

## Effects of pre- and postharvest factors on browning in Braeburn

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### Abstract

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The effects of several pre- and postharvest factors on apple cv. Braeburn browning disorder (BBD) incidence using a nine-factor experimental design has been investigated. The design allowed the determination of the effects of single factors as well as their interaction with growing season and storage time. BBD increased in severity with storage duration. BBD incidence was reduced with calcium and potassium fertilizers application, while it was increased when triazoles were used. Delayed controlled atmosphere (DCA) application resulted in less BBD in storage, while treatment with 1-methylcyclopropene (1-MCP) increased BBD incidence. More BBD was observed in fruit stored at above optimal CO<sub>2</sub> levels. BBD incidence was increased when O<sub>2</sub> concentration in CA was increased from 1 kPa to 3 kPa (optimum CA) or 6 kPa. Finally, the various factors showed a different effect for different growing seasons and storage time. These findings suggest a possible mechanism for the development of BBD. Further work should focus on extending the experimental design to include the interactions between the different pre- and postharvest factors.

**Keywords:** Braeburn browning disorder; fertilizers; triazoles; controlled atmosphere; 1-methylcyclopropene

*Malus domestica* Borkh., cv. Braeburn is a popular apple cultivar worldwide. To facilitate year-round availability of fruit, apples are stored at low temperature under controlled atmosphere (CA) conditions. Under the commercial storage conditions, apples can be stored well as a result of their reduced metabolic rate. The storability of cv. Braeburn is, however, limited by the development of internal browning (ELGAR et al. 1999). Affected fruit show patches of brown tissue which can result in cavity formation

(ELGAR et al. 1998). The combination of brown flesh and off flavours makes brown apples unacceptable in the market, resulting in economic losses.

Different apple varieties vary in their susceptibility to browning (STREIF 2008). Cv. Braeburn has a high skin resistance (RAJAPAKSE et al. 1990) turning the skin into a stronger diffusion barrier as compared to some other apple cultivars. Together with a dense cellular organization this results in relative high internal CO<sub>2</sub> concentration (HERREMANS et

al. 2013). These factors contribute to the sensitivity of cv. Braeburn to browning in storage (LAU 1998).

Preharvest factors influencing browning include seasonal changes and the application of agrochemicals. Cv. Braeburn fruit grown in cold seasons show more browning incidence than fruit from warmer seasons, and this might be attributed to increased respiration rate and reduced skin and tissue diffusivity in fruit from cold seasons (LAU 1998). Calcium plays an important role in maintaining the postharvest quality of fruit. It stabilizes the cell membranes and delays fruit senescence (MARINOS 1962). More browning was observed in cv. Braeburn fruit grown in soils with low calcium levels (RABUS, STREIF 2000). Potassium is also an important nutrient associated with browning in apple. JOHNSON (2009) showed that triazole application induced browning in cv. Cox apples.

Postharvest factors that affect the quality of pome fruit include picking date, gas levels, temperature and delayed controlled atmosphere (DCA) application (BLANPIED 1975; LAMMERTYN et al. 2000). Late season apples show more browning incidence (ELGAR et al. 1999). This can be explained by the high metabolic rate and fast ripening in addition to increased skin and tissue resistance to gas exchange in late season fruit (LAU 1998). ELGAR et al. (1998) showed that delaying the CA application reduces browning incidence in cv. Braeburn apples. This is perhaps due to higher energy levels in fruit subjected to delayed CA (SAQUET et al. 2003). During CA storage, O<sub>2</sub> concentration is decreased to reduce cellular respiration while avoiding fermentation. Depending on the fruit's tissue properties local hypoxic regions might still arise inducing fermentation providing insufficient energy to repair damage to cellular membranes (on cv. Conference pear: FRANCK et al. 2007; on apple: HO et al. 2011, 2013). As a result of de-compartmentalization, phenolic compounds come into contact with polyphenol oxidase and are oxidized to brown pigments (MAYER 2006). During CA storage CO<sub>2</sub> concentration is often slightly increased to further reduce the fruit's metabolic rate (McGLASSON, WILLS 1972). However, care should be taken as too high CO<sub>2</sub> concentration is a strong trigger of browning in cv. Braeburn apples (ELGAR et al. 1998). Cold storage ensures that metabolic reactions are reduced (BOHLING, HANSEN 1983), yet, too low temperatures are to be avoided as they can lead to cold-related fruit injuries. The postharvest application of the ethylene action inhibitor, 1-methylcyclo-

propene (1-MCP) is often used to preserve fruit firmness and total acid content (DEELL et al. 2003). LAFER (2008) showed that 1-MCP treated cv. Braeburn apples had an increased incidence of browning compared to the untreated group.

Previous research has often focused on the effect of individual factors on browning development; however, it is more likely that the occurrence of browning is the result of complex interactions among those factors. VERLINDEN et al. (2002) showed that the probability of core breakdown in cv. Conference pears depended on several variables in a more complicated way than assumed before. Reviewing the browning disorders in pears, FRANCK et al. (2007) suggested that it is unsatisfactory to evaluate the effect of single factors individually.

In this study, we aimed to investigate the effects of several pre- and postharvest factors, and their interactions with the growing season and the storage duration, on the development of BBD. For this purpose, a balanced experimental design consisting of different combinations of optimal and sub-optimal levels of the chosen factors was applied.

## MATERIAL AND METHODS

**Experimental design.** In this work, a two-level, nine-factor, fractional factorial design was utilized. A fractional factorial design was important to reduce the number of possible combinations that results from full factorial designs. As a result, only information about the effects of single factors in addition to the interaction with storage time and growing season could be deduced. The experimental design as depicted in Table 1, consisted in 2010 of 12 combinations of 3 preharvest factors (calcium, potassium and triazoles), harvest date and 5 postharvest factors (DCA, O<sub>2</sub>, CO<sub>2</sub>, temperature and 1-MCP). In 2011, storage conditions were chosen based on the results of 2010; no delayed CA was used and the storage temperature was kept at 1°C for all tests, resulting in a total of 8 different combinations. The levels of the nine factors used were coded as 0 and 1.

**Fruit growing and harvesting.** Braeburn, a browning susceptible cultivar, and Jonagold, a browning insensitive cultivar, were grown and picked in the orchard of the experimental tree fruit research station (RSF-PCFruit) in Sint-Truiden, Belgium. Fruits were grown under 8 different con-

Table 1. Overview of the different treatment combinations used for the growing and storage of cvs Braeburn and Jonagold apples during the storage seasons 2010 and 2011

Test ID	2010										2011										Explanation labels (0/1)	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Calcium	1	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	1	1	0	1	no	/
Potassium	0	1	1	0	1	1	1	0	0	0	1	0	0	0	1	0	1	0	1	1	no	/
Triazoles	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	1	0	1	1	1	no triazoles	/
Harvest	0	1	0	1	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	early	/
Delay	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	no	/
O <sub>2</sub>	0	0	0	1	0	1	1	0	1	1	1	0	1	0	1	0	0	1	0	1	optimal	/
CO <sub>2</sub>	1	0	0	0	1	0	1	1	0	1	1	0	1	1	0	0	0	0	1	1	optimal	/
Temp	1	1	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	optimal	/
1-MCP	1	1	1	0	0	0	1	0	1	1	0	0	0	1	1	1	0	0	0	1	no	/

\*2010 season; \*\*2011 season

ditions, as defined by the fertilization regimes for calcium and potassium and the use of triazoles and growth regulators. Calcium and potassium were applied in a minimal or maximal amount of fertilizer, both in the soil and on the leaves. Maximal triazole application was used for some fruit, while fruit without triazole treatment received other fungicides and growth regulators.

Cv. Braeburn apples (Hillwell rootstock, 9<sup>th</sup> and 10<sup>th</sup> growth year) were harvested on two different dates (early – 19/10/2010 and late – 08/11/2010) in 2010 and in 2011 (early – 07/10/2011 and late – 26/10/2011). Jonagold apples (Novajo rootstock, 7<sup>th</sup> growth year) were harvested on two different dates (early – 10/09/2010 and late – 08/10/2010) only in 2010. Early and late harvest dates of both cultivars were determined by the Flanders Centre of Postharvest Technology based on a combination of firmness, starch, sugars and acids measurements.

**Controlled atmosphere storage.** Five different postharvest factors were considered in the experimental design for season 2010. These factors were: time of DCA application, O<sub>2</sub>, CO<sub>2</sub>, temperature and 1-MCP treatment (Table 1). The commercial storage conditions for Jonagold (1°C, 1 kPa O<sub>2</sub> and 2.5 to 3 kPa CO<sub>2</sub> with 1 day DCA) and for cv. Braeburn apples (1°C, 2.5–3 kPa O<sub>2</sub> and 0.7 kPa CO<sub>2</sub> with 21 days DCA) were used as optimal conditions. Different combinations of the optimal and suboptimal levels of these factors were applied. Some fruits were directly stored at CA, while others were kept in air at 1°C prior to CA application. Suboptimal levels were as follows: in the case of O<sub>2</sub>, 3 kPa above optimum in 2010 and 2 kPa below optimum in 2011; in the case of CO<sub>2</sub>, suboptimal level was 3 kPa above the optimum; for storage temperature, suboptimal level consisted of raising the temperature from 1°C to 4°C. In the case of 1-MCP, some fruit received commercial application, while others were stored without previous treatment with 1-MCP.

**Internal browning assessment.** In 2010, cvs Braeburn and Jonagold apples were rated for browning at harvest and after three and six months of storage using 30 apples per condition. As in 2010 browning was already detected early in storage, assessments in 2011 were done more frequently: at harvest, after two and four weeks, and after two, four and six months of storage. Slices (~1 cm thick) were cut from the middle of the fruit perpendicular to the longitudinal axis, and pictures were taken. Included in the pictures was a colour-calibration card that was used for correcting for light differ-



Fig. 1. Cross-section of apples with internal browning (left picture, BI of 22.6) and without browning (right picture, BI = 4.89). Calibration card: class 1 (yellow, BI = 1) to class 10 (brown, BI = 100)

ences between images (Fig. 1). Browning was rated by using an in-house developed Matlab R2010 (Mathworks, Natick, USA) program in which the colour of each pixel of the cortex (excluding the core, the skin, and the reddish coloured outer layer directly underneath the skin) was compared to the yellow to brown colours from the calibration card. A brown index (BI) was calculated as the average of the squared colour indices of all pixels from the cortex, so that the contribution of any brown spot in the apple slice to the average BI is increased. As the colour scale is based on 10 classes (yellow = 1 to brown = 10) the calculated BI ranges from 1 to 100.

**Data analysis.** To determine the effects of the factors as well as the interactions between them, data was subjected to ANOVA. Only differences (between treatment levels) for which the model was significant ( $P < 0.05$ ) were considered for further analysis. For browning analysis, only the 10 brownest apples from each condition were considered. By choosing the average of the 10 brownest apples instead of the complete 30 fruit replicates per treatment, the analysis was made more sensitive to small changes in browning incidence, and thus the difference between the treatments was accentuated.

## RESULTS AND DISCUSSION

The effects of the various pre- and postharvest factors on browning in cv. Braeburn apples are shown in Figs 2 and 3. The figures only present the results that showed statistically significant differences ( $P < 0.05$ ) between the different treatment levels. When a factor showed no significant year

effect, it was represented by the overall average of the two seasons.

### Cultivar effect

Under all pre- and postharvest treatment combinations used, cv. Jonagold did not develop browning during storage (data not shown). This is consistent with previous research that showed cv. Jonagold to be resistant to the development of internal browning during storage (SAQUET et al. 2000). Braeburn cv., on the other hand, developed browning during storage with higher incidence in 2011 than in 2010 (Fig. 2a).

### Fertilization effects

Fruit that received max. calcium treatment developed less BBD as compared to those without calcium (Fig. 2b). RABUS and STREIF (2000) showed that the use of calcium fertilizers resulted in a lower susceptibility of cv. Braeburn apples to browning in storage. As calcium stabilizes the membranes (MARINOS 1962), the risk of decompartmentalisation, potentially bringing phenolic compounds in contact with polyphenol oxidases, is reduced. Besides, calcium was also shown to be a necessary factor for the process of membrane repair (STEINHARDT et al. 1994). Potassium competes with calcium absorption from the soil which might therefore lead to higher browning incidence (NEILSEN, NEILSEN 2009). However, in our study, max. potassium treatment resulted in decreased BBD incidence in 2011 fruit, while 2010 fruit showed only a slight decrease (Fig. 2c).

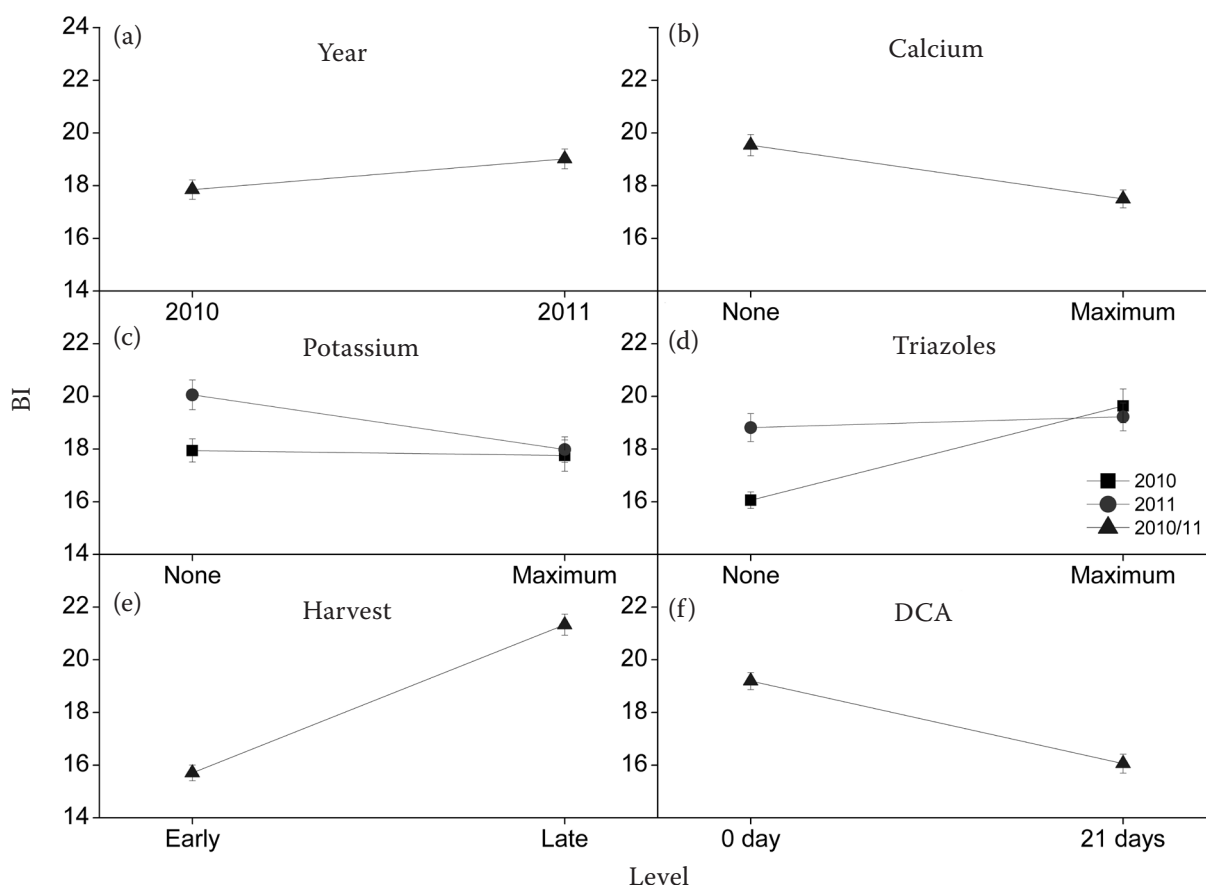


Fig. 2. Effects of the different growing treatments, harvest date, delayed application of controlled atmosphere (DCA), and season on browning index (BI) in cv. Braeburn apples in 2010 and 2011

Measurements were done at harvest and after CA storage. Values are means  $\pm$  standard error. Only statistically significant differences ( $P < 0.05$ ) between the 2 treatment levels are shown

### Triazole effects

In 2010, treatment with max. triazole resulted in fruit with more BBD (Fig. 2d). This is consistent with a study by JOHNSON (2009) on Cox's Orange Pippin apples, where triazoles resulted in higher browning incidence. Triazoles inhibit brassinosteroids (TADAO et al. 2003), which are plant hormones that induce ethylene synthesis (ARTECA et al. 1988). Reduced ethylene levels result in lower ATP levels which can lead to the impairment of the membrane repair mechanism; thus contributing to more browning incidence. On the other hand, the higher respiration rate upon ethylene action is responsible for decreased  $O_2$  concentration in the internal fruit tissue with a subsequent increase in browning incidence (HO et al. 2011). In 2011, however, fruit that received max. triazole treatment only showed a slight increase in BBD incidence (Fig. 2d).

### Harvest date effects

Late harvest apples showed more BBD than early harvest apples (Fig. 2e). This is similar to the increased incidence of browning in cv. Braeburn apples reported by RABUS and STREIF (2000). This might be due to the high respiration rate and the increased resistance to gas transport in late harvest fruit, thus resulting in decreased  $O_2$  and increased  $CO_2$  concentration in the internal tissue (LAU 1998; HO et al. 2011).

### CA effects

Delaying the CA application resulted in less BBD incidence (Fig. 2f). This is in line with previous research on cv. Braeburn (ELGAR et al. 1998). This decrease in browning incidence might be due to the

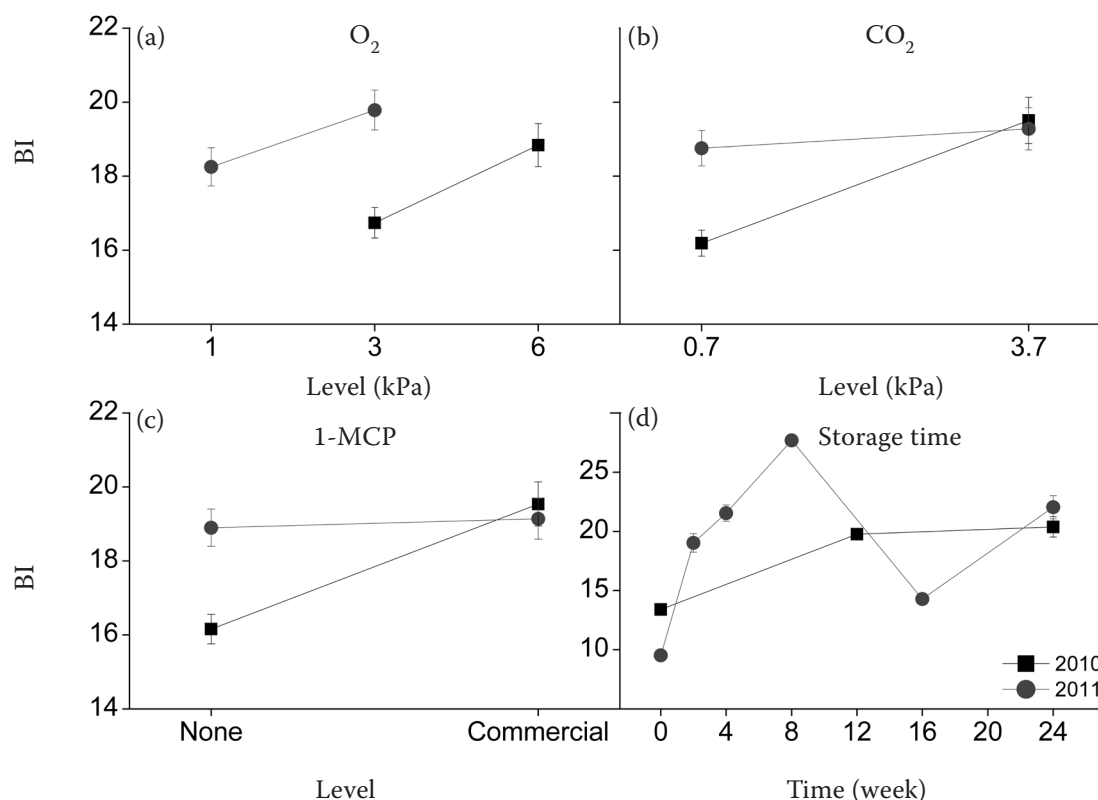


Fig. 3. Effects of O<sub>2</sub>, CO<sub>2</sub>, 1-MCP and storage time on browning index (BI) in cv. Braeburn apples in 2010 and 2011 (a) – O<sub>2</sub> concentration in the storage atmosphere (1.3 and 6 kPa); (b) – CO<sub>2</sub> concentration in the storage atmosphere (0.7 and 3.70 kPa); (c) – 1-MCP treatment (untreated/commercial application); (d) – storage duration (2, 4, 8, 12, 16 and 24 weeks of CA storage). Values are means ± standard error. Only statistically significant differences ( $P < 0.05$ ) between the 2 treatment levels are shown

increased energy level after delaying the CA application (SAQUET et al. 2003). Increasing the concentration of O<sub>2</sub> from 3 to 6 kPa in 2010 season resulted in increased BBD incidence. Reduction in BBD incidence was observed in 2011 when the O<sub>2</sub> concentration was lowered from 3 kPa to 1 kPa (Fig. 3a). This might be due to the fact that although the increased O<sub>2</sub> level in the range studied (1–6 kPa) leads to higher respiration rates, and thus more explain adenosine triphosphate (ATP) production, the potential production of ROS (a respiration by-product) also increases. The net balance between the two processes is of such nature that the incidence of BBD was higher when the O<sub>2</sub> concentration was increased.

More BBD incidence was observed in both seasons when CO<sub>2</sub> concentration was raised (0.7 to 3.7 kPa). This increase, however, was less pronounced in 2011 (Fig. 3b). These results are in agreement with previous reports that showed more internal browning in cv. Braeburn apples when CO<sub>2</sub> concentration was increased to 3 kPa (LAU 1998; ARGENTA, MATTHEIS 2010). High CO<sub>2</sub> levels shift the metabolism from the

Krebs cycle to fermentation resulting in inadequate energy for the maintenance of membranes which eventually might lead to browning development. Fermentation also produces acetaldehyde and ethanol which were found to be associated with higher internal browning in apples (LEE et al. 2012b).

### 1-MCP effects

1-MCP treatment resulted in fruit with higher BBD incidence in 2010. However, in 2011 the browning incidence was similar in both treated and untreated apples (Fig. 3c). The increased BBD incidence with 1-MCP treatment is similar to the results reported by LAFER (2008) on cv. Braeburn apples. 1-MCP inhibits ethylene production resulting in lower energy levels, thus leading to impairment of the maintenance of cellular membranes. Besides, 1-MCP treated fruit show increased polyphenol oxidase (PPO) activity (JUNG, WATKINS 2011), which is the main enzyme responsible for browning reactions.

### Storage time effects

BBD was observed in cv. Braeburn fruit early in storage, and the incidence increased with storage time reaching the highest level after 8 weeks of storage. Surprisingly, the fruits that were analysed after 12 and 16 weeks showed less browning as compared to those after 8 weeks of storage. After 24 weeks of storage, a further increase in BBD incidence was observed (Fig. 3d). This compares to previous reports which showed that browning incidence increased with storage duration (CLARK, BURMEISTER 1999; LEE et al. 2012a).

### CONCLUSION

The objective of this work was to study the effects of several pre- and postharvest factors on browning in cv. Braeburn apples. Treatments with calcium and potassium reduced the incidence of BBD, while the incidence was increased when triazoles were applied. Reduced BBD incidence was also observed when the application of CA was delayed. On the other hand, 1-MCP treatment increased the risk of BBD development. The concentration of gases in the storage atmosphere played an important role, increasing BBD incidence when higher concentrations of O<sub>2</sub> or CO<sub>2</sub> were applied. Finally, the response of fruit to the various pre- and postharvest treatments varied between the growing seasons and with the storage duration. These findings could suggest a possible mechanism for the development of internal browning in cv. Braeburn apples. Further work should focus on extending the experimental design to include more factors (e.g., crop load, different orchards), and the interaction terms between the different pre- and postharvest factors.

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