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Solvent effect on quercetin antioxidant capacity

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Abstract

In this work, a study of the evolution of antioxidant activity during quercetin degradation under different solvent and temperature conditions is undertaken. In particular, quercetin solutions in ethanol, methanol or water–ethanol mixture were stored at 22, 37 and 60 °C, and analysed for antiradical activity by the DPPH· assay.

An initial increase and a following decrease in antiradical activity were observed for quercetin solutions in ethanol and methanol. Maximum antioxidant activity was higher and occurs in a shorter time as storage temperature was increased or solvent polarity was decreased. By contrast, a progressive decrease in antioxidant activity was observed in quercetin hydro-alcoholic solutions. Differences in antioxidant activity evolution were attributed to the development of selected reaction pathways: polymerisation was the prevalent oxidative reaction pool in ethanol or methanol solutions, whilst oxidative cleavage was favoured in hydro-alcoholic ones.

Keywords: Antiradical activity; Phenols; Oxidation; Polymerisation; Solvent; Temperature

1. Introduction

Phenols are present in important amounts in a great variety of plant matrices, such as fruits and leaves (Heim, Tagliaferro, & Bobilya, 2002; Hsieh & Kinsella, 1989; Peterson & Dwyer, 1998). Besides improving the stability of lipid-containing foods (Candlish & Das, 1996; Chen et al., 1991; Karastogiannidou, 1999; Schwarz et al., 2001), phenols are known to exert potential beneficial effects on human health by lowering the incidence of coronary heart disease (CHD), thrombotic and atherogenic processes (Ames, Shigena, & Hagwn, 1993; Aruoma, 1994; Facino et al., 1996; Hagerman et al., 1998; Huong, Matsumoto, Kasai, Yamasaki, & Watanabe, 1998; Kuehnau, 1976; Middleton & Kandaswami, 1993), as well as acting as antiviral agents against some diseases, such as diarrhea, arthritis, influenza and poliomyelitis (Esquenazi et al., 2002; Parmar et al., 1996; Serkedjieva & Hay, 1998). Since they represent common dietary constituents, the study of their properties has been shown to be fundamental in order to establish their effective role in human health. In fact, a large number of scientific reports indicates that phenol effects could be related to their strong antioxidant activity. These results have created a new perspective concerning the potential of dietary flavonoids in preventing serious diseases. However, despite the general acceptance of their health protecting capability, a clear correlation between phenol content in fruit and vegetables and their effect in vivo has not yet been reported.

Recent experimental evidence indicates that processing and storage conditions are expected to strongly affect content and biological activity of these molecules. Their fate upon processing is of great importance, considering that they can easily undergo chemical or enzymatic oxidation (Amiot, Forget-Richard, & Goupy, 1996; Nicoli, Calligaris, & Manzocco, 2000). Although phenol degradation is generally believed to be responsible for a depletion of antioxidant activity, this is not always the case. In fact, it has been observed that antioxidant properties of food, characterised by a high phenol content, such as tea beverages, apple derivatives and small fruits, progressively increased upon storage

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(Kalt, Forney, Martin, & Prior, 1999; Manzocco, Anese, & Nicoli, 1998). Similar results have also been obtained in model systems containing selected phenols, which were subjected to enzymatic or chemical oxidation. In both cases, oxidation promoted an initial increase and a following decrease in the antioxidant activity, so that a maximum value was observed (Cheigh, Umrn, & Lee, 1995; Nicoli et al., 2000). The peculiar evolution of the antioxidant activity during phenol oxidation has been attributed to the formation of partially polymerised phenols, which exhibit higher antioxidant activity than non-oxidised phenols or tannins formed in the advanced steps of the reaction. In fact, beyond a certain level of molecular complexity (more than four monomer residues), the antioxidant activity of polyphenols would decrease as a consequence of steric hindrance (Lu & Yeap Foo, 2000; Saint-Cricq de Gaulejac, Vivas, Freitas, & Burgeois, 1999).

It must be considered that phenol degradation occurs in plant foods under a wide variety of conditions. The latter are strongly dependent on both plant matrix and technological treatment applied. For instance, phenol degradation is expected to proceed differently upon storage of processed vegetables at chilling temperatures or during fermentation and ageing of alcoholic beverages. Hence, also the evolution of the antioxidant properties, as a consequence of phenol degradation in plant foods, is likely to be influenced by several factors. It might be expected that solvent properties and technological conditions (e.g. temperature/time combinations) could exert a critical role. In addition, synergy or counterbalancing effects among these variables, in determining the overall antioxidant activity, can not be underestimated. However, little information is available about the influence of these parameters on the changes in antioxidant properties during phenol degradation.

In the light of these considerations, the aim of this work was a comparative study of the evolution of antioxidant properties of phenol-containing model systems during degradation order different solvent and temperature conditions. Quercetin, which is one of the most abundant flavonoids in plant matrices (Bandoniene, 2002; Nianzeng, Chen, Mitchell, & Young, 2001; Pedrielli, Pedulli, & Skibsted, 2001; Russo, Toscano, & Uccella, 2000; Wollenber, 1982), being widely present in olives, tomatoes, apples, tea and red wine, was taken as an example. In particular, quercetin solutions in different solvents, containing ethanol, methanol or water, were used to carry out this study.

The selection of the solvents is supported by their different chemical characteristics. The choice of water and ethanol is justified by their abundant presence in all fruit and vegetable derivatives containing quercetin (from fruit juices to alcoholic beverages) (Fernández de Simón, 1995; Stecher, Huck, Popp, & Bonn, 2001; Stewart et al., 2000). Obviously, methanol is not a large component of these products but it represents a chemical intermediate between water and ethanol. For this reason it was chosen as a possible solvent which could give insights about the evolution of antioxidant activity under "intermediate" conditions.

Besides, the utilization of water, ethanol and methanol, as usual extraction solvents for polyphenolic compounds from several food materials, is important in order to manipulate or optimize the antioxidant capacity of these extracts (Moure et al., 2000; Rubilar, Pinelo, Sineiro, & Nũñez, 2003). Since properties of these solvents are well-known, they may allow us to model the changes in antioxidant activity. This is a necessary step in order to understand the antioxidant behaviour of complex matrices such as food systems, where antioxidants are subjected to a number of interactions with substances having very different chemical characteristics.

Solutions were stored at different temperatures (22, 37 and 60 °C). In this way it was also possible to study the effect of temperature on the antioxidant capacity of model systems; 22 and 37 °C are normal storage temperatures for foods, in which low storage temperatures are not required. In fact, most natural and processed foods, typically characterized by high polyphenol content, are subjected to storage at room temperature (Jinhe, hagenmaier, & Baldwin, 2002; Shashi, Tripathi, & Thakur, 2002). Furthermore, 37 °C is the intermediate temperature between the minimum value chosen (22 °C) and the extreme one, 60 °C, a temperature commonly reached during several technological operations involving plant matrices containing important amounts of phenols. Examples are spirit distillation, blanching of fruits and vegetables, concentration of plant matrices, brewing and malting.

2. Materials and methods

2.1. Sample preparation

Ethanol (Carlo Erba, Milano, Italy), methanol (Carlo Erba, Milano, Italy) or 10% (w/w) ethanol–water solutions containing 10^{-4} M quercetin (Q-0125, Lot 90K1746, Sigma, St. Luis, Mo, USA), were prepared. The pH values of the alcoholic and aqueous mixtures were 6.7 and 6.5, respectively. Five hundred ml capacity screw-capped flasks containing 200 ml of quercetin solution were maintained in water baths at 22, 37 and 60 °C. At different lengths of time, 5 ml aliquots the solutions were removed and immediately analysed.

2.2. Analytical determinations

2.2.1. Optical density

Chemical oxidation was followed by measurement of optical density at 380 nm using a Uvikon 860 spectro-

photometer (Kontron Instruments, Milano, Italy). This wavelength is in the spectral region of quercetin maximum absorption.

2.2.2. Antiradical activity

The antiradical activity was measured, following the methodology described by Brand-Williams, Cuvelier, and Berset (1995), wherein the bleaching rate of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH·) is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH· absorbs at 515 nm, but upon reduction by an antioxidant or a radical species its absorption decreases.

A volume of 1.80 ml of 6.1×10^{-5} M DPPH· methanol solution was used. The reaction was started by the addition of 200 μl of samples. The bleaching of DPPH· was followed at 515 nm (Uvikon 860, Kontron Instruments, Milano, Italy) at 25 °C for 20 min. In all cases, the DPPH· bleaching rate was proportional to the sample concentration added to the medium. The following equation was chosen in order to obtain the reaction rate of DPPH· bleaching, k (Manzocco et al., 1998; Nicoli et al., 2000):

$$\frac{1}{A^3} - \frac{1}{A_0^3} = 3kt, \tag{1}$$

where A_0 is the initial optical density and A is the optical density at increasing time, t. The antiradical activity was expressed as k ($OD^{-3}\,min^{-1}$ mg of dry $matter^{-1}$).

2.3. Statistical analyses

The results reported in this work are the averages of at least three measurements and the coefficients of variations, expressed as the percentage ratio between standard deviations and the mean values, were found to be less than 7 for antiradical activity and 6 for optical density.

3. Results and discussion

The changes in the antiradical activity of an ethanol solution containing 10^{-4} M quercetin during storage at different temperatures are shown in Fig. 1. A fast and remarkable increase in the antiradical activity of the solution stored at 60 °C was observed during the first four days of the reaction. When storage time was prolonged, the antiradical capacity was progressively decreased so that a maximum antioxidant activity value was observed. Although in different time scales and with different maximum values, similar antiradical activity evolution was also observed at 37 and 22 °C. These data are in agreement with the typical behaviour of some phenols, whose chemical oxidation promotes the formation of partially polymerised compounds. The latter

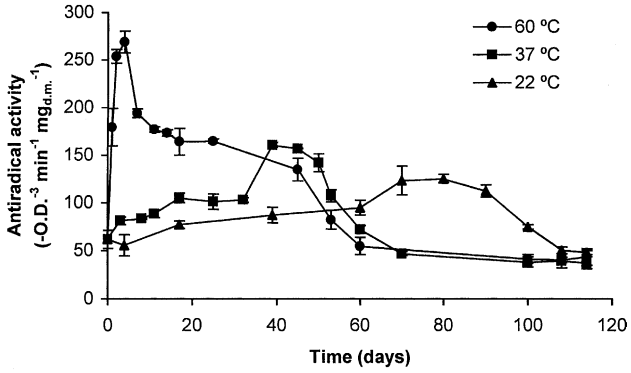

Fig. 1. Antiradical activity of quercetin ethanol solution as a function of storage time at 22, 37 and 60 °C.

have been shown to exert higher antioxidant activities than both non-oxidised phenols and tannins formed in the advanced steps of the reaction (Lu et al., 2000; Saint-Cricq de Gaulejac et al., 1999).

Notwithstanding the common evolution of antioxidant activity at the different temperatures, Fig. 1 clearly shows the critical role of this parameter in determining the maximum antioxidant capacity value and the relevant reaction time. In fact, as storage temperature was increased, the maximum value was observed to be higher and to occur in shorter time. Moreover, the period of maintenance of maximum antiradical activity was longer at lower temperatures. Due to the peculiar evolution of the antioxidant activity and to the marked effect of temperature, after 60 days of storage, the antioxidant capacity of the quercetin ethanol solution at 22 °C was higher than that detected at 37 and 60 °C. Data not shown reveal similar results for quercetin model systems in methanol and (9:1 w/w) water–ethanol. This result could contribute to explain unexpected fluctuations in grape juice antioxidant activity reported by Talcott and Lee (2002). These authors actually observed that red and white grape juices stored for two months at 20 °C exerted higher antioxidant activities than those stored at 37 °C, despite no decline in single polyphenolic compounds.

Changes in optical density at 380 nm of quercetin ethanol solution during storage at the different temperatures are plotted in Fig. 2. Comparing Figs. 1 and 2, it can be observed that the increase in antioxidant activity did not correspond to any appreciable change in optical density. By contrast, after reaching the maximum value, the decrease in antioxidant activity was associated with a concomitant decrease in optical density. Such an event corresponds to the formation of a complex mixture of compounds whose overall antiradical activity and optical density is lower than that of the reaction products previously formed. It can be inferred that, when the optical density starts to decline, further increase in antioxidant activity should not be expected. In other

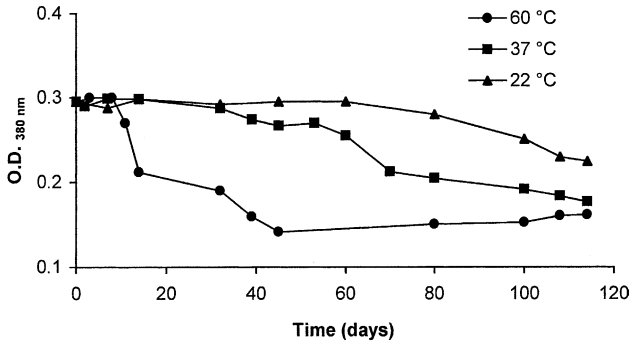

Fig. 2. Optical density of quercetin ethanol solution as a function of storage time at 22, 37 and 60 °C.

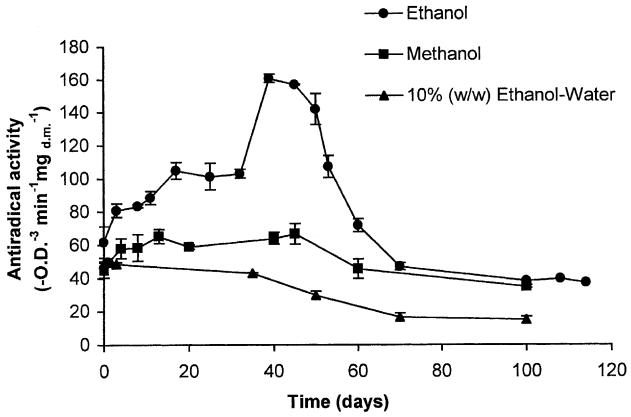

Fig. 3. Antiradical activity of quercetin in ethanol, methanol or 10% (w/w) ethanol–water solutions as a function of storage time at 37 °C.

words, such a decrease in optical density would indicate that the reaction phase, associated with the development of the maximum antioxidant potential, at a given temperature, is over. This phenomenological parallelism yields certain information about antioxidant behaviour of quercetin solutions obtained by optical density measurements, these being much easier and quicker to assess than measurement of antioxidant capacity. Future studies will reveal whether these observations in quercetin can be extrapolated to other phenolic models or more complex food systems, using optical density as a qualitative indicator of the antioxidant capacity of such systems.

The antioxidant capacities of solutions containing 10^{-4} M quercetin in ethanol, methanol and 10% (w/w) ethanol–water solution are shown in Table 1. Polarity of the solvent is also reported. Antioxidant capacity was popularly affected by the solvent. In particular, as polarity was increased, the same amount of quercetin exerted a lower antiradical activity. These results are in agreement with studies carried out by Valgimigli, Banks, Ingold, and Lusztyk (1995), emphasising that hydrogen bonding may induce dramatic changes in the H-atom donor activities of phenolic antioxidants. In this way Pedrielli et al. (2001) observed that quercetin antioxidant activity was much higher in the non-hydrogen-bonding solvent, chlorobenzene, than in the "water-like" solvent, *tert*-butyl alcohol. Likewise, van der Berg, Haenen, Van der Berg, and Bast (1999) reported that the antioxidant activity of quercetin in ethanol solution was near twice higher than that observed in the organic solvent Triton X-100, which presents an important polar group.

Table 1
Antiradical activity of quercetin in ethanol, methanol or 10% (w/w) ethanol–water solutions

Solvent	Antiradical activity[a] ($OD^{-3}\,min^{-1}\,mg^{-1}$)	Polarity[b]
Ethanol	61.8 ± 9.4	5.2
Methanol	46.9 ± 4.8	6.6
10% (w/w) ethanol–water solution	41.2 ± 1.25	9.0

Polarity of solvents is also reported.
[a] Mean ± SD($n = 3$).
[b] From Chemtech (1972) 359–363.

Evolution of antiradical activity of quercetin dissolved in the different media was detected during storage at different temperatures. Fig. 3 shows an example of the data obtained relevant to experiments carried out at 37 °C. Considerable differences in antioxidant behaviour were observed as a function of the dissolving media. While an increase in antiradical activity was detected in both ethanol and methanol systems, a progressive decrease was observed in the hydro-alcoholic one. Data not shown also showed no changes in optical density in the hydro-alcoholic solution, suggesting a different reaction pathway for quercetin degradation in aqueous systems from that occurring in alcoholic ones.

Table 2 compares the maximum antiradical activity and the relevant reaction time observed, during storage at 22, 37 and 60 °C, of quercetin dissolved in different media. The time at which optical density at 380 nm starts to decrease is also reported.

As observed at 37 °C (Fig. 3), also at 22 and 60 °C, the maximum antioxidant activity was in the order ethanol > methanol > hydro-alcoholic solution. In addition, maximum value in ethanol–water solution was observed for freshly prepared solution as, at all temperatures, a progressive decrease in antioxidant activity was always detected. Data relevant to the increase (ethanol and methanol media) or decrease (ethanol–water solution) in antioxidant activity upon quercetin degradation were elaborated to obtain their kinetic rate constants. The changes in antioxidant activity were found to follow the zero-order kinetic model for all temperatures and solvents considered. Rate constants of antioxidant activity change are shown in Table 3 and were used to calculate apparent activation energies according to the Arrhenius equation:

Table 2
Maximum antiradical activity, time needed to reach maximum antiradical activity (time max) and time needed for optical density decrease (time $OD_{380\,nm}$ decrease) observed during quercetin storage at 22, 37 and 60 °C in different solvents

Solvent	Temperature	Maximum antiradical activity ($OD^{-3}\,min^{-1}\,mg^{-1}$)	Time max (days)	Time $OD_{380\,nm}$ decrease (days)
Ethanol	22	126 ± 4.4	80	90
	37	160 ± 2.4	39	45
	60	269 ± 11.4	4	7
Methanol	22	59.6 ± 7.5	90	100
	37	66.8 ± 6.2	50	60
	60	102 ± 10.0	5	8
10% (w/w) ethanol–water solution	22	41.2	0	0
	37	41.2	0	0
	60	41.2	0	0

Table 3
Zero-order rate constants of changes in antioxidant activity observed during quercetin storage at 22, 37 and 60 °C in different solvents (determination coefficients in brackets)

Solvent	Rate of changes in antiradical activity (day^{-1})			Apparent E_a (kJ)
	22 °C	37 °C	60 °C	
Ethanol	0.009 (0.93)	0.019 (0.84)	0.372 (0.99)	80.9 (0.94)
Methanol	0.004 (0.88)	0.009 (0.93)	0.40 (0.87)	100.0 (0.93)
0% (w/w) ethanol–water solution	−0.009 (0.60)	−0.014 (0.85)	−0.028 (0.89)	24.7 (0.99)

Apparent activation energies (E_a), calculated according to Eq. (1), are also reported.

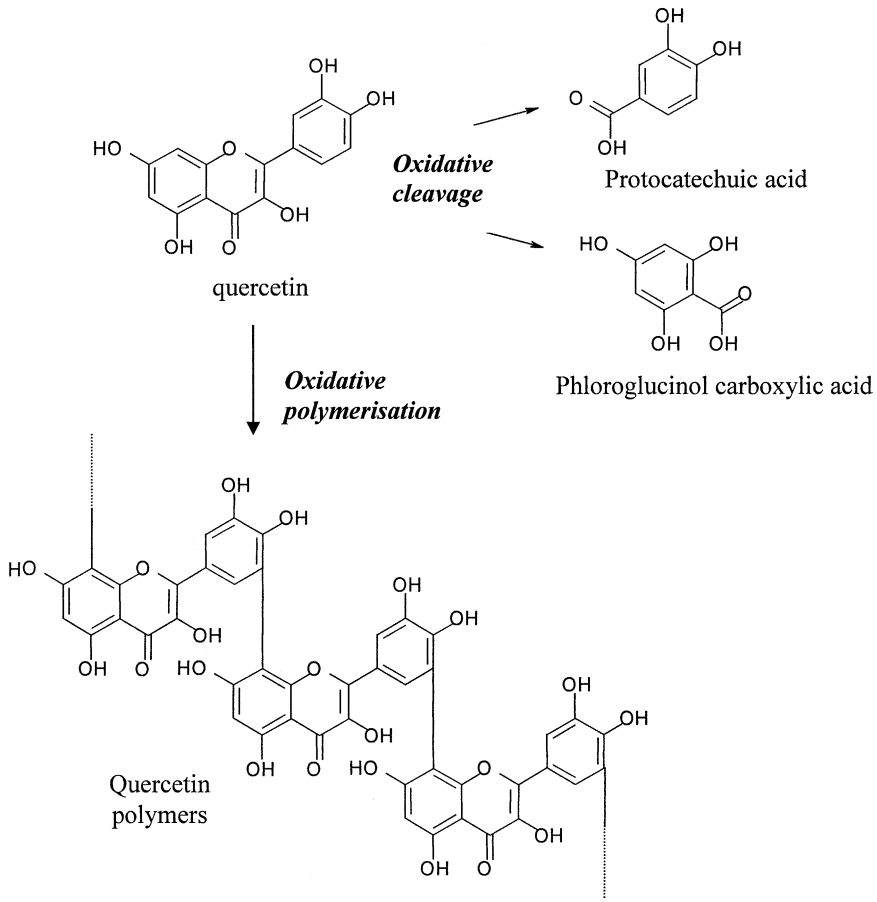

Fig. 4. Oxidative cleavage and oxidative polymerisation of quercetin.

$$k = k_0 e^{-E_a/RT}, \quad (2)$$

where k is the zero-order rate constant of the changes in antioxidant activity, T is temperature (K), E_a is the activation energy, R is the universal gas constant and k_0 is an experimental constant.

Table 3 shows that, in ethanol and methanol solvents, the rates of the changes in antioxidant activity are characterised by similar temperature dependence, whilst a much lower E_a value was detected for oxidation occurring in the hydro-alcoholic media. These results clearly indicate that, while phenol polymerisation develops, even to different extents, in ethanol and methanol solutions, aqueous media prevent its occurrence. In fact, due to the low solvating capacity of water and to its high hydrogen-accepting properties, quercetin molecules hardly co-ordinate to form complex polymers and the most probable reaction pathway becomes that of oxidative degradation (Fig. 4). As previously reported by Makris and Rossiter (2000), heating of quercetin in aqueous solutions causes cleavage of its skeleton, leading to the formation of characteristic fragments, such as protocatechuic acid and phloroglucinol carboxylic acid (Fig. 4). The latter are known to be less efficient antioxidants than the parent molecule quercetin (Makris & Rossiter, 2001), thus explaining the progressive decrease in antioxidant activity observed in the hydro-alcoholic media (Fig. 3 and Table 3).

4. Conclusions

Results obtained in this study clearly showed that quercetin oxidative degradation promotes peculiar changes in the antioxidant capacity, which are temperature-and solvent-dependent. While antioxidant activity evolution is affected by temperature according to the Arrhenius model, solvent may contribute to select the main reaction pathway. In particular, when quercetin was allowed to react in ethanol or methanol solutions, the formation of complex polymers was the prevalent oxidative reaction pool, leading to an initial increase and a following decrease in antiradical activity. By contrast, a decrease in antioxidant activity during quercetin degradation was observed in hydro-alcoholic solutions. It can be inferred that, polymerisation of quercetin molecules was prevented by the presence of water due to its high hydrogen-accepting capability, which makes oxidative cleavage the most probable reaction pathway.

It can be concluded that the antioxidant capacity of phenol-containing foods is expected to greatly change during processing as a function of the technological conditions adopted. However, it is noteworthy that plant foods contain numerous phenolic substances whose oxidation concomitantly occurs, potentially contributing to unexpected changes in antioxidant activity. Certainly, the ability of phenols to resist oxidative cleavage and polymerise, leading to an improvement in the overall antioxidant activity of plant foods, is highly associated with their structure. This work shows that, in the case of quercetin activity is also strictly dependent on solvent nature and temperature/time conditions. Indeed, further factors, such as pH, as well as the presence of oxygen and oxidative agents or enzymes, could play important roles. Currently, work is in progress on complex mixtures of phenols, which will further clarify their behaviour during processing. It is expected that knowledge of the reactivity of phenol compounds under different conditions will greatly facilitate interpretation of epidemiological data relevant to processed fruit and vegetables, thus providing clues to understanding their complex biological activity.

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