stachyose level. In cotyledons the oligosaccharide contents also decrease during germination, so the mass ratio of sucrose to oligosaccharides in cotyledons increased in this period (Fig. 5A). Desiccation process of germinated embryo axes and cotyledons evoked the significant increase of sucrose content and less significant of raffinose, glucose and galactose (Figs. 3 and 4). The changes in sugar contents after desiccation were noted in embryo axes and cotyledons in both germination periods. They had effect on the values of sucrose to oligosaccharides and oligosaccharide to monosaccharide ratios (Fig. 6A and B). Nevertheless the ratios of oligosaccharide to monosaccharide in fresh and desiccated embryo axes and cotyledons of germinated seeds where desiccation tolerance was lost, were evidently lower than those in desiccation tolerant ones (Fig. 6B). The

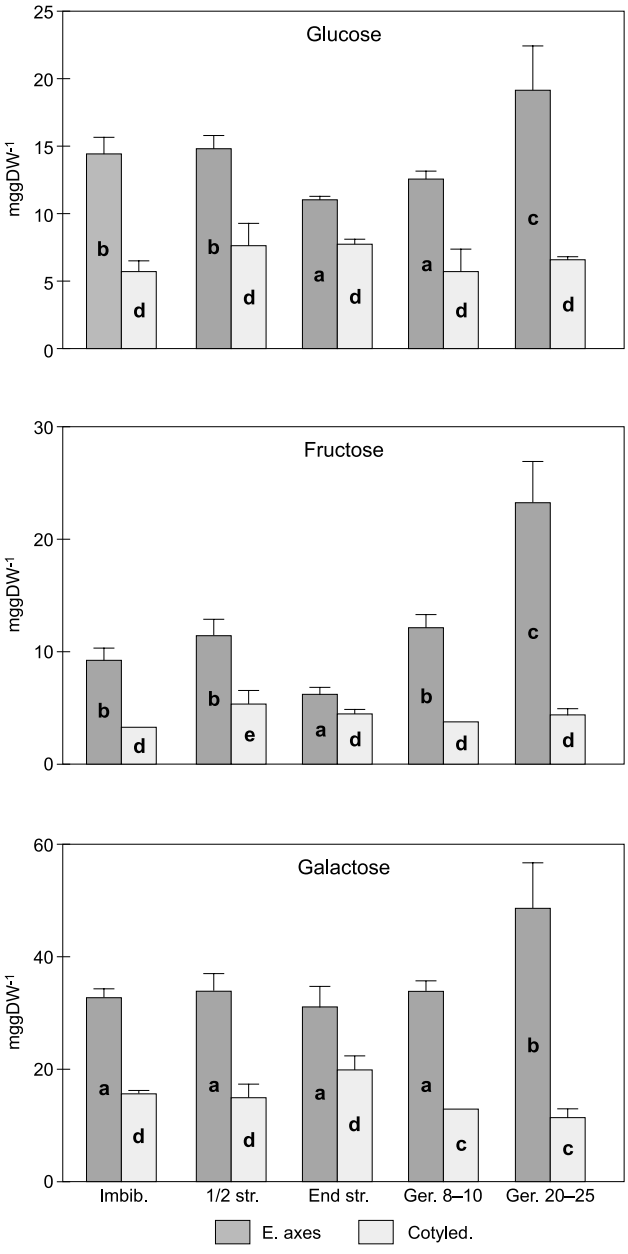

Fig. 1. Monosaccharide contents in embryo axes and cotyledons of Norway maple (*Acer platanoides*) seeds after imbibition, half and end of cold stratification and during radicle protrusion to 8–10 mm and 20–25 mm. Different letters in the same seed parts are significantly different at 95% level by Duncan's test

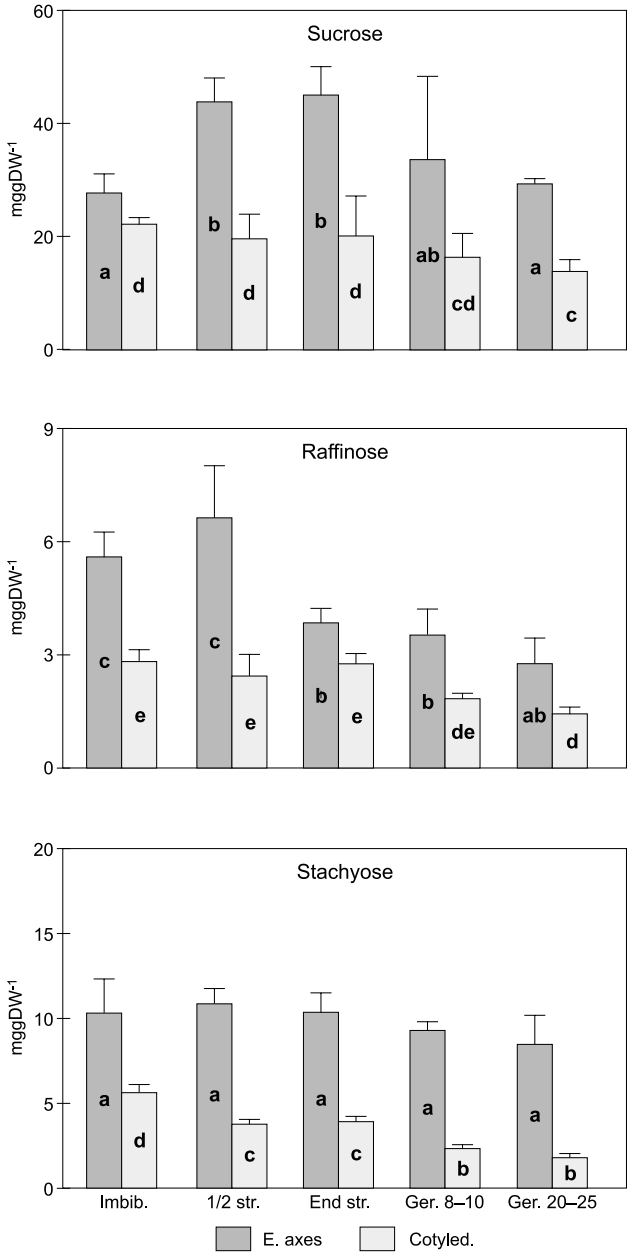

Fig. 2. Sucrose and oligosaccharide contents in embryo axes and cotyledons of Norway maple (*Acer platanoides*) seeds after imbibition, half and end of cold stratification and during radicle protrusion to 8–10 mm and 20–25 mm. Different letters in the same seed parts are significantly different at 95% level by Duncan's test

sugar content in embryo axes and cotyledons of Norway maple seeds before and after dehydration and the ability to synthesize sugar during the process of dehydration did not agree with the lack of desiccation tolerance in seed parts. The cotyledons remained tolerant to desiccation in spite of degradation of sucrose and oligosaccharides and increase sucrose/oligosaccharide ratio (Figs 2 and 5A). The results of Lin et all. (1998) on some species of crop seeds showed that in some of them the loss of tolerance to desiccation during germination was connected with decrease of sucrose and oligosaccharide contents and their reciprocal ratios, but in some not. Presented results also indicated that the loss of desiccation tolerance in germinated Norway maple seeds depends not only on sucrose and oligosaccharide decline. Generally, only monosaccharide concentrations and their ratio to oligosaccharides were strictly coincident with desiccation tolerance of seed parts before and after dehydration.

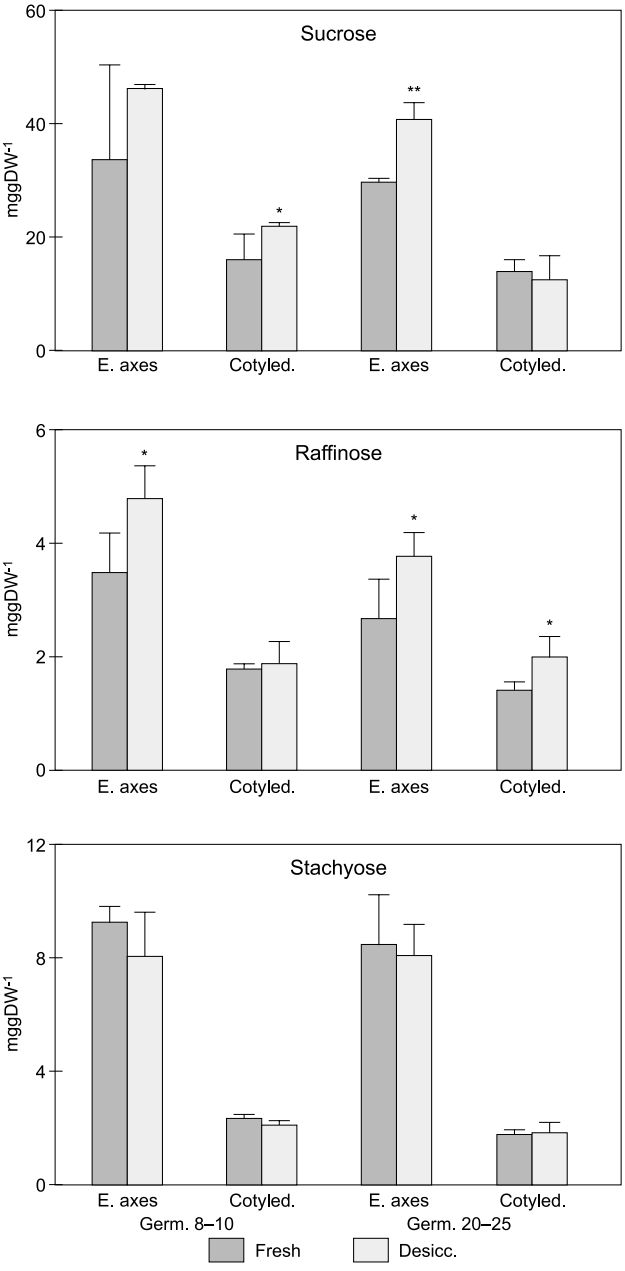

Fig. 3. Sucrose and oligosaccharide contents in germinated embryo axes and cotyledons of Norway maple (*Acer platanoides*) seeds, during radicle protrusion to 8–10 mm and 20–25 mm, before and after desiccation. Probability significance level: *, $P < 0.05$; **, $P < 0.01$

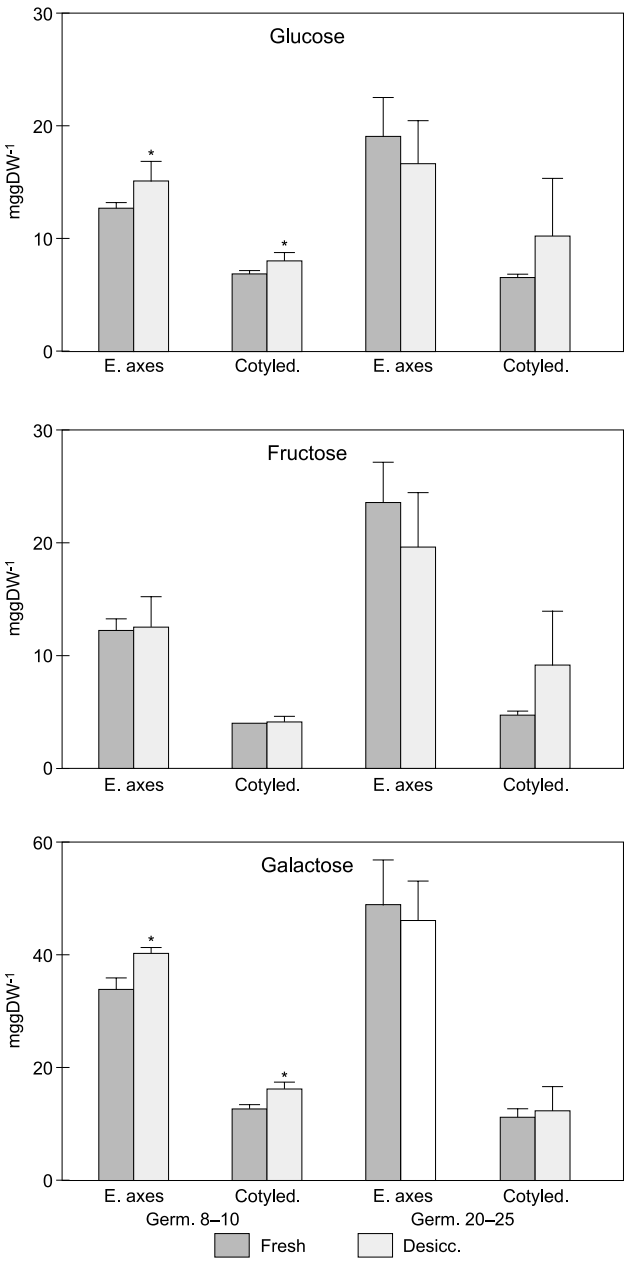

Fig. 4. Monosaccharide contents in germinated embryo axes and cotyledons of Norway maple (*Acer platanoides*) seeds, during radicle protrusion to 8–10 mm and 20–25 mm, before and after desiccation. Probability significance level: *, $P < 0.05$; **, $P < 0.01$

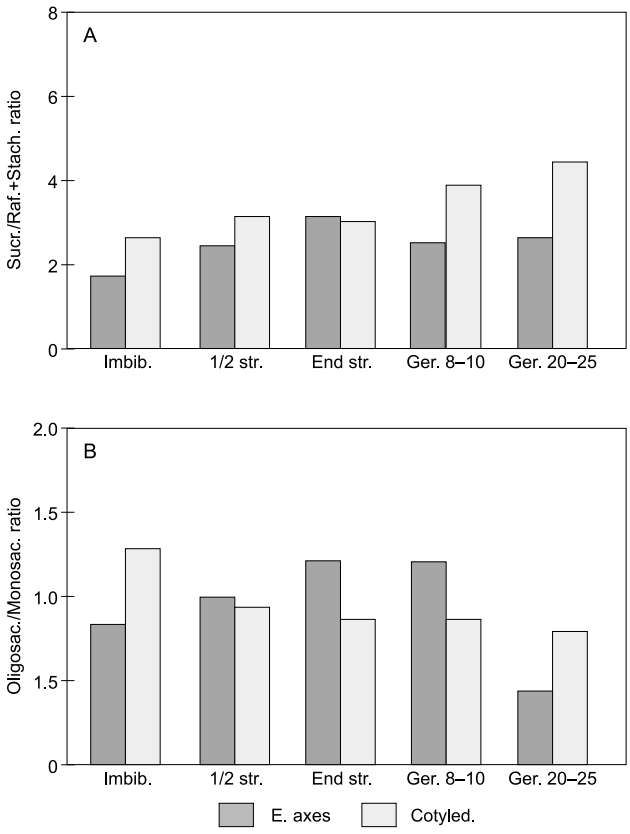

Fig. 5. Sucrose to oligosaccharide (A) and oligosaccharide to monosaccharide (B) ratios in embryo axes and cotyledons of Norway maple (*Acer platanoides*) seeds after imbibition, half and end of cold stratification and during radicle protrusion to 8–10 mm and 20–25 mm

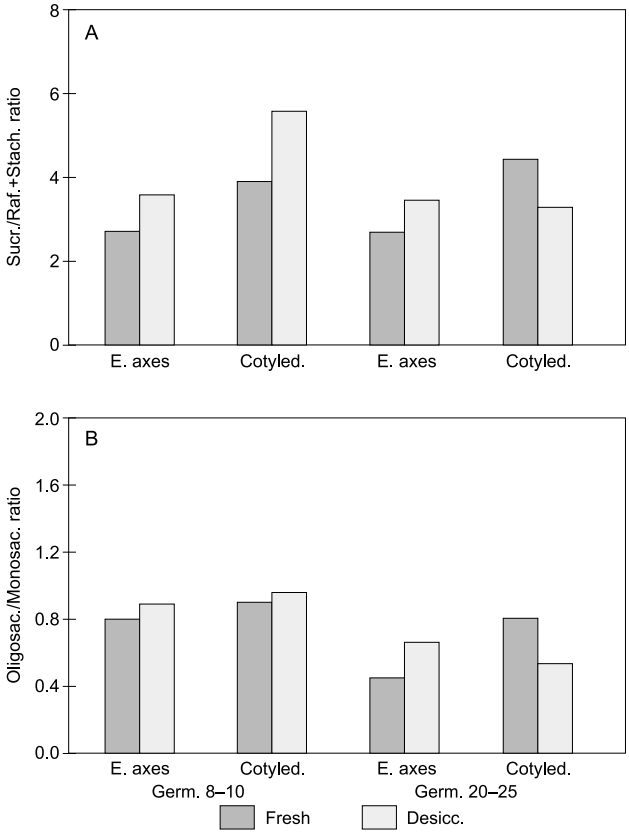

Fig. 6. Sucrose to oligosaccharide (A) and oligosaccharide to monosaccharide (B) ratios in embryo axes and cotyledons of Norway maple (*Acer platanoides*) seeds, during radicle protrusion to 8–10 mm and 20–25 mm, before and after desiccation

Physiological factors presumably exist as the primary regulator responsible for the lack or desiccation tolerance of germinating seedlings. Desiccation tolerance in orthodox seeds appears during their development and a gradual reduction in metabolism occurs, as water is lost from seed tissues. Upon imbibition and stratification there is a reactivation of metabolism leading to dormancy breaking and then to the synthesis of new components, cell division and growth until autotrophy is established. Seed maturation and seed germination are two distinct physiological stages in area of gene expression and metabolism (Kermode 1995). Drying of developing seed terminate maturation mode, but drying of imbibed seeds do not terminate germination. During desiccation of germinating seeds sucrose and oligosaccharides could help withstand dehydration but the loss of viability may be effect of stress involving free radical-mediated deteriorations. The increase in reducing sugars could contribute to cell damage during dehydration (Leprince et al. 1994) due to their reactions with amines and proteins and DNA (Koster and Leopold 1988). Autooxidation of reducing sugars is connected with production of hydroxyl radicals (Wolff and Dean 1987). Free radicals are the cause of lipid peroxidation and membrane damages. Thus, the desiccation tolerance of germinated seeds is rather related to the mechanism to prevent damages resulting of free radical action than sucrose and oligosaccharide relations.

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