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Full Length Research Paper

Effects of various freezing and thawing techniques on pork quality in ready-to-eat meals

Kwang-II Kim¹, Jun-Bo Shim², Seon-Mi Yoo³, Sang-Gi Min², SangYoon Lee¹, Yeon-Ji Jo² and Mi-Jung Choi¹*

¹Department of Bioresources and Food Science, Konkuk University, Seoul, Korea. ²Department of Bioindustrial Technologies, Konkuk University, Seoul, Korea. ³Department of Agro-food Resources, National Academy of Agricultural Science, Rural Development Administration, Jeonju, Korea.

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Meat rapidly decomposes and discolours due to oxidation and enzyme activity; therefore, it must be frozen when stored. This study investigates the effects of different freezing and thawing processes on pork quality. Pork meat was frozen by natural convection freezing (NCF, -38°C), individual quick-freezing (IQF, -45°C), or liquid nitrogen freezing (LNF, -100°C). Freezing was completed when the thermocouple temperature reached -12°C. The meat was then placed in a general showcase at -24°C for 24 h. Thawing was conducted by natural convection thawing (NCT, 25°C) or running water thawing (RWT, 10°C). The cooking loss and drip loss contents of the samples did not significantly differ, whereas the thawing loss contents were higher in the NCF sample than that in the other samples. Compared to fresh meat, the L, a, and b colour values decreased and the total colour difference (ΔE) was similar in the samples subjected to IQF/RWT. The pH values of all the samples except for the one subjected to NCF were significantly increased than that in fresh meat (p < 0.05). IQF/RWT Treatment resulted in the highest water-holding capacity and maintained homogenous tissue similar to fresh pork; however, the shear force value was lower than those in the other frozen/thawed samples. These results suggest that the IQF/RWT process was optimal for pork.

Key words: Freezing, thawing, pork, shear force, pH.

INTRODUCTION

Optimization of processed meat quality attributes, such as colour, pH, water-holding capacity (WHC), shear force, thawing, and cooking loss, are important to the meat processing industry (Mortensen et al., 2006). The meat quality attributes that are influenced by freezing processes depend on many factors, including the temperature, time, air speed, and rate of freezing and thawing (Giddings and Hill, 1978; Nicholson, 1973). Freezing is the most effective process for the preservation of food quality. Freezing and thawing can greatly affect the structural and chemical properties of muscle foods, including muscle fibers, lipids, and proteins, all of which have the potential to significantly influence the quality properties of meat and vegetable

*Corresponding author. E-mail: choimj@konkuk.ac.kr. Tel: +82-2-450-3048. Fax: +82-2-450-3726.

Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution License 4.0</u> International License products (Miller et al., 1980).

Freezing has many advantages for the preservation of meat, but it can results in the destruction of muscle fibers due to the formation of ice crystals of various sizes according to the freezing rate (Hong et al., 2005b). This may lead to problems during thawing, such as drip loss, various WHC contents, decreases in the gel-forming potentials of muscle fiber proteins, and reductions in the space within the myofibrils (Sakata et al., 1995; Huff-Lonergan and Lonergan, 2005). Classic freezing and thawing procedures change the texture and cooking properties of food, and this is probably due to the destruction of the membrane structure and concentration changes in the solute (Londahl, 1997). The development of new methods for the freezing and thawing of foods is required in food industries (Massaux et al., 1999).

Several novel freezing techniques, such as individual quick freezing (IQF) and liquid nitrogen freezing (LNF), have been developed in recent years. IQF is an improvement of classical air blasts freezing, which generally entails temperatures of -18°C or lower (Fennema et al., 1975). In IQF, small food pieces are frozen in an air blast freezer at temperatures that are lower (-30 to -50°C) than that used for traditional freezing. IQF can freeze individual or bulk samples of various food groups, such as meat, vegetables, and fruits, in less time (Jo et al., 2014). Cryogenic freezing with liquid nitrogen results in high freezing rates, even at the center of the product, and faster freezing time compared with conventional air freezing (Zhou et al., 2010). However, the cost of the cryogenic liquid is high, and this system has the disadvantage of freeze cracking, which causes critical and irreversible damage (Lovatt et al., 2004).

While freezing is a simple and effective way for preserving food, the thawing of frozen food is also important in the process. During food thawing, thermal treatments can damage the chemical, physical, and microbiological properties of food (Hong et al., 2009; Boonsumrej et al., 2007). Minimum thawing times can reduce microbial decomposition, the deterioration of food product quality, and water loss from dripping or dehydration (Taher and Farid, 2001). Most meat thawing is performed within the temperature ranges of -5 to -1°C, and only a small fraction of thawing is performed within the temperature range of -24 to -5°C (Heldman, 1975). Thawing can play an important role in membrane decomposition as well as affect the sensory properties of the food (Nilsson and Ekstrand, 1995). The freeze-thaw process has negative effects on the physicochemical properties and overall quality of the food (Jeong et al., 2011). Therefore, guidelines for the conditions for the optimal processing for freezing and thawing need to be established.

The freeze-thaw process may affect the quality of meat differently depending on the species. Universally, frozen storage is necessary to increase shelf life because pork meathasone of the shortest shelf lives among meat products due to fast microbial growth and lipid oxidation (Wulf et al., 1995).

The objective of this study was to investigate the changes in the physicochemical properties, microstructure, and quality of pork meat that result from different freezing [natural convection freezing (NCF), IQF, and LNF] and thawing [natural convection thawing (NCT) and running water thawing (RWT)] processes.

MATERIALS AND METHODS

Materials and sample preparation

Pork (crossbreed of Landrace × Yorkshire × Duroc, 6 month old hogs) samples (eye of round) were obtained from a commercial market (48 h postmortem; pH, 5.7–5.9). The fat and connective tissue were removed, and the pork was cut into a rectangular shape (1 × 1 × 5 cm, 90 ± 0.5 g) parallel to the muscle fiber direction. For the fresh (unfrozen) pork, parts of the samples were placed into a showcase at 4°C for 24 h. After freezing treatment, the sample was vacuum packaged in a polyethylene bag, individually. A thermocouple (k-type) was inserted into the center position of each sample in order to monitor the temperature of the samples during freezing and thawing.

Freezing and thawing process

NCF was performed at -38°C in a showcase, whereas IQF was conducted with the use of a -45°C air blast freezer (SEO JIN Freezer Co., Ltd., Goyang-City, Korea). For LNF, the samples were sprayed in a cryo-chamber system (150 \times 30 \times 50 cm [L \times W \times H], HyunDae FA, Korea) with four circular spray nozzles (MS TECH CO., LTD., Sungnam-City, Korea) with a spray angle of 60° and a flow rate of liquid nitrogen vapor of 9.0 L/min. The samples were cryogenically frozen (-100°C) for 2 min 30 s. The freezing was finished when the temperature of the thermocouple reached -12°C. Each freezing treatment sample was divided into two groups and vacuum packaged in a polyethylene bag that was placed in a general showcase at -24°C for 24 h. The thawing process was performed with two methods so that one group was thawed in running water (RWT) at 10°C and the natural convection thawing (NCT) treatment was kept at 25°C. Thawing was finished when the temperature center position reached 4°C. The temperature-time profiles of all of the samples were observed by connecting the thermocouple with a mobile corder (MV-100, Yokogawa Electric Corporation, Tokyo, Japan).

pH measurements

The pH values of the prepared samples were measured with a pH meter [S-220, Mettler-Toledo (Schweiz) GmbH, Greifensee, Switzerland]. Five grams of each sample was mixed with 45 mL of distilled water and homogenized at 12,000 rpm for 1 min with a homogenizer (HP-91, SMT Co. Ltd., Japan).

Thawing loss and cooking loss

After the thawing treatment, the pork surface exudate was removed with a tower, and the samples were weighed. The thawing loss was determined by calculating the difference in the pre-freezing and post-thawing weights. After determining the thawing loss, the samples were bagged in polyethylene pouches and thermally treated in an 80°C water bath (DX9, Hanyoung Nux Co., Ltd., Namgu, Incheon, Korea) until the core temperature reached 75°C. Cooking loss was calculated as the difference in the weights from before cooking to after cooking.

Thawing loss (%) =
$$\frac{W_1 - W_2}{W_1} \times 100$$

 W_1 : weight of the sample after freezing (g) W_2 : weight of the sample after thawing (g)

Cooking loss (%) =
$$\frac{W_1 - W_2}{W_1} \times 100$$

 W_1 : weight of the sample after freezing and thawing (g) W_2 : weight of the sample after thermal treatment (g)

Water-holding capacity (WHC)

WHC was measured with modification of the method of Hong et al. (2005a). One gram of each thawed pork sample was weighed and then placed into a centrifuge tube with absorbent cotton. The samples were centrifuged with a centrifuge separator (1736R, LABOGENE, Korea) at 1,500 × g for 10 min at 4°C. After centrifuging, the pork was removed from the tube, and the weights of the centrifuge tubes were determined before and after the drying. The WHC was expressed as the percentage of moisture content in the meat.

$$WHC(\%) = (1 - \frac{W_3 - W_2}{W_1}) \times 100$$

 W_1 : weight of the sample after centrifuging (g) W_2 : weight of the tube, including the cotton, after centrifuging (g) W_3 : weight of the tube after the sample was removed and after centrifuging (g).

Shear force measurement

The samples from each batch were cut into cuboids $(1 \times 1 \times 5 \text{ cm})$. The shear force of the pork samples was determined before and after cooking in quintuplicates with a texture analyzer (CT3, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) that was equipped with a v-type plain probe. The texture analysis conditions were as follows: compression type, 10 kg force load cell; test speed, 2.5 mm/s; target distance, 15 mm; and trigger loads of 900 g and 650 g on uncooked and cooked samples, respectively. The test was repeated at least 16 times. The maximum peak force (kg) was used as the indicator of the texture parameter.

Colour measurement

The colour change of each sample was determined with a colourimeter (CR-400, Konica Minolta Inc., Tokyo, Japan) that was calibrated with a white standard plate ($L^* = +97.83$, $a^* = -0.43$, $b^* = +1.98$). The CIE L^* , a^* , and b^* values were determined as indicators of brightness (L), red to green colour (a), and yellow to blue colour (b). To measure the colour changes, four pieces of pork were arranged in the direction of their longest length. The total colour difference (ΔE) was numerically calculated by determining the colour difference between the fresh meat and the treated samples with the following equation:

$$\Delta \mathbf{E} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Light microscopy

Light microscopy of fresh and frozen pork tissue was conducted on 0.2 cm-thick sections of formalin-fixed, paraffin-embedded samples stained with hematoxylin and eosin (H and E; BBC

Biochemical, USA), using autostainer (Leica autostainer XI ST5010 Autostainer XL, Leica Microsystems Ltd., Korea).

Statistical analysis

All of the reported values are the average of three (or more) experiments. Analysis of variance and Duncan's tests were conducted at the 95% confidence level ($p \le 0.05$) with SPSS 20.0 software (IBM Corporation, Armonk, NY, USA) in order to determine the significance of the differences in the results.

RESULTS AND DISCUSSION

Temperature-time profile

Figure 1 shows the time-temperature profiles of the pork treatment during freezing and thawing. The freezing time for the core temperature of the pork to reach -12°C was 58 min in the NCF treatment. IQF treatment showed rapid freezing compared to NCF, and the freezing time was estimated as 18 min. The LNF-treated pork showed the most rapid temperature drop among the freezing treatments, and the center temperature reached -12°C in 3 min. These results were in accordance with the freezing temperature of each treatment. Based on Boonsumrej et al. (2007), cryogenic freezing was favorable as a rapid freezing technique compared to air-blast freezing or commercial freezing, and this was in agreement with the results of our study.

For the thawing method, the overall thawing times of RWT and NCT were well differentiated (Figure 1b). All of the RWT treatments thawed within 10 min compared to the NCT treatments that took longer than 30 min. Although the NCT treatment was conducted at a higher temperature (25°C) than the RWT treatment (10°C), the results indicated that RWT was more advantageous than NCT for the rapid thawing. In addition, the thawing times of the samples differed according to the type of freezing that was applied. In the RWT treatments, the rapidity of the thawing occurred in the order of IQF, NCF, and LNF, and the same order was observed for the NCT treatments. Although the reason why the thawing rate was affected by freezing type was unclear in the present study, this study demonstrated that IQF that was followed by RWT was the best condition for the meat freezing and thawing processes.

pН

The freezing and thawing treatments affected the pH of



Figure 1. Time-temperature profiles of the (A) freezing and (B) thawing processes. The freezing and thawing treatment included natural convection freezing (NCF), individual quick-freezing (IQF), liquid nitrogen freezing (LNF), natural convection thawing (NCT), and running water thawing (RWT).

the pork, as depicted in Figure 2. Irrespective of the thawing methods, NCF treatment resulted in pH values of 5.46 - 5.47, which was not significantly different from the 5.46 pH of the fresh control. However, pork that was frozen by IQF and LNF showed a significantly higher pH than that of the control (p < 0.05), and a particular increase in pH was noticeable with LNF. For the thawing methods, the NCT treatments resulted in higher pH values compared to the RWT treatments (p < 0.05). Consequently, the highest pH (5.58) was obtained with LNF treatment that was combined with NCT (p < 0.05).

Various studies have reported inconsistent relationships of muscle pH and freezing/thawing treatment. Leygonie et al. (2012) and Devine et al. (1995) have reported that frozen/thawed meat had a slightly lower pH than that of the fresh state due to the electrolyte exudate from the muscle tissue. Muela et al. (2010) postulated that the pH of fresh meat and frozen/thawed meat did not differ significantly.

Alternatively, Kim and Lee (2011) reported that frozen/thawed meat had a higher pH than fresh control meat because of partial denaturation of the muscle proteins. Those authors also insisted that the pH of treated meat is an important indicator of the physical properties of the muscle proteins. In the present study, it was clear that the impact of the treatment conditions on

Freezing treatment ¹	Thawing methods ²	Thawing loss	Cooking loss	Water holding capacity
Control			15.74±0.617 ^b	85.33±3.32 ^a
NCF		6.75±0.322 ^{a,3}	19.07±1.556 ^a	80.5±1.87 ^{bc}
IQF	NCT	4.48±1.23 ^b	18.9±0.795 ^ª	79.18±2.14 ^{bc}
LNF		4.16±1.268 ^b	20.39±0.72 ^a	82.43±0.51 ^{ab}
NCF		4.67±1.282 ^b	21.69±1.842 ^a	77.68±1.87 ^{3c}
IQF	RWT	4.8±1.425 ^{ab}	19.97±2.613 ^a	85.19±1.230 ^a
LNF		3.75±1.21 ^b	20.18±1.448 ^a	79.65±1.575 ^{bc}

Table 1. Effects of the freezing and thawing treatments on the water binding properties.

¹Freezing treatment: NCF (natural convection freezing), IQF (individual quick freezing), and LNF (liquid nitrogen freezing). ²Thawing treatment: NCT (natural convection thawing) and RWT (running water thawing). ³⁾Each value is expressed as the mean \pm standard deviation of multiple measurements (n = 5). ^{a-c}Means within the same column with different superscript letters differ significantly (p < 0.05).

the physical state of the meat proteins was remarkable. Despite the finding that the LNF treatment was for a short period (2.5 min), an extreme temperature condition would result in cold denaturation of the muscle proteins, which would thereby increase the pH of the meat. Furthermore, thawing at relatively high temperature (25°C of NCT) was not favorable to minimize the quality loss of frozen muscle comparing to that thawed at low temperature (10°C of RWT). For the protein state, the application of LNF required the optimization of the proper operating conditions, such as the processing temperature and time.

Water-binding properties

The water-binding properties of the frozen/thawed pork are given in Table 1. With the exception of NCF that was followed by NCT treatment, the overall thawing loss of the pork ranged from 3.75 to 4.80%, which was not significantly different among the treatments. However, the NCF/NCT treatment had the highest thawing loss (6.75%) among the treatments (p < 0.05). The highest thawing loss resulting from the NCF/NCT treatment was possibly related to the slow freezing and thawing rates. The NCF treatment involved a slow freezing rate by which the pork tissue would be more damaged than with the other freezing methods. Considering the decreased thawing loss of LNF that was followed by NCT treatment, the freezing rate appeared to influence the thawing loss of the pork rather than the thawing rate. However, it was obscure why LCF showed a small amount of thaw drip with RWT treatment. One possible explanation is that the frozen rapidly mean was not affected by the thawing methods, while rapid thawing was necessary when the meat was frozen slowly.

The cooking loss of the fresh control was 15.7%, and the loss was significantly lower than the losses from the freezing/thawing treatments (p < 0.05). Among the treatments, no significant differences in cooking loss were found, and the loss ranged from 18.9% to 21.7%. There was no doubt that the tissue damage that was caused by

ice crystallization and recrystallization attributed to the high cooking loss compared with fresh meat. For frozen/thawed meat, Mortensen et al. (2006) reported that a low freezing temperature and a high thawing temperature tended to result in high thawing loss and cooking loss. However, sample size is another important factor that affects the cooking loss of meat samples (Leygonie et al., 2011). In the present study, the meat was sampled in small strips that are used for home meal replacement products. The small size of the samples was compensated for by the insignificance of the cooking loss of the frozen/thawed treatments.

Compared to 85.3% of fresh control, the WHC of the treatments was slightly or significantly lower than the control and ranged from 77.7 to 85.2%. Jo et al. (2014) found that the WHC of muscle freezing/thawing treatment is lower than that of fresh controls, and this is in accordance the findings of this study. Miller et al. (1980) noted that the WHC percentage decreases for pork and beef meat because of damage to the muscle tissue from being frozen. Ngapo et al. (1999) showed that damaged cell membranes cause the drip exudate from the intracellular space to the extracellular space, which results in the easy release of drips from the muscle tissue. In this study, LNF that was followed by NCT (82.4%) and IQF that was followed by RWT treatment (85.2%) did not show significant differences in WHC compared with a control, while other treatments had lower WHCs than controls (p < 0.05). With respect to food hygiene, the thawing process is supposed to be conducted at a relatively low temperature in a short time, and, thus, RWT would be better than NCT in order to minimize the water binding properties of the treated meat. Therefore, these results suggest that IQF is the best application for meat freezing.

Shear force

Figure 3 depicts the shear force of the cooked pork. The fresh pork had a shear force of 2.41 kg, and the frozen/



Figure 2. Effects of the freezing and thawing treatments on the pH of pork. The pH values of the control (fresh pork) and after the freezing and thawing treatments of natural convection freezing (NCF), individual quick-freezing (IQF), liquid nitrogen freezing (LNF), natural convection thawing (NCT), and running water thawing (RWT). Each value is expressed as the mean ± standard deviation of multiple measurements (n = 5). ^{a–d}Means with different superscript letters are significantly different (*p* < 0.05).



Figure 3. Effects of the freezing and thawing treatments on the shear force of cooked pork. The control (fresh pork) and experimental samples were subjected to freezing and thawing with natural convection freezing (NCF), individual quick-freezing (IQF), liquid nitrogen freezing (LNF), natural convection thawing (NCT), and running water thawing (RWT). Each value is expressed as the mean ± standard deviation of multiple measurements (n = 5). ^{a-d}Means with different superscript letters differ significantly (*p* < 0.05).

thawed treatment values ranged from 2.31 to 3.31 kg. Regardless of the thawing methods used, the shear force

of meat that was treated by IQF did not significantly differ than the control. However, the LNF and NCF treatments

Freezing treatment ¹	Thawing treatment ²	L*	a*	b*	Total colour difference	(∆ <i>E</i>)
Control		59.16±1.7 ^{a,3}	8.02±0.94 ^a	7.1±0.76 ^b		
NCF	NCT	54.52±1.46 ^b	7.93±1.03 ^a	8.74±1.68 ^a	5.34±1.37 ^a	
IQF		55.07±2.24 ^b	6.18±0.63 ^b	5.62±0.96 ^c	4.98±1.90 ^a	
LNF		54.39±2.48 ^b	8.05±1.03 ^a	6.66±0.96 ^{bc}	5.03±2.36 ^a	
NCF	RWT	54.84±2.29 ^b	7.74±1.06 ^a	6.75±1.22 ^{bc}	4.69±2.10 ^a	
IQF		56.04±3.04 ^b	6.97±0.90 ^{ab}	7.14±0.75 ^b	4.19±1.75 ^ª	
LNF		53.76±1.24 ^b	7.37±1.28 ^a	5.52±0.55 [°]	5.81±1.27 ^a	
NCF		54.84±2.29 ^b	7.74±1.06 ^a	6.75±1.22 ^{bc}	4.69±2.10 ^a	

Table 2. Effects of the freezing and thawing treatments on the CIE colour of pork.

¹Freezing treatment: NCF (natural convection freezing), IQF (individual quick freezing), and LNF (liquid nitrogen freezing). ²Thawing treatment: NCT (natural convection thawing) and RWT (running water thawing). ³Each value is expressed as mean \pm standard deviation of multiple measurements (n = 5). ^{a-c}Means within the same column with different superscript letters differ significantly (p < 0.05).

that were followed by RWT treatment had significantly higher shear forces compared with controls (p < 0.05). Shanks et al. (2002) and Lagerstedt et al. (2008) reported that meat toughness was decreased by the freezing and thawing process. The decrease in the shear force might have been due to the loss in cell membrane durability that occurred as a result of ice crystal formation and the reduction in the shearing (Lui et al., 2010). The tenderization of meat can occur as a result of the activation of enzymes, such as those involved in proteolysis, and the loss of physical structure by ice crystal formation (Leygonie et al., 2012). In these investigations, however, the shear force was measured prior to the thermal processing. Alternatively, Lagerstedt et al. (2008) reported that the shear force of frozen meat is closely related to the storage period and storage condition. In addition, the frozen/cooked meat showed a higher shear force than the fresh/cooked meat did (Kolczak et al., 2005). In this study, IQF treatment resulted in the best tenderness of the meat, and the IQF was favorable for applying it as a quick freezing technique.

Colour

The CIE colour parameters of the treatments were compared with those of fresh controls (Table 2). All of the freezing and thawing treatments had lower L, a, and b values compared with those of the control, with the exception of b in which the NCF/NCT samples showed a significantly lower b* compared with the control (p < 0.05). Lind et al. (1971) obtained similar results in that the freezing rates did not have a significant effect on colour. The b value of the IQF/RWT samples among the frozen/ thawed samples. The total colour difference tended to be lower than those of the other samples, but the difference was not significant. The results can be explained by the IQF/RWT value being similar to those of fresh pork meat.

The hue angle of the meat product has been used to indicate the colour stability of fresh and processed meats (Brewer and Harbers, 1992). Leygonie et al. (2012) reported that the CIE L*, a*, and Chroma values in the visual test were significantly decreased in the frozen/ thawed samples. These results suggest that the meat product should exhibit an overall browner and more somber appearance because of rapid oxidation of the myoglobin after freezing/thawing.

Light microscopy

The light microscopy images of the pork samples are shown in Figure 4. For the raw pork (control), transverse sections of the myofibrils have most uniform shape and the myofibrils were maintained their integrity. However, segmental muscle and segmental coagulation necrosis in longitudinal section were observed after NCF and NCF treatments. This result could be explained by Jo et al. (2014) in which this phenomenon may result from ice crystal formation and recrystallization. Alternately, IQF maintained the condition of myofibrils, although their density was not as intense as that of the raw meat. Furthermore, the condition of myofibrils did not change after different thawing treatments. Based on Mortensen et al. (2006), cell structure of frozen muscle tissue was closely depending on freezing rate. Rapidly frozen muscle tissue showed broadly intact structure with partial damages, whereas the tissue frozen slowly showed completely damaged cell structure. Therefore, our results could demonstrate that tissue damage during freezing and thawing is inevitable, and confirm that tissue damage was more influenced by the freezing methods than thawing methods.

Conclusion

This study compared the effects of different freezing and



Figure 4. Histological appearance of the frozen and thawed pork samples following the different freezing and thawing processes. (A) Control (raw pork), (B and E) natural convection freezing, (C and F) individual quick-freezing, (D and G) cryogenic freezing (B, C and D), natural convection thawing, and (E, F and G) running water thawing.

thawing treatments on the quality of pork. The factors of temperature, time, and the rates of freezing and thawing influenced the changes in the meat quality attributes, such as colour, thawing loss, WHC, and shear force. The results of this study suggested that IQF/RWT treatment is an effective process by which the meat quality is maintained.

Conflict of interests

The authors did not declare any conflict of interest.

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