Effect of Storage Time on Ascorbic Acid and Total Phenolic Contents and Colour of Blanched, Boiled and Steamed Cauliflowers (*Brassica oleracea* L. ssp. *botrytis*)

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**ABSTRACT**

The effect of storage time on ascorbic acid content (AAC), total phenolic content (TPC) and colour of blanched, boiled and steamed cauliflowers (*Brassica oleracea* L. ssp. *botrytis*) was investigated. Blanching and steaming retained more AAC in cauliflower as compared to boiling. Storage time showed no significant changes in AAC measured at 30 min interval up to 2.5 h in all samples with the exception of boiled sample at 2.5 h in which significant reduction was observed. Blanching resulted in a higher TPC in cauliflower as compared to boiling or steaming. There were no significant differences between the TPC of boiled and steamed cauliflowers. Storage time showed no significant changes in TPC measured at 30 min interval up to 2.5 h in all samples. As for the colour measurement, there were significant reduction in the **L***, **a*** and **b*** values of the blanched, boiled and steamed cauliflowers as compared to raw cauliflower but there were no significant changes in these colour values measured at 30 min interval up to 2.5 h. This study demonstrated that blanched, boiled or steamed cauliflower can be stored up to 2 h with no significant changes in the AAC, TPC, **L***, **a*** and **b*** colour values.

**Keywords**: Blanching, boiling, cooking, room temperature storage, steaming, thermal processing, vitamin C

**INTRODUCTION**

The Brassicaceae is a plant family that contains about 340 genera and 3700 species and is of huge economic importance (Pedras & Yaya, 2010). Cauliflower (*Brassica oleracea* L. ssp. *botrytis*) belongs to this family. Examples of other plants under the Brassiceae family are cabbage, brussel...
sprouts and broccoli (Kadam et al., 2005; Pellegrini et al., 2010). Cauliflower has been mainly cultivated in the Northern Europe and England and has been extended to United States, India and China as well. Optimum temperatures for growth of cauliflower are 15-20°C with maximum and minimum temperature of 25°C and 8°C, respectively (Tsao & Lo, 2003). The texture of fresh cauliflower is firm with slight spongy texture and minor bitter flavour contributed by the presence of glucosinolates. The enzymatic derivatives of glucosinolates are biologically active (Kapusta-Duch et al., 2016). Cauliflower contains a number of phytochemicals and vitamins such as vitamin C and K which are good sources of antioxidant associated in reducing risk of cardiovascular diseases and cancer (Byers & Perry, 1992; Singh et al., 2007). The total phenolic and ascorbic acid contents change during ripening process of fruits and vegetables; as ripening progresses, the total soluble solid (TSS) increases (Guleria, 2000). Broccoli is a highly perishable vegetable and colour is one of the main external quality attributes of broccoli (Schouten et al., 2009).

Exposure of most of the food to temperature above ambient conditions causes physical and chemical changes in texture, flavour, colour or nutrients due to oxidation and degradation of nutrients. Nonetheless, thermal processes such as blanching, boiling and steaming are important as these processes contribute in preserving and producing food that is safe for consumption by ensuring that most pathogenic and spoilage-causing microorganisms are destroyed. This would also lead to creating an environment that does not support the growth of spoilage microorganism (Ramaswamy & Chen, 2002). The losses of nutrients in cauliflower can be reduced by ensuring that thermal heat applied on food is at the optimum level and over processing of food is avoided. There is a relationship between the temperature and duration of exposure on the loss of nutrients. Low temperature with long duration time causes less damage as compared to high temperature with short duration (Goullieux & Pain, 2005). Common cooking processes treated on cauliflowers are blanching, boiling and steaming. The differences in each processes is the duration of time exposed to boiling water except for steaming which does not have a direct contact to boiling water but rather the steam from the boiling water is transferred as heat to the food. The advantage of steaming over the other two thermal processes is that it reduces the contact of water with food which decreases the loss of nutrients via leaching (Fellows, 2009). Blanching is a thermal process done by briefly immersing the samples into boiling water for approximately 3 to 5 min. The main purpose of this process is to inactivate the enzymes such as peroxides that affect the sensory quality and nutritional values during storage. Therefore, to ensure minimal losses, this process should be carried out really quick and at most 5 min (Parreño & Torres, 2011). Besides that, these thermal processes can cause browning in cauliflower due to the activity of polyphenol oxidase which catalyzes the oxidation of phenols to quinones that
produce brownish red pigments as seen on fruit or vegetables browning (Pellegrini et al., 2010; Roy et al., 2007; Vamos-Vigyazo, 1981). Vegetables are usually cooked and may be left at room temperature for a period of time before consumption. There is currently no reported study on the stability of ascorbic acids, phenolic compounds and colour of cauliflowers after thermal processing or cooking. The objective of this study was to determine the effect of storage time on ascorbic acid content, total phenolic content and colour of blanched, boiled and steamed cauliflowers (Brassica oleracea L. ssp. botrytis).

MATERIALS AND METHODS

Materials

White cauliflowers with total soluble solids of 7.4 ± 0.2° Brix measured using a refractometer (Atago, Minato-ku, Japan) were obtained from a local supermarket in Shah Alam, Selangor, Malaysia. All chemicals and solvents were of analytical grade and purchased either from Sigma-Aldrich (St. Louis, U.S.A.) or Merck (Darmstadt, Germany).

Cooking Processes

Blanching. Blanching was carried out according to the method of Volden et al. (2009) with some modifications. Florets were immersed into water at 96°C with a ratio of [weight of sample (g): volume of water (mL), 1: 10] for 3 min. Then, the samples were removed and left to drain for 1 min.

Boiling. Boiling was carried out according to the method of Volden et al. (2009) with some modifications. Florets were immersed into a pot of boiling water with a ratio of [weight of sample (g): volume of water (mL), 1: 5] for 10 min. Then, the samples were removed from the pot of boiling water and left to drain for 1 min.

Steaming. Steaming was carried out according to the method of Volden et al. (2009) with some modifications. Florets (500 g) were placed in a steamer above 1 L of boiling water for 10 min. Then, the samples were removed from the steamer and left to cool for 1 min.

Storage Studies

Single layer of blanched, boiled or steamed samples were left exposed at 25°C for 2.5 h and samples were taken for analysis at 30-min interval time.

Sample Preparation

The sample preparation was carried out according to the method of Choo et al. (2014) with some modifications. For the ascorbic acid content, 5 g of florets were cut, crushed and mixed with 50 mL of 4% (w/v) metaphosphoric acid. It was mixed for 15 min followed by filtration of the mixture under vacuum and transferred into a 100 mL volumetric flask. For the total phenolic content, 70 g of florets were cut and crushed into paste-like state with addition of 100 mL of water using a Waring blender for 1 min (with intermittent stops to minimize heating at every 10 s interval between 30 s
of blending). The homogenized sample was transferred into a 250 mL volumetric flask and top up to the mark using 50% ethanol. The mixture was mixed for 15 min and then filtered under vacuum. All the filtered extracts obtained were stored at -20°C. The ascorbic acid content and colour were measured immediately after extraction.

**Ascorbic Acid Content (AAC)**

The AAC in cauliflower was measured using the iodine titration method of Suntornsuk et al. (2002). Firstly, starch solution was prepared by mixing 1g of starch into 200 mL of boiling water. The solution was immediately removed from heat and left to cool.

The iodine titration was performed by mixing 25 mL of juice extracted from cauliflower into a 250 mL Erlenmeyer flask. Then, 25 mL of 2N sulphuric acid, 50 mL of water and 3 mL of starch indicator were added into the flask and mixed well. The sample was titrated using 0.001N of iodine solution. Each 1 mL of 0.1N iodine used is equivalent to 8.806 mg of ascorbic acid. The AAC was expressed in terms of milligram of ascorbic acid in 100 g of sample.

**Total Phenolic Content (TPC)**

TPC in cauliflower was determined using Folin-Ciocalteu’s reagent according to the method of Lim et al. (2007). Sample extracted (0.3 mL) was placed into test tubes followed by addition of 1.5 mL of Folin-Ciocalteu’s reagent (10% v/v) and 1.2 mL of sodium carbonate (7.5% w/v). Then the test tubes were covered using parafilm, vortexed and left to stand for 30 min at room temperature before the absorbance was measured at 765 nm against a blank reagent. If the sample absorbance exceeded 1, appropriate dilution was required to give absorbance reading less than 1. TPC is expressed in terms of gallic acid equivalents in mg per 100 g of sample. As ascorbic acid contributes to the formation of blue molybdenum-tungsten complex, absorbance originating from it is corrected by measuring an ascorbic acid calibration curve.

**Colour Measurement**

Colour determination of cauliflower was carried out using a Hunter colorimeter (Hunter Associates Laboratory, Virginia, U.S.A). L* designates the lightness of the sample, 100=white, 0=black, a* indicates redness when positive, greenness when negative, b* indicates yellowness when positive, blueness when negative.

**Statistical Analysis**

All experiments were carried out in independent triplicates. The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test using SAS software package (SAS Institute Inc, Cary, U.S.A.). The statistical significance was evaluated at p<0.05.

**RESULTS AND DISCUSSION**

**Ascorbic Acid Content (AAC)**

The AAC of the raw cauliflowers (Table 1) in this study is in accordance to the AAC
reported for raw cauliflowers (Korus, 2010; Mazzeo et al., 2011). Thermal processing caused significant reduction in AAC of cauliflowers as compared to that of raw cauliflower (Table 1). The decrease of AAC after processing was due to heat degradation as ascorbic acid is heat sensitive which is easily destroyed under high heat temperature. Among the thermal treatment, the AAC of boiled cauliflowers was the lowest (Table 1). This is in accordance to the study of Volden et al. (2009). Ascorbic acids are water soluble; therefore, ascorbic acids in the cauliflower can easily leach from the cauliflower into the boiling water. In addition, less water and longer duration are usually used for boiling of vegetables as more energy is required in the process compared to blanching (Ferracane et al., 2008; Miglio et al., 2008). There were no significant changes in the AAC of raw, blanched and steamed samples during storage for 2.5 h (Table 1). These indicate that the TPC of the raw, blanched and steamed cauliflowers remained stable during this period of time. However, there was a significant reduction (p<0.05) of the AAC of boiled sample stored at 150 min or 2.5 h as compared to immediately after boiling. This corresponds with the AAC of boiled sample was the lowest among the thermal treatment (Table 1) and after storage for 2.5 h, further reduction occurred. This also suggests that to avoid a significant loss in AAC, boiled cauliflower should be consumed before 2.5 h.

### Total Phenolic Content (TPC)

The TPC of cauliflowers (Table 2) in this study was lower than that reported by Volden et al. (2009). The difference in TPC might be due to the different environmental growth conditions such as temperature, light intensity, humidity, water availability, wind strength and rainfall that would greatly affect the growth of plants (Solomon et al., 2002). Raja et al. (2011) reported the presence of gallic acid, ferulic acid, chlorogenic acid and catechin whereas Lee at al. (2011) reported the presence of caffeic acid, p-coumaric acid, ferulic acid and sinapic acid in cauliflowers. There was

<table>
<thead>
<tr>
<th>Storage time (min)</th>
<th>Raw</th>
<th>Blanched</th>
<th>Boiled</th>
<th>Steamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>59.4±1.8&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>51.6±1.1&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>39.3±0.8&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>52.1±1.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>59.4±1.1&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>50.9±0.9&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>38.5±0.9&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>51.2±0.8&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>59.7±1.1&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>52.6±1.5&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>38.5±0.9&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>50.9±0.8&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>59.0±1.1&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>52.6±1.5&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>37.8±0.9&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>51.2±1.1&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>60.4±0.4&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>52.3±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>38.1±1.3&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>51.2±1.5&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>150</td>
<td>59.9±0.7&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>52.1±0.7&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>37.3±0.7&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>51.2±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results were presented as mean ± standard deviation
<sup>Aa</sup>Values with different superscript letters within a column indicate significant differences at p<0.05
<sup>ab</sup>Values with different superscript letters within a row indicate significant differences at p<0.05
no significant difference between the TPC of raw and blanched cauliflowers and the TPC of these cauliflowers were significantly higher \((p<0.05)\) than boiled and steamed cauliflowers (Table 2). Furthermore, the TPC of steamed cauliflowers was not significantly different from that of boiled cauliflowers. Factors such as temperature, exposure time and volume of water used would affect the total loss of phenolic content in vegetables (Ismail et al., 2004; Natella et al., 2010; Turkmen et al., 2005). The reduction of TPC in boiled cauliflower is in accordance with the study of Mazzeo et al. (2011) on boiled frozen cauliflower and Watchel-Galor et al. (2008) on boiled cauliflower.

Steamed frozen cauliflowers (Mazzeo et al., 2011) and steamed cauliflowers (Watchel-Galor et al., 2008) were reported to have higher TPC compared to the raw samples. These authors suggested that steaming process caused tissue softening which enhanced the availability of the compounds to be extracted and led to the production of redox-active secondary plant metabolites products. These results are in contrast to that obtained in this study and by Pellegrini et al. (2010) on steamed frozen cauliflowers. The discrepancy between the TPC observed for cauliflower as a result of steaming was suggested by Mazzeo et al. (2011) to probably related to a different size of cauliflower. There were no significant changes in the TPC of raw, blanched, boiled and steamed samples during storage for 2.5 h (Table 1). These indicate that the TPC of the raw and thermal processed cauliflowers remained stable during this period of time.

**Colour Measurement**

The L*, a* and b* values of cauliflower (Tables 3-5) in this study is in accordance with those reported by Pellegrini et al. (2010). The L* value of frozen cauliflowers were lower than fresh cauliflowers (Pellegrini et al., 2010). The three thermal processes reduced the L* value of cauliflowers with steamed cauliflowers with the lowest L* value (Table 3). These thermal treatment may have induced the development of browning in cauliflowers. Low temperature

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**Table 2**

*Total phenolic contents of raw, blanched, boiled and steamed cauliflowers*

<table>
<thead>
<tr>
<th>Storage time (min)</th>
<th>Raw</th>
<th>Blanched</th>
<th>Boiled</th>
<th>Steamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.1±3.2Aa</td>
<td>36.0±3.6Aa</td>
<td>17.9±5.9Ab</td>
<td>18.8±5.9Ab</td>
</tr>
<tr>
<td>30</td>
<td>32.6±1.8Aa</td>
<td>27.8±3.4Aa</td>
<td>16.8±5.8Ab</td>
<td>15.5±7.2Ab</td>
</tr>
<tr>
<td>60</td>
<td>34.0±5.9Aa</td>
<td>28.9±7.5Ab</td>
<td>16.7±5.9Ab</td>
<td>16.6±6.5Ab</td>
</tr>
<tr>
<td>90</td>
<td>31.9±4.9Aa</td>
<td>31.1±2.1Aa</td>
<td>17.9±5.9Ab</td>
<td>17.0±4.5Ab</td>
</tr>
<tr>
<td>120</td>
<td>29.9±5.0Aa</td>
<td>28.6±6.7Aa</td>
<td>15.4±5.9Ab</td>
<td>16.7±6.4Ab</td>
</tr>
<tr>
<td>150</td>
<td>33.3±6.0Aa</td>
<td>27.8±2.9Ab</td>
<td>16.5±6.6Ab</td>
<td>16.2±7.4Ab</td>
</tr>
</tbody>
</table>

Results were presented in mean ± standard deviation

AValues with different superscript letters within a column indicate significant differences at \(p<0.05\)

abValues with different superscript letters within a row indicate significant differences at \(p<0.05\)
storage of cauliflowers at 4°C also resulted in decreased L* values (Berrang et al., 1990). These are attributed to the development of browning in cauliflowers (Nunes, 2008). Decrease in L* value indicates lower brightness intensity but at the same time, there was an increase in –a* value in the three thermal processed cauliflowers which indicates a shift towards green, moving away from the redness (Table 4). These results are in accordance to the studies of Mazzeo et al. (2011) on frozen cauliflowers and Pellegrini et al. (2010) on both fresh and frozen cauliflowers. Blanching and boiling reduced the b* values of cauliflowers but there was no significant difference between raw and steamed cauliflowers. As for the effect of storage, there were no significant changes in the L*, a* and b* values of the three thermal processed cauliflowers during storage for 2.5 h (Tables 3-5). These indicate that colour of the raw and thermal processed cauliflowers remained stable during this period of time.

Table 3
Colour (L*) of raw, blanched, boiled, steamed cauliflowers

<table>
<thead>
<tr>
<th>Storage time (min)</th>
<th>Raw</th>
<th>Blanched</th>
<th>Boiling</th>
<th>Steaming</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>82.5±0.4&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>79.3±1.0&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>77.7±0.8&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>77.3±1.4&lt;sup&gt;Ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>82.5±0.8&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>77.5±1.2&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>79.9±3.5&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>77.5±1.8&lt;sup&gt;Ab&lt;/sup&gt;</td>
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<td>60</td>
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</tr>
<tr>
<td>90</td>
<td>82.5±0.6&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>77.7±1.6&lt;sup&gt;Ab&lt;/sup&gt;</td>
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<td>120</td>
<td>82.4±0.2&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>78.9±0.6&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>77.4±0.9&lt;sup&gt;Ac&lt;/sup&gt;</td>
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<tr>
<td>150</td>
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<td>77.9±0.6&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>77.1±1.6&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
</tbody>
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<sup>A</sup>Values with different superscript letters within a column indicate significant differences at p<0.05
<sup>abc</sup>Values with different superscript letters within a row indicate significant differences at p<0.05
L* designates the lightness of the sample; 100=white, 0=black

Table 4
Colour (a*) of raw, blanched, boiled, steamed cauliflowers

<table>
<thead>
<tr>
<th>Storage time (min)</th>
<th>Raw</th>
<th>Blanched</th>
<th>Boiled</th>
<th>Steamed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.6±0.3&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-0.4±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>-1.9±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>-1.8±0.3&lt;sup&gt;Ab&lt;/sup&gt;</td>
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<tr>
<td>30</td>
<td>0.7±0.6&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-0.6±0.3&lt;sup&gt;Ab&lt;/sup&gt;</td>
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</tr>
<tr>
<td>60</td>
<td>0.5±0.4&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-0.7±0.6&lt;sup&gt;Ab&lt;/sup&gt;</td>
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<td>-1.7±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>0.4±0.4&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-0.5±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>-1.6±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>-0.9±0.1&lt;sup&gt;Ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>0.8±0.4&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-0.4±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>-1.8±0.2&lt;sup&gt;Abc&lt;/sup&gt;</td>
<td>-1.9±0.5&lt;sup&gt;Ac&lt;/sup&gt;</td>
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<tr>
<td>150</td>
<td>0.7±0.4&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-0.8±0.4&lt;sup&gt;Ab&lt;/sup&gt;</td>
<td>-1.5±0.2&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>-1.1±0.5&lt;sup&gt;Ac&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results were presented as mean ± standard deviation
<sup>A</sup>Values with different superscript letters within a column indicate significant differences at p<0.05
<sup>ab</sup>Values with different superscript letters within a row indicate significant differences at p<0.05
a* indicates redness when positive; greenness when negative
CONCLUSION
There were no significant changes in the AAC, TPC, L*, a* and b* colour values of blanched, boiled or steamed cauliflowers during storage up to 2 h. These results serve as a food preparation guide for consumers to avoid nutrient loss after food preparation. Longer storage time can be investigated in the future. Besides measuring AAC, TPC and colour values, other bioactive compound or antioxidant activity in cauliflower can be measured.

ACKNOWLEDGEMENT
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