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Effects of anti-browning agents on wound responses of fresh-cut mangoes

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Abstract

The effects of different anti-browning compounds (ascorbic acid, citric acid, L-cysteine and glutathione) on L^*-value, hue angle, brown scores and brown pigments of fresh-cut mangoes cv. 'Nam Dok Mai' were investigated at four concentrations (0% as control, 0.5%, 1.5% and 2.5%). The browning attributes of treated fresh-cut mangoes were modelled to express the processes and to analyse the data obtained. The results revealed the similar changing tendency of L^*-value and hue angle. They decreased in time during storage at 10°C, while the brown scores and amount of brown pigment increased. All processes were found to follow a first order reaction mechanism of decay or growth. A validation experiment was conducted by dipping fresh-cut mangoes in a different anti-browning solution of ascorbic acid, citric acid, L-cysteine or glutathione at 1.5% concentration and distilled water as control. The estimated values for the browning attributes using proposed models had good agreement with the measured values. In this validation experiment, other changes namely respiration rate, and enzymes related to browning (polyphenol oxidase (PPO) and peroxidase (POD)) were determined. Treatment with L-cysteine or glutathione was effective in suppressing tissue metabolism and enzyme activity, while citric acid significantly inhibited the growth of microorganisms as indicated by total plate count (TPC) (data not shown).

Introduction

Fresh-cut fruit is a strong and ever growing segment of food products. Usually, the quality of fresh-cut product is evaluated based on appearance such as colour at the time of purchase. However, fresh-cut process increases the metabolic activity and the de-compartmentalisation of enzymes and substrates in tissues inducing changes in flesh colour (Ahvenainen, 1996). The most important enzyme associated with discoloration of fresh-cut fruit is polyphenol oxidase (PPO), which is typically present in the majority of plant tissues (Garcia and Barret, 2002). PPO can induce the browning occurrence by catalyzing the oxidation of phenol to o-quinones which are polymerized to produce brown pigments. Some properties of PPO in mango and product have been reported (Yoruk and Marshall, 2003; Guerrero-Beltran *et al.*, 2005). In addition, peroxidase (POD) is another main enzymatic browning (Yingsanga *et al.*, 2008) that is commonly associated with injury and wound response related to the hydrogen peroxide (H_2O_2) (Toivonen and Brummell, 2008).

Postharvest techniques maintaining the quality of fresh-cut fruit have been investigated by several researchers (Abe and Watada, 1991; Watada *et al.*, 1996; Paull and Chen, 1997; Buta *et al.*, 1999; Pittia *et al.*, 1999; Gonzalez-Aguilar *et al.*, 2005, Djioua *et al.*, 2009). To extend the shelf life of fresh cut mangoes, many methods have been proposed, including physical and chemical treatments. Control of enzymatic browning in fresh-cut mangoes has been achieved through different pre-treatments, based on individual anti-browning agents or mixtures of them: calcium ascorbate with citric acid and N-acetyl-L-cysteine (Plotto *et al.*, 2010), citric acid (Chiumarelli *et al.*, 2011), ascorbic acid with citric acid and calcium chloride (Robles-Sanchez *et al.*, 2009) and 4-hexylresorcinol with potassium sorbate and D-isoascorbic acid (Gonzalez-Aguilar *et al.*, 2000). None of these studies, however, concern the modelling of the effects anti-browning agents on colour change in fresh-cut mangoes.

The aim of the research was therefore to investigate the role of anti-browning agents on the browning of fresh-cut mangoes to describe, quantify and optimise their effectiveness in preventing the development of browning reactions in this product. For model validation, finally, the colour change of fresh-cut mangoes dipped with each anti-browning

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agent at 1.5% concentration was measured and compared with the estimated values. Respiration rate, and enzymes related to browning (polyphenol oxidase (PPO) and peroxidase (POD)) were also daily determined.

Materials and Methods

Fruit source and preparing

'Nam Dok Mai' mango (*Mangifera indica* L.) was obtained from a commercial grower in Nakhon Pathom Province, Thailand (north-west of Bangkok). Fruit were selected at the commercial maturity stage and allowed to ripen at room temperature for 3 days. Ripe mangoes with a flesh firmness of 5.95±0.56 N were selected for processing. Before cutting, fruit were washed with running tap water at room temperature followed by submersion into 150 ppm sodium hypochlorite for 2 min. They were then air-dried at room temperature. After that, each fruit was halved and the fleshy sections were cut with a sharp knife one in the longitudinal direction and three in cross sectional direction.

Preliminary experiment

Each half slice of 'Nam Dok Mai' mango was separately dipped in a different anti-browning solution of ascorbic acid, citric acid, L-cysteine or glutathione with concentrations of 0 (control), 0.5, 1.5 and 2.5%, (w/v). After air drying, they were stored at 10°C and 90-95% RH for 4 days. Colour data, brown colour (score) and brown pigment were recorded daily. The results from this experiment were used to estimate the rate constant in the considered model.

Model development

Normally, colour changes of selected products under postharvest storage conditions and processing have been reported and modelled by a first order kinetic model (Lau *et al.*, 2000; Schouten *et al.*, 2009; Sothornvit and Kiatchanapaibul, 2009). Also the other quality characteristics such as firmness and nutritional content have been modelled according to a first order mechanism (Tijskens *et al.*, 1998, Nourian *et al*., 2003, Piagentini *et al.*, 2005, Villanueva *et al*., 2005). In this research, changes in browning attributes of fresh-cut mangoes over time can be extracted following the fundamental rules of chemical kinetic. The model that describes the colour degradation and evolution over the storage period was an exponential decay (1) and growth (2) equations.

$$Q_t = Q_0 \cdot e^{-k \cdot t} \quad (1)$$

$$Q_t = Q_0 \cdot e^{k \cdot t} \quad (2)$$

Where Q_t is the measured value of colour variables at time t, Q_0 the initial value of colour variables at time zero, t the storage time (day), and k reaction rate constant (day^{-1}).

Validation experiment

To verify the acceptability of the models, a validation experiment was carried out by separating fresh-cut mangoes into five groups. The first group consisted of control samples (without dipping) and the others were individually dipped in a different anti-browning solution of ascorbic acid, citric acid, L-cysteine or glutathione at 1.5% concentration. After that, all of each group was respectively stored at 10°C and 90-95% RH. The quality measurements were conducted daily for 4 days.

Colour measurement

Changes in flesh colour were assessed using a Minolta chromameter (model DP-300, Osaka, Japan), expressing the colour parameters in the CIELAB system (L^*,a^*,b^*). The equipment was calibrated against a standard white calibration plate. Flesh colour of each peeled half fruit per replicate was measured at 3 points: at the top, middle, and bottom of each fleshy section. For each treatment colour data were daily recorded for four replicates in each series.

Browning assessment (score)

The extent of the total browning (flesh colour) was visually assessed every day for each cutting area according to the following scale: 1 = no browning (excellent quality); 2= slight browning; 3 = <25% browning; 4 = < 50% browning; 5 = >50% browning (poor quality). Brown colour was expressed as average value. The evaluated index at level higher than 3 was considered unacceptable for marketing.

Brown pigment

Brown pigment of fresh-cut mangoes was daily measured according to Jiang *et al.* (1999). Five gram of flesh sample in each of four replicates were finely extracted with 17 ml of 60% methanol (v/v) in a 0.1 M sodium phosphate buffer (pH 6.8) and 0.5 g of polyvinylpyrolidone (PVP). The extracts were centrifuged at 4500 rpm for 20 min. The supernatant was filtered through Whatman No.1 paper, collected and diluted to 1:4 ratio with sodium phosphate buffer. The absorbance was measured with a spectrophotometer at 410 nm (UV-1600, Shimadzu Co., Japan).

Respiration rate

Respiration rate of fresh-cut mangoes was

measured using a closed system method according to Kang and Lee (1997) and Techavuthiporn *et al.* (2008). The sample was weighed and placed in a gas-tight plastic chamber. The CO_2 concentration in the chamber was measured at the start and after about 30 min. A 1-ml gas sample was withdrawn through the septum using gas-tight syringe and injected into the gas chromatograph (GC) (Model GC-8A, Shimadzu, Japan). The gas sample was separated by WG-100 column and analysed by a thermal conductivity detector (TCD). Respiration rate was expressed in term of CO_2 production (mg kg^{-1} h^{-1}).

Enzyme assay and protein determination

For the determination of polyphenol oxidase (PPO) and peroxidase (POD), one flesh sample (2 g) from each half side was homogenized at 4°C with 20 ml of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.25 g of polyvinylpyrrolidone (PVP, Sigma). The sample was filtered and centrifuged at 19,000 rpm for 20 min at 4°C. The supernatant was used as crude enzyme extract.

PPO activity was assayed with 4-methylcatechol as substrate according to the method of Jiang (2000). 0.5 ml supernatant (the crude enzyme extract) was added to 2.0 ml 0.05 M sodium phosphate buffer (pH 7.0) and 0.5 ml 0.1 M 4-methylcatechol. The change in absorbance at 410 nm was recorded automatically after 5 min of reaction at 25°C.

POD activity was determined against guaiacol as substrate: 25 µl crude enzyme extract was mixed with 2.78 ml 0.05 M sodium phosphate buffer (pH 7.0), 0.1 ml 0.02 M H_2O_2 and 0.1 ml 0.02 M guaiacol. The assay mixture was incubated at room temperature (25°C). The change in absorbance at 470 nm was followed by spectrophotometer (Zhang *et al.*, 2005). Protein concentration was determined in the extracts according to the dye-binding assay of Bradford (1976).

Statistical analysis

All the measurements were done in 4 replicates in each experiment. Results are presented as means and standard deviation. The values of the rate constant (k) in Eq. (1) and (2) were estimated by the least squares method using Excel (Excel 2002, Microsoft Co., Japan). To evaluate the efficiency of prediction of the model, a mean relative percentage deviation in modulus (P) was estimated (Boquet *et al.*, 1978).

Results and Discussion

Model development and verification

Experimental results obtained in this work (L^*-value, hue angle, browning scores and pigment) were described by a first-order kinetic model: Eq. 1 was used for expressing changes in L^*-value and hue angle, while Eq. 2 was used for expressing changes in brown scores and brown pigment. The kinetic parameters and the confidence intervals at 95% were estimated by the least squares method as shown in Table 1. All experiment data were successfully fitted to the proposed equations (Eq. 1 and 2). The estimated parameters considerably lowered for higher concentrations used, which indicates that colour change in cut mangoes is affected by the application of anti-browning agents.

The measured and predicted colour parameter of control and fresh-cut mangoes dipped in various anti-browning agents at the concentration 1.5% are shown in Figure 1. Measured values of L^*-value (Figure 1a) and hue angle (Figure 1b) decreased with time, while brown scores (Figure 1c) and brown pigment increased (Figure 1d). Irrespective of the anti-browning agent applied, the trend in all colour aspects was the same.

The P values (a mean relative percentage deviation in modulus) of changes in those browning attributes were investigated. The obtained standard errors of estimates, indicated in Table 1, were not higher than 10%, except for the control of brown pigment parameter. Comparing results of different anti-browning agents used at 1.5% concentration, the overall value obtained from L-cysteine is greater, which would be said that brown colour of cut surface of mangoes is more suppressed. This study revealed that brown colour of cut mango might be the most sensitive to L-cysteine. L-cysteine may react with quinines to give colorless product owing to the high nucleophilicity of the thiol group and directly inhibit the activity of PPO (Ali *et al*., 2015).

Effects of anti-browning agents on colour attributes

L^*-value (lightness) of fresh-cut mangoes dipped with different kinds of anti-browning agent is presented in Figure 1a. During storage, L^*-values decreased continually both in control and treated cut mangoes. Anti-browning agents decreased browning when compared to control samples. Among the compounds tested, L-cysteine and glutathione showed the highest inhibitory activity on changing of cut surface lightness. In general, all compounds applied significantly suppressed the colour change (L^*-value), in accordance with their anti-browning activity in cut apples (Son *et al*., 2001).

Figure 1b shows changes in hue colour of fresh-cut mangoes treated with different anti-browning agents during storage for 4 days. Measurement of

Table 1. The estimated parameters k and the standard error of estimates (S.E.) in Eq. (1) and (2) for dipped fresh-cut mangoes at different concentration and anti-browning agents

Treatments	k_L±S.E. (day^{-1})[a]	k_H±S.E. (day^{-1})[a]	k_{BS}±S.E. (day^{-1})[a]	k_{BP}±S.E. (day^{-1})[a]
Control	0.0209±0.0012	0.0158±0.0008	0.2604±0.0474	0.2616±0.0187
Ascorbic acid				
0.5%	0.0132±0.0012	0.0132±0.0002	0.3313±0.0567	0.1566±0.0231
1.5%	**0.0088±0.0007**	**0.0090±0.0004**	**0.2146±0.0777**	**0.1272±0.0062**
2.5%	0.0066±0.0010	0.0079±0.0003	0.2231±0.0572	0.1186±0.0129
Citric acid				
0.5%	0.0213±0.0023	0.0120±0.0014	0.1859±0.0466	0.1981±0.0212
1.5%	**0.0210±0.0030**	**0.0098±0.0012**	**0.1642±0.0971**	**0.1559±0.0177**
2.5%	0.0164±0.0023	0.0097±0.0007	0.1443±0.1053	0.1386±0.0062
L-Cysteine				
0.5%	0.0068±0.0019	0.0095±0.0011	0.1776±0.0521	0.1427±0.0263
1.5%	**0.0053±0.0008**	**0.0077±0.0011**	**0.1342±0.0400**	**0.1188±0.0127**
2.5%	0.0025±0.0002	0.0041±0.0001	0.1385±0.0213	0.0653±0.0047
Glutathione				
0.5%	0.0180±0.0010	0.0107±0.0004	0.1866±0.0487	0.1993±0.0095
1.5%	**0.0094±0.0011**	**0.0089±0.0004**	**0.1676±0.0262**	**0.1311±0.0115**
2.5%	0.0061±0.0007	0.0087±0.0004	0.1329±0.0229	0.0973±0.0167

[a] k_L and k_H represent the estimated rate constant of decreasing L^*-value and Hue angle in Eq. (1) and k_{BS} and k_{BP} represents the estimated rate constant of increasing brown colour (score) and brown pigment in Eq. (2) The possibility of all obtaining results (p-value) was lower than 0.01

hue value (the occurrence of browning or loss of yellow colour) clearly showed varying degrees of suppression of browning on cut surfaces resulting as a function of the dipping used. The control resulted in significantly lower hue values than dipping treatments. Lower level indicated greater browning. The dip with sulphur-containing amino acids was more effective to prevent colour changes.

Scores of surface browning on cut mangoes decreased markedly with storage time. A rapid increase in brown score of control was observed. All dipping treatments resulted in better sensory acceptance than non-dipped cut mangoes (Figure 1c). L-cysteine and glutathione were the most effective compounds by sensory analysis for colour.

Brown pigment increased during the period of storage time at 10°C (Figure 1d). The absorbance of brown pigment at 410 nm of the control samples was higher than the samples treated with anti-browning agents. The degree of brown pigment inhibition depended on the anti-browning agent. Comparing the inhibitory efficiencies of different tested anti-browning on brown pigment formation, the result showed that dipping in ascorbic acid or citric acid was less effective than in L-cyesteine and glutathione.

Effects on other quality attributes

Respiration rates of fresh-cut mangoes during storage at 10°C are presented in Figure 2. As a result of wounding by cutting, the CO_2 production rate increased the first day after cutting. In treated slices, however, the increase was less than in non-treated slices. The production declined in all treatments until the second day and then remained constant throughout storage without significant differences among the treatments. The higher level of CO_2 production rate in control can be related to tissue stress which is caused by the processing such as cutting (wounding). Similar behaviour was reported by Ribeiro *et al.* (2007): samples treated with anti-browning agent showed a lower respiration rate than reported for many non-treated fresh-cut produces (Gonzalez-Aquilar *et al*., 2004; Rocculi *et al*., 2006; Petri *et al.*, 2008; Chiumarelli *et al*., 2011).

Figure 3 shows the changes in PPO activity in the non-treated and treated samples as influenced by the anti-browning agents during storage at 10°C for 4 days. The activity continued to decrease throughout

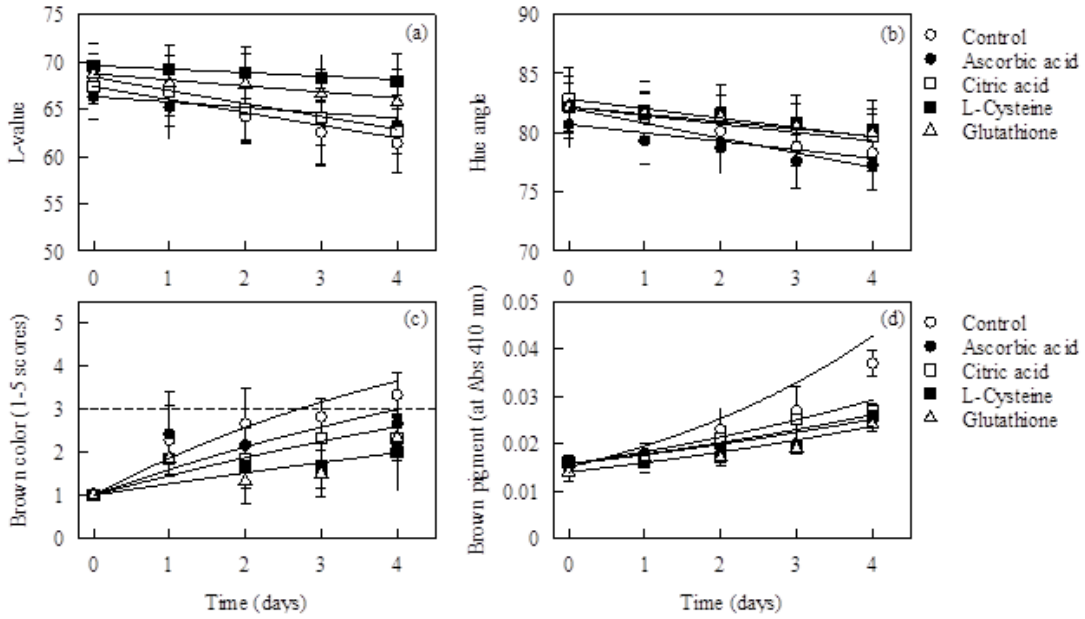

Figure 1.Predicted and measured L^*-value (a), Hue angle (b), brown colour (score) (c) and brown pigment (d) of fresh-cut mangoes stored at 10°C for 4 days. After processing, the cut mangoes were treated with anti-browning agents at 1.5% concentration. Solid line represents predicted values according to Eq. (1) for L^*-value and Hue angle and Eq. (2) for brown colour and brown pigment. Each vertical bar represents standard deviation (n = 4)

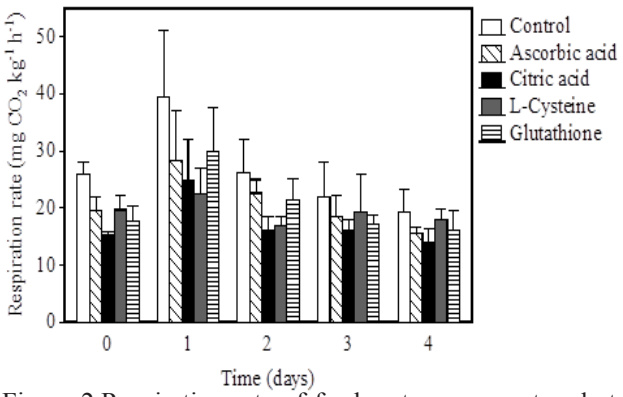

Figure 2.Respiration rate of fresh-cut mangoes stored at 10°C for 4 days. After processing, the cut mangoes were treated with anti-browning agents at 1.5% concentration. Each vertical bar represents standard deviation (n = 4)

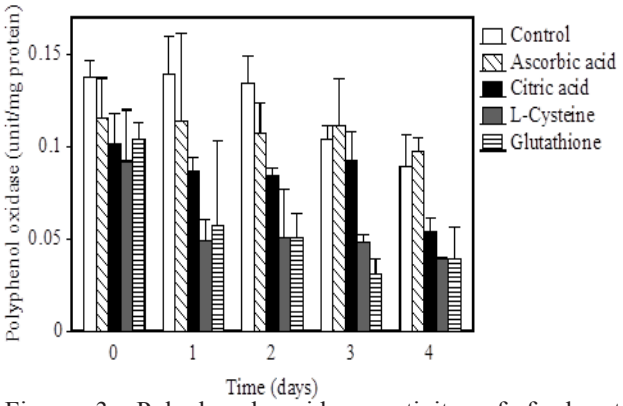

Figure 3. Polyphenol oxidase activity of fresh-cut mangoes stored at 10°C for 4 days. After processing, the cut mangoes were treated with anti-browning agents at 1.5% concentration. Each vertical bar represents standard deviation (n = 4)

the storage period regardless of the treatments. The activity in control showed a significant higher level than those dipped with anti-browning agents. The result of PPO activity related with the colour data (colour data, brown score and brown pigment) which showed a larger change in control sample (Figures 1a to 1d). The PPO activity of fresh-cut mangoes was most effectively inhibited by L-cysteine and glutathione followed by citric acid and ascorbic acid. Apparently, the reduction of PPO activity by anti-browning agents involves more than one mechanism related to the prevention of browning of cut surface of mango. Similar effects have been reported for many products: Andres *et al.* (2002) revealed that cut apples treated with a solution of ascorbic or citric acid showed a 2-3 time reduction in activity of PPO. Ascorbic acid and cysteine were found to be competitive inhibitors to PPO in lettuce, while citric acid was found to be a non-competitive inhibitor (Altunkaya and Gokmen, 2008). Jiang *et al.* (1999) demonstrated the effect of glutathione on PPO activity and browning of litchi fruit.

Ascorbic acid controls the activity of PPO by reduction of the formed o-quinones back to

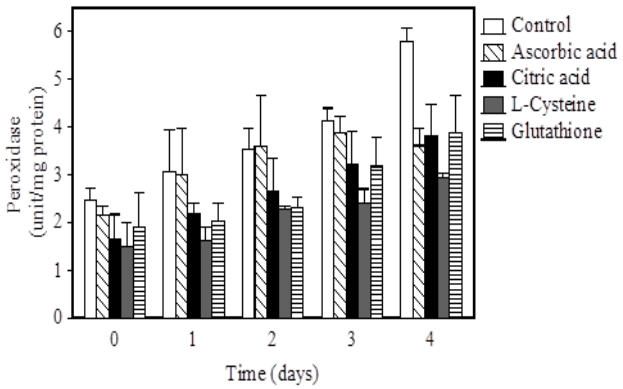

Figure 4.Peroxidase activity of fresh-cut mangoes stored at 10°C for 4 days. After processing, the cut mangoes were treated with anti-browning agents at 1.5% concentration. Each vertical bar represents standard deviation (n = 4)

their phenolic substrates oxidizing itself into dehydroascorbic acid (Oms-Oliu *et al.*, 2010). Citric acid, on the other hand, exerts a double inhibitory effect, by lowering the pH to below the optimum level for PPO activity and by chelating copper at the active site of PPO (Son *et al.,* 2001). L-cysteine and glutathione would react with o-quinones during the initial phase of enzymatic reactions to yield colourless product (Oms-Oliu *et al.*, 2010). However, L-cysteine and glutathione treatments may have different effects on PPO activity depending on the source of the enzyme (Billaud *et al.,* 2004).

In addition to PPO, POD has also been identified to play some role in browning reactions in fresh-cut mangoes and other products (Tomas-Barberan and Espin, 2001). POD activity in fresh-cut mangoes continuously increased right from the start of the storage period. All anti-browning solutions presented relatively low activity of POD during storage at 10°C in comparison to the control, which fluctuated with the highest value on day 4 of storage (Figure 4). However, no significant difference in POD activity among the anti-browning agents could be detected.

Conclusions

Several factors are involved in browning reaction in fresh-cut mangoes. To investigate the effect of anti-browning agent on fresh-cut mangoes, four solutions were tested for inhibitory activity. It indicates that the degree of changes in flesh colour of cut mangoes is related to the concentration of the different anti-browning agents. Among the compounds tested, L-cysteine and glutathione were the most effective to inhibit browning. A simple exponential model can be used to describe the L^*-value, hue angle, brown scores and brown pigment during storage. Moreover, the use of agents in this study could reduce the respiration rate, which provides a decrease on metabolic activities, suppressing the enzyme activities and delaying the quality losses in term of colour of fresh-cut mango pulp.

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