Quality Attributes of Fat-free Sausage Made of Chicken Breast and Liquid Egg White

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Abstract

We developed a type of sausage made of chicken breast and liquid egg whites for consumers interested in weight management. To determine the quality of the product, its chemical characteristics, fatty acid composition, free amino acid contents, and nucleotides contents were evaluated during 4 weeks of storage. Sensory evaluation was conducted by both general consumers and body-builders. The sausage was proposed as a fat-free product as fat content was 0.12% based on the Korean Indication Standard of Animal Origin Food. Protein content was 13.42% and calorie value was 61.50 kcal/100 g of the sausage. In sensory evaluation, the mixture of chicken breast and egg whites stuffed into the same casing had an adverse effect on taste, color, texture and overall acceptance while the product that contained egg white stuffed separately into the outer casing enclosing the chicken breast (double layer) improved these attributes. The developed double-layer sausage can last for at least 4 weeks of storage without quality deterioration of flavor-related compounds, such as fatty acids and nucleotides.

Key words: fat-free, sausage, chicken breast, egg white

Introduction

Obesity is a problem in modern society associated with chronic diseases including cardiovascular disease, cancer, diabetes, and musculoskeletal disorders (Kim et al. 2011; Ladabaum et al. 2014) and lowering fat intake has been recommended to reduce obesity prevalence (Bray & Popkin 1998; Cho et al. 1997). Furthermore, high protein and low calorie diets can decrease weight and health risk while maintaining muscle mass and strength (Cho et al. 1997; Hernandez-Alonso et al. 2015). Chicken meat is an excellent source of protein and n-3 polyunsaturated fatty acids (PUFA) and it contains relatively lower fat and cholesterol than other animal origin foods (Jung et al. 2015; Seo et al. 2015). Milicevic et al. (2015) studied the physicochemical and functional properties of chicken meat and reported that protein composition was higher in chicken breast (19.59-23.62%) compared to that in chicken leg (16.72-19.93%) while fat content was lower in the breast (1.32-6.78%) than in the leg (4.33-15.29%). This means that chicken breast is more suitable for health-related purposes, having high protein and low fat. Additionally, egg contains high protein (12.14%) and high biological value (93.7) while egg white has no fat and low calories (Lee SK 2005). Therefore, we believe that the combination of chicken breast and egg white would be a good approach to produce flavorful products, which cater to the requirements of consumers interested in weight management as well as health benefits through diet.

However, the consumption pattern for chicken breast is limited to cooking intact cuts (boiling, roasting, smoking, or steaming), which does not favor general consumers due to the flavor and too lean texture. Thus, we developed a type of sausage made of chicken breast and liquid egg white and tried to improve the
texture. The quality attributes of the sausage were evaluated during 4 weeks of storage.

Materials and Methods

1. Sausage manufacture

Chicken breast (Cherrybro Co., Ltd., Jincheon, Korea) and frozen liquid egg white (Ganong Bio Co., Ltd., Pocheon, Korea) were purchased from a local market (Jeungpyeong, Korea). The egg white was thawed in a refrigerated condition prior to sausage manufacture and the chicken breast was ground using a grinder and a bowl cutter. Ingredients such as ice water, salt, phosphate, ascorbic acid, spice mix, red pepper, garlic, carrot, and onion were added to the chicken breast then mixed with the egg white in a bowl cutter, and the batter was stuffed in a pork natural casing (Table 1). No fat or salt was added. The sausages were cooked until their internal temperature reached 85°C, cooled, and stored in a refrigerated condition. The chemical composition (%) and changes in the fatty acid composition (%), free amino acid contents (mg%), and nucleotides contents (mg%) of the fat-free sausage were evaluated during 4 weeks of storage. We prepared another double layer product using 2 different casings [a sheep natural casing (an inner casing, 22 mm diameter) and separated the egg white in a PVDC casing (an outer casing, 50 mm diameter) as shown in Fig. 1].

2. Chemical characteristics

The chemical composition and calorie of the sausage were assessed using slightly modified AOAC methods (1995). Briefly, the moisture content was obtained by drying the sample (3 g) placed in an aluminum dish at 104°C for 15 hr. Crude protein contents were measured with the Kjeldahl method (VAPO45, Gerhardt Ltd., Idar-Oberstein, Germany). Crude fat contents were determined using the Soxhlet extraction system (TT 12/A, Gerhardt Ltd., Idar-Oberstein, Germany). Crude ash content was measured with igniting 2 g of each sample in a furnace at 600°C overnight. Carbohydrate contents were calculated by subtraction of moisture, protein, fat, and ash from total 100% basis. The chemical composition was employed for the calculation of the number of calories in the sausage and the calories were expressed as kcal/100 g.

3. Fatty acid composition

Lipids were extracted from meat samples according to the method described by Folch et al. (1957). BF3-methanol (1 ml, Sigma-Aldrich, St. Louis, MO, USA) was added to 100 μL of lipid extract and incubated at 70°C for 30 min. After cooling, 2 mL of hexane and 5 mL of distilled water were added, mixed thoroughly and left overnight for phase separation. The top (hexane) layer containing methylated fatty acids was analyzed using a gas chromatograph (HP 7890, Agilent Technologies, Santa Clara, CA, USA). A split inlet (split ratio, 100:1) was used to inject the samples into a column (DB-Wax; 50 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, CA, USA). The oven temperature was 200°C while the inlet and detector temperatures were 250°C. Helium was used as the carrier gas at a constant flow rate of 0.79 mL/min. The data was integrated within chemstation software (Agilent Technologies, Santa Clara, CA, USA).

4. Free amino acid contents

Defatted meat samples (2.5 g) were mixed with 10 mL of 2% trichloroacetic acid solution and homogenized (T25, Ika Works, Staufen, Germany) at 13,500 rpm for 1 min. The homogenate was then centrifuged and filtered through a 0.45 μm membrane filter. The filtrate was derivatized using the Waters AccQ-Tag method (1993, Millipore Co-Operative, Milford, MA, USA) and 5 μL was injected into a RP-HPLC (AccQ · Tag™ column; 3.9 × 150 mm). The column temperature was set to 37°C and a fluorescent detector (Waters™ 2475, Millipore, Billerica, MA, USA) was used with 250 nm excitation and 395 nm emission wavelengths. The separation was performed using buffers: A (Waters AccQ · Tag eluent) and B (60%, v/v, acetonitrile). The accuracy and repeatability of this analysis were ensured by the inclusion of a control sample of known amino acid composition prior to hydrolysis.
5. Nucleotide contents

Meat samples (5 g) were mixed with 20 mL of 0.7 M perchloric acid and homogenized (T25, Ika Works) to extract nucleic acids. The homogenized samples were then centrifuged at 3,000 rpm for 15 min (Continent 512R, Hanil Co., Ltd, Incheon, Korea) and filtered through a filter paper (Whatman No. 4, Whatman PLC., Kent, UK). The supernatant was then adjusted to pH 5 with 7 N KOH, transferred into a volumetric flask and brought to a volume of 50 mL with 0.7 M perchloric acid (pH 5). After 30 min of cooling, the samples were centrifuged at 3,000 rpm for 15 min and the supernatants were filtered through a membrane filter (0.2 μm) into a glass vial. The samples were injected into a high performance liquid chromatography (HPLC; Ultimate 3000, Thermo Fisher Scientific Inc., Waltham, MA, USA) system. The analytical conditions for HPLC included a Synergi™ Hydro-RP 80 Å (250×4.6 mm, 4 μm particles; Phenomenex Inc., Seoul, Korea), 20 mM potassium phosphate, monobasic (pH 5) conditions, with a flow rate at 1.0 mL/min. The injection volume was 10 μL and elution time was 25 min. The column temperature was maintained at 30°C and detection was monitored at a wavelength of 254 nm. Each content was calculated through a standard curve obtained using a standard adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine, and hypoxanthine (Sigma-Aldrich) and calculated using the area for each peak.

6. Sensory evaluation

Sensory evaluation was conducted by 49 panelists that consisted of 15 general consumers and 34 body-builders. The samples used were A (the sausage with chicken breast and egg white mixed in the same casing), B (the sausage made of chicken breast in the inner casing and egg white in the outer casing), and C (market product, meatball composed of 87.43% chicken breast). The different chicken breast meat products were compared using a 7-point hedonic scale to evaluate the preference for the products according to the following criteria: like extremely (7), neither like nor dislike (4), and dislike extremely (1). The sensory parameters used in the present study were flavor, odor, color, texture, and overall acceptance.

7. Statistical analysis

Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and when significant differences were detected, the differences among the mean values were identified by Duncan's multiple range test using SPSS software with the confidence level at P<0.05 (SPSS 12.0, Statistical Package for Social Sciences, SPSS Inc., USA).

Results and Discussion

1. Chemical composition

Compared to normal commercial products, relatively higher moisture (84.17%) and lower fat (0.12%) contents were observed in the sausage produced in the present study (Table 2). Andres et al. (2006) made low-fat chicken sausages containing 76.61% of moisture and 0.61% of fat while Lin et al. (2002) evaluated the quality of chicken sausage and found 66.5% of moisture and 18.5% of fat contents in the products. As fat was not added to the sausages made of chicken and egg white (Table 1), the chemical composition in Table 2 seems reasonable. Even considering the non-addition of fat during the manufacturing process (Table 1), the moisture content still seems higher compared to the results obtained by Andres et al. (2006). Phosphate addition at 0.27% and 81.73% chicken breast may increase the pH, resulting in improved water holding capacity for the sausage. In the preliminary study, the chicken breast used during the sausage manufacture in the present study was found to contain high levels of arginine (682.26-799.04 mg%, data not shown) and this may be one of the reasons for the high moisture content even after cooking. Similarly, Kim et al. (2014) suggested that L-arginine could be one of the substitutes for phosphate as the moisture content of pork sausage was increased from 56.9% to 61.8% with 0.5% addition of arginine. Qin et al. (2015) reported

Table 1. Ingredient composition (%) of fat-free sausage containing chicken breast and egg white

| Inner casing |          |          |          |
|--------------|----------|----------|----------|
| Chicken breast | 81.73    | Ice water | 9.08     |
| Salt         | -        | Phosphate | 0.27     |
| Ascorbic acid | 0.02     | Spice mix | 0.91     |
| Red pepper   | 1.48     | Carrot   | 0.95     |
| Garlic       | 2.80     | Onion    | 2.76     |

| Outer casing | Egg white | 100.00 |

by Duncan's multiple range test using SPSS software with the confidence level at P<0.05 (SPSS 12.0, Statistical Package for Social Sciences, SPSS Inc., USA).
increased water holding capacity for chicken breast with 0.15, 0.20, and 0.25% L-arginine due to protein aggregation \((P < 0.05)\).

With 0.12% fat, the protein content (13.42%) was relatively high and the calorie content was calculated as 61.50 kcal/100 g, suggesting that the sausage made of chicken and egg white is a fat-free, high-protein, and low calorie meat product at retail (Table 2) according to the Korean indication standard of animal origin food (MFDS, 2015). The contents of ash and carbohydrate were insignificant in the present study as the sausage were low in those constituents (0.84% and 1.47%, respectively).

2. Fatty acid composition

Table 2. Chemical characteristics of fat-free sausage containing chicken breast and egg white

| Moisture (%)| Protein (%) | Fat (%) | Ash (%) | Carbohydrate (%) | Calorie (kcal/100 g) |
|-------------|------------|---------|---------|------------------|----------------------|
| 84.17±0.57  | 13.42±0.46 | 0.12±0.04| 0.84±0.08| 1.47±0.08        | 61.50                |

Variation in fatty acid composition is important because it can lead to changes in the firmness, shelf-life, and flavor of meat (Wood et al. 2003). The fatty acid composition of the sausage containing chicken breast and egg white was not significantly changed during 4 weeks of storage (Table 3). Oleic acid was highest among the fatty acids followed by palmitic acid (C16:0) and linoleic acid (C18:2). The high concentration of UFA was attributed to the high amount of oleic acid and linoleic acid in chicken breast (data not shown), indicating that the sausage can be a good source of polyunsaturated fatty acids (PUFA). In addition, the ratio of PUFA to saturated fatty acids (SFA) ranged from 0.53 to 0.57, which is above the 0.4 ratio recommended by the Department of Health in the UK (Wood et al. 2003).

Table 3. Changes in fatty acids composition (%) of fat-free sausage containing chicken breast and egg white during 4 weeks of storage

| C12:0 | 0.04±0.00 | 0.04±0.00 | 0.04±0.00 | 0.04±0.00 |
| C14:0 | 0.72±0.01 | 0.72±0.01 | 0.72±0.01 | 0.72±0.01 |
| C14:1 | 0.21±0.01 | 0.21±0.01 | 0.21±0.01 | 0.21±0.01 |
| C16:0 | 25.50±0.13 | 25.50±0.13 | 25.50±0.13 | 25.50±0.13 |
| C16:1 | 5.50±0.07  | 5.50±0.07  | 5.50±0.07  | 5.50±0.07  |
| C18:0 | 6.20±0.06  | 6.20±0.06  | 6.20±0.06  | 6.20±0.06  |
| C18:1 | 36.50±0.55 | 36.50±0.55 | 36.50±0.55 | 36.50±0.55 |

\(1^{st}\) SFA: saturated fatty acid, \(2^{nd}\) UFA: unsaturated fatty acid, \(3^{rd}\) MUFA: monounsaturated fatty acid, \(4^{th}\) PUFA: polyunsaturated fatty acid
However, the actual health beneficial effect of the fatty acids may be minimal in the present study because the fat content was less than 0.5% in the final product.

3. Free amino acid contents

Free amino acids are important for aroma development and their contents showed a significant decrease during storage, especially at week 1 (Table 4). Rabie et al. (2014) analyzed the evolution of each amino acid as well as the total free amino acid contents in sausages made of horse meat, beef, and turkey meats. The concentration of total amino acids in the horse and beef sausages increased while that in the turkey sausage decreased from 48.01 mg/kg to 17.03 mg/kg within the 28 day period. The decrease of total free amino acid contents in the turkey sausage was attributed to microbial uptake and conversion to biogenic amines due to the amino acid decarboxylase activity of the microorganisms (Rabie et al. 2014; Virgili et al. 2007). However, the sausage in the present study did not show microbial growth (data not shown) and the decrease of total free amino acid could be explained by water separation from the sausage detected during 4 weeks of storage. In addition, the major free amino acid in the sausage was threonine (77.10-89.71 mg%) followed by arginine (44.78-54.78 mg%), lysine (26.35-33.92 mg%), and glutamic acid (18.63-22.40 mg%). These free amino acid values were different from those of raw chicken breast [arginine (682.66-799.04 mg%), histidine (37.57-88.46 mg%), threonine (25.08-36.71 mg%), and lysine (34.62-48.49 mg%), data not shown] due to the egg white and the other ingredients added while manufacturing the sausage.

4. Nucleotide contents

After slaughter, adenosine triphosphate (ATP) becomes IMP, which is degraded to inosine, hypoxanthine, and further, ribose (Lee et al. 2015). IMP is a principal nucleotide in postmortem muscle and postmortem changes improve the taste and flavor of meat (Maga JA 1983; Ishiwatari et al. 2013). In our results, the initial IMP concentration was as low as 28.98 mg% compared to those reported in fresh meat, however, there was no significant change during storage (Table 5). Ishiwatari et al. (2013) simulated the decomposition kinetics of IMP during the cooking of meat at different temperatures and reported that the enzyme activity related to IMP formation decreased above 40°C. We can assume

Table 4. Changes in free amino acid contents (mg%) of fat-free sausage containing chicken breast and egg white during 4 weeks of storage

|      | 0      | 1      | 2      | 3      | 4      |
|------|--------|--------|--------|--------|--------|
| Ala  | 14.05±0.75<sup>a</sup> | 11.95±0.58<sup>a</sup> | 12.12±0.65<sup>a</sup> | 11.79±0.23<sup>a</sup> | 11.92±0.55<sup>a</sup> |
| Arg  | 54.78±2.00<sup>a</sup> | 48.01±2.50<sup>a</sup> | 48.29±2.66<sup>a</sup> | 44.78±1.44<sup>a</sup> | 47.71±2.13<sup>a</sup> |
| Asp  | 9.54±0.32<sup>a</sup> | 8.61±0.44<sup>a</sup> | 8.50±0.32<sup>a</sup> | 8.01±0.33<sup>a</sup> | 8.48±0.30<sup>a</sup> |
| Cys  | 3.15±0.14<sup>a</sup> | 3.19±0.12<sup>a</sup> | 2.97±0.11<sup>a</sup> | 2.40±0.17<sup>a</sup> | 2.90±0.08<sup>a</sup> |
| Glu  | 22.40±0.54<sup>a</sup> | 21.71±1.16<sup>a</sup> | 20.55±1.03<sup>a</sup> | 18.63±0.73<sup>a</sup> | 20.08±0.64<sup>a</sup> |
| Gly  | 8.38±0.31<sup>a</sup> | 7.28±0.34<sup>a</sup> | 7.44±0.36<sup>a</sup> | 7.25±0.12<sup>a</sup> | 7.34±0.34<sup>a</sup> |
| His  | 14.41±0.56<sup>a</sup> | 12.33±0.57<sup>a</sup> | 12.16±0.56<sup>a</sup> | 11.84±0.16<sup>a</sup> | 12.38±0.51<sup>a</sup> |
| Ile  | 6.42±0.16<sup>a</sup> | 5.86±0.18<sup>a</sup> | 5.91±0.19<sup>a</sup> | 5.83±0.06<sup>a</sup> | 5.92±0.20<sup>a</sup> |
| Leu  | 9.36±0.25<sup>a</sup> | 8.39±0.32<sup>a</sup> | 8.53±0.33<sup>a</sup> | 8.30±0.10<sup>a</sup> | 8.46±0.32<sup>a</sup> |
| Lys  | 33.92±5.27<sup>a</sup> | 29.70±6.53<sup>a</sup> | 27.94±2.57<sup>a</sup> | 27.88±4.88<sup>a</sup> | 26.35±2.72<sup>a</sup> |
| Met  | 6.12±0.12<sup>a</sup> | 5.65±0.13<sup>a</sup> | 5.71±0.17<sup>a</sup> | 5.65±0.05<sup>a</sup> | 5.69±0.17<sup>a</sup> |
| Phe  | 7.37±0.17<sup>a</sup> | 6.80±0.19<sup>a</sup> | 6.84±0.19<sup>a</sup> | 6.71±0.07<sup>a</sup> | 6.87±0.23<sup>a</sup> |
| Pro  | 7.58±0.26<sup>a</sup> | 6.81±0.24<sup>a</sup> | 6.85±0.30<sup>a</sup> | 6.78±0.05<sup>a</sup> | 6.87±0.25<sup>a</sup> |
| Ser  | 13.13±0.47<sup>a</sup> | 11.54±0.55<sup>a</sup> | 11.69±0.62<sup>a</sup> | 11.15±0.22<sup>a</sup> | 11.53±0.46<sup>a</sup> |
| Thr  | 89.71±3.19<sup>a</sup> | 77.10±3.79<sup>a</sup> | 79.64±4.40<sup>a</sup> | 78.59±2.24<sup>a</sup> | 79.01±4.16<sup>a</sup> |
| Tyr  | 9.03±0.28<sup>a</sup> | 8.24±0.25<sup>a</sup> | 8.24±0.31<sup>a</sup> | 8.18±0.09<sup>a</sup> | 8.33±0.22<sup>a</sup> |
| Val  | 7.87±0.26<sup>a</sup> | 7.00±0.27<sup>a</sup> | 7.06±0.31<sup>a</sup> | 6.95±0.08<sup>a</sup> | 7.05±0.30<sup>a</sup> |

<sup>1)</sup> Values with different superscripts in the same row (−) are significantly different (P<0.05).
Table 5. Changes in nucleotides contents (mg%) of fat-free sausage containing chicken breast and egg white during 4 weeks of storage

|                          | Storage period (week) |
|--------------------------|-----------------------|
|                          | 0                     | 1       | 2                     | 3                     | 4                     |
| Adenosine monophosphate  | 2.90±0.67<sup>ab</sup> | 4.57±0.49<sup>b</sup> | 4.85±1.05<sup>a</sup> | 4.42±0.76<sup>b</sup> | 3.91±0.45<sup>ab</sup> |
| Inosine monophosphate    | 28.98±1.84<sup>b</sup> | 27.11±3.70<sup>b</sup> | 32.60±4.97<sup>a</sup> | 29.39±2.72<sup>b</sup> | 27.37±3.54<sup>b</sup> |
| Inosine                  | 76.78±2.31<sup>a</sup> | 68.74±4.46<sup>d</sup> | 69.59±2.28<sup>d</sup> | 72.31±2.25<sup>bc</sup> | 74.19±1.69<sup>ab</sup> |
| Hypoxanthine             | 10.11±1.28<sup>ab</sup> | 8.53±1.25<sup>bc</sup> | 8.10±0.88<sup>c</sup> | 9.58±1.07<sup>ab</sup> | 9.81±1.25<sup>ab</sup> |

<sup>1)</sup> Values with different superscripts in the same row (***) are significantly different (P<0.05).

that IMP was decomposed during the sausage manufacturing process in the presence of heat. Thus, low initial contents at week 1 and no changes during 4 weeks of storage were observed.

5. Sensory evaluation

Sausage B was a double layered sausage made by stuffing chicken breast mixture in the inner casing and egg white in the outer casing (Fig. 1) while sausage A was prepared by mixing chicken breast and egg white together and stuffing them into the same casing. Sausages A and B and market product C (meatball made by chicken breast) were provided to the sensory panelists grouped into general consumers and body-builders. The mixture of chicken breast and egg white in the same casing had an adverse effect on sensorial properties, except odor, whereas the double layer sausage showed an improvement in these properties (Table 6). Sensory score on taste, odor, color, texture and overall acceptance for sausage B had no significant difference compared to those of C. The results for the texture of the sausage were varied between general consumers and body-builders. Because the body-builders had more experience with eating meat products made of chicken breast than general consumers, the body-builders did not differentiate between sausages A and B and market product C. However, the general consumer group did not like the texture of sausage A (P < 0.05).

Conclusion

From the results, we concluded that the sausage made of chicken breast and egg white is a fat-free, high-protein, and low calorie meat product. The double layered sausage (the sausage B) had significantly improved texture compared to the sausage made by mixing the chicken breast and egg white together in one casing. Further, the sausage could be a product in the retail market for consumers seeking alternative foods for weight management due to minimal fat, no added salt, and desirable sensory qualities. The flavor-related compounds of the sausage did not change significantly during 4 weeks of refrigerated storage.

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Table 6. Sensory evaluation of fat-free sausage containing chicken breast and egg white compared to market product

|                          | General consumers | Body-builders |
|--------------------------|-------------------|---------------|
|                          | A<sup>1)</sup>    | B             | C             | A             | B             | C             |
| Taste                    | 3.27±1.22<sup>b</sup> | 5.73±1.28<sup>a</sup> | 5.60±0.99<sup>a</sup> | 4.62±1.41<sup>b</sup> | 5.38±1.30<sup>a</sup> | 5.56±0.93<sup>a</sup> |
| Odor                     | 4.80±0.68<sup>a</sup> | 5.00±0.85<sup>a</sup> | 5.07±1.10<sup>a</sup> | 4.53±1.26<sup>b</sup> | 4.79±1.45<sup>ab</sup> | 5.18±1.06<sup>a</sup> |
| Color                    | 4.73±0.88<sup>b</sup> | 5.53±1.06<sup>a</sup> | 5.40±0.99<sup>b</sup> | 4.59±1.21<sup>b</sup> | 5.26±1.26<sup>ab</sup> | 5.15±1.16<sup>b</sup> |
| Texture                  | 3.33±1.05<sup>b</sup> | 5.47±0.99<sup>a</sup> | 5.20±1.01<sup>a</sup> | 4.06±1.25<sup>a</sup> | 5.00±1.37<sup>a</sup> | 5.26±1.21<sup>a</sup> |
| Overall acceptance       | 4.07±0.80<sup>b</sup> | 5.60±1.06<sup>a</sup> | 5.47±0.99<sup>a</sup> | 4.66±1.19<sup>b</sup> | 5.28±1.18<sup>a</sup> | 5.36±1.24<sup>a</sup> |

<sup>1)</sup> Sausage made of chicken breast and egg white (A) mixed in the same casing; (B) separated in the outer casing or (C) market product at retail.

<sup>2)</sup> Values with different superscripts in the same row (***) are significantly different (P<0.05).
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