CHARACTERISTICS OF INTERACTIONS BETWEEN SOME TEXTURE PROPERTIES AND COMPOSITION OF CARRAGEENAN GELS AS A RESULT OF ITS DEFINED DIVERSIFIED FREEZING AND THAWING TREATMENT

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ABSTRACT

Model samples of carrageenan gels based on water, milk and juice were air-blast frozen and frozen by immersion in glycol and in liquid nitrogen. The gel freezing rate was determined on the basis of the kinetics of freezing. Carrageenan gel samples were characterized by evaluation of its thawing drip loss and hardness determined with compression and penetration tests. Freezing in liquid nitrogen ensured the highest freezing rates. Thawing drip loss of gels significantly depended on the carrageenan content, pH of the solution, freezing method and freezing rate. The resulting relationships are linear functions with high determination coefficients. The results of compression and penetration tests prove the significant effect of the carrageenan content and pH on gel hardness. The higher carrageenan content in a sample, the higher compression force and penetration of the gel. Gel freezing resulted in lower hardness. Freezing conditions had a significant effect on the properties tested. The correlation between compression forces and penetration depending on the carrageenan content and the freezing method was described using regression equations with high determination coefficients. Gels based on milk and juice with 2.2% carrageenan content are recommended for immersion freezing at rates above 5.0 cm·h⁻¹.

Keywords: freezing, carrageenan, gels, drip loss, hardness.

INTRODUCTION

Carrageenan is a sulfated polysaccharide extracted from red seaweed (Rhodophyceae), which is applied widely as a food additive. Carrageenan is classified into tree types as kappa (κ), iota (ι) – gel forming or lambda (λ) – which does not form a gel [25, 29, 30]. κ-Carrageenan consists of a repeating unit composed of the disaccharide, β-(1-3)-D-galactose-4-sulfate and α-(1-4)-3.6-anhydro-D-galactose. ι-Carrageenan possesses two sulfate groups in a disaccharide repeat unit; β-(1-3)-D-galactose-4-sulfate and α-(1-4)-3.6-anhydro-D-galactose-2-sulfate. λ-Carrageenan consists of β-(1-3)-D-galactose-2-sulfate and α-(1-4)-D-galactose-2.6-disulfate including three sulfate groups. All carrageenan types dissolve in hot water (above 70 °C). Carrageenans dissolve in hot milk yielding gels upon cooling whose strength and consistency depend on concentration and affinity to calcium ions [14, 30]. κ-Carrageenans are readily soluble in aqueous sucrose solutions, while τ-carrageenan is scarcely soluble [27]. The gelation mechanism of carrageenan is based on the formation of double helix structure [25, 29].

The stability of carrageenan gels depends on pH, temperature, time, presence of metal ions, protein content, presence of other colloids, common salt and sugars. Increased pH of the medium leads to higher gelation temperature of κ-carrageenan and higher hardness of gels [25, 27]. An example of this is the effect of pH and carrageenan concentration on whey protein rheology properties [12]. The viscosity of whey protein gels consid-
erably gels were obtained at 0.3% polysaccharide concentration and pH 7. Gels formed from κ-carrageenan are hard, brittle, slightly opalescent and prone to syneresis. When gums, such as guar gum, are added to κ-carrageenan, its elasticity increases [11, 21, 29].

In the food industry, κ-carrageenan is used as a gelling, thickening, stabilizing and water-binding agent in various food products, such as instant products, dessert, sauces, milk, yogurt and meats. Carrageenans are used in meat processing as water-binding agents, fat-replacer, stabilisers for stuffing and meat emulsions, as functional additives for meat restructuring and in low-fat finely-ground sausages [1, 3, 25]. In dairy industry, they are used to stabilise condensed milk, creams and milk desserts, puddings, yoghurts [13, 31], processed cheese and emulsions [23, 25]. They are widely used in ice cream and other frozen food production in which they provide appropriate texture by reducing the growth of ice crystals and ensure higher product stability when storage temperature changes [25]. Also, they are used in baking and confectionary industry. The addition of carrageenan to dough in an amount of 0.1% improves the bread texture. The synergy of carrageenan and lecithin and milk proteins in dough leads to a higher strength of dough and improves loaf volume, shape and texture [19].

Positive effects of carrageenan as a cryoprotectant have been documented for frozen pork [8, 9] and poultry [20], ice cream [5], frozen dough [17], and survivability of lactic acid bacteria and yeasts [10].

The aim of this study was to analyse the effect of various freezing methods on selected properties of κ-carrageenan gels prepared in water, milk and juice. The scope of investigation included preparation of model gel samples, freezing them and determination of its hardness based on compression and penetration tests.

**MATERIAL AND METHODS**

In this study κ-carrageenan (E 407-Satiagel™ of 10-Cargill, France) was used. The model studies comprised carrageenan gels prepared on the basis of water (pH 7.0), milk (Mlekpol, protein 3.2 g, carbohydrates 4.7 g, fat 3.2 g/100 g milk, pH 6.6) and orange juice (Sokpol ekstrakt 10° brix, pH 3.7) with the carrageenan mass share of 1.0, 1.3, 1.6, 2.0, 2.2% to solvent weight ratio. The preparation of colloid solution (sol) consisted in measuring out adequate amount of cold solvent (juice, water or milk) and addition of carrageenan powder under vigorous agitation. The mixture was heated to temperature 80 °C. The prepared solutions were distributed into containers of 3.0 cm diameter and 14 cm³ volume (for the compression tests) and the containers of 3.0 cm diameter and 10 cm³ volume (for the penetration tests). The obtained solidified gel samples – not frozen (control – P0) and those after the thermal treatment were investigated.

The gels were frozen using: air method (Freezer Whirlpool, Italy, natural convection conditions, temperature -33 °C) and immersion freezing (Immersion cryostat Wiggen Hauser, Germany, in glycol, temperature -35 °C) or liquid nitrogen immersion (Dewar MVE Millennium 2000, USA – Cryopreservation System, temperature -196 °C). The freezing process was continued until the temperature in the thermal centre of the prepared model was -18 °C. During the freezing, the sample thermal centre temperature was recorded by means of a multi channel digital thermometer equipped with NiCrNi thermoelements with measurement accuracy of ±0.05 K. All the measurements were preceded by the verification of the thermometer readings, taking the temperature of distilled water-ice bath. The recorded temperatures in time were converted to the Excel spreadsheet to obtain the freezing curves which served for determination of the mean linear freezing rate according to the Recommendations of the International Institute of Refrigeration [16, 18].

\[
\frac{\delta}{\tau} = \frac{w}{\tau}
\]

where: \(w\) – mean linear freezing rate (cm·h⁻¹), \(\delta\) – thickness of frozen layer (cm), \(\tau\) – its freezing time (h).

After freezing, the samples were stored for 24 h in a cabinet freezer at temperature –33 °C. Then the samples were thawed at room temperature (+20 °C) until +10 °C was obtained in the centre of sample. Temperature of the sample center during thawing was measured and recorded with a multi channel digital thermometer equipped with NiCrNi thermoelements.

After thawing, the samples were dried with tissue paper and weighed each time to evaluate the amount of drip loss (\(L_d\)). The material weight
changes caused by the drip loss depended on the freezing technique. Thawing drip loss was determined as a difference between the sample weight before and after the thawing process [2].

\[ L_D = \frac{m_i - m_r}{m_i} \times 100\% \]  \hspace{1cm} \text{(2)}

where: \( m_i \) – material mass before freezing (kg), \( m_r \) – material mass after thawing (kg).

The unfrozen gel samples and those subjected to freezing process underwent the compression and penetration testing using the LFRA texture analyzer BROOKFIELD (Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts).

During the compression test the probe of 40 mm diameter cylinder was employed, mandrel displacement rate was 1.0 mm·s\(^{-1}\), initiation force 0.5 N, the compression test depth up to 50% of the sample height. Whereas at the penetration test, a cylinder probe of 7 mm diameter with a cone base angle of 45° was used; mandrel displacement rate was 1.0 mm·s\(^{-1}\), initiation force – 0.5 N, penetration depth was up to 50% of the sample height. The obtained research results were analyzed to establish the peak force of compression and penetration of gels [6].

The statistical analysis of the results was conducted by the variance analysis with Statistica 6 (StatSoft) software. Tukey tests was used to determine significant difference (\( p \leq 0.05 \)). The results of the statistical analysis were presented as comments in the text. The data were fitted using regression equations. The degree of fit was judged by the \( R^2 \) coefficient.

**RESULTS AND DISCUSSION**

Significant differences were noted in the freezing time of samples with regards to a freezing technique employed and freezing conditions provided. It was also found that the freezing time of samples did not depend significantly on the type of the prepared gels. The analysis of freezing curves has proved that the linear freezing rate relies on the freezing conditions and its value is very similar for all the gels, irrespective of the type of solvent.

Freezing process of the gel samples appeared to be the slowest in the air freezing conditions at -33 °C (\( w = 0.6 \text{ cm·h}^{-1} \)). The main cause of poor performance under these conditions was predominantly the low efficiency of heat transfer process between the surroundings and the material. The gel freezing process performed in the immersion cryostat with ethyl glycol as a refrigerating medium intensified the process and consequently, the freezing rate reached \( w = 5.0 \text{ cm·h}^{-1} \). Importantly, immersion freezing with liquid nitrogen ensured the highest freezing rate \( w = 35.7 \text{ cm·h}^{-1} \).

Drip loss is the synthetic indicator of the reversibility of gel freezing processes. Thawing drip loss determined by gel weight changes after thawing depending on the freezing method is shown in Table 1 (with significance of differences between means).

Changes in thawing drip loss, which indicate gel structure damage, were between 0 and 50.10% depending on the freezing method used. The largest significant (\( p < 0.05 \)) weight losses due to thawing drip were noted for water gels (\( \kappa \)-carrageenan content 1.0%) subjected to air-blast freezing at a freezing rate of 0.6 cm·h\(^{-1}\) (50.10%) and to immersion freezing in liquid nitrogen at a freezing rate of 35.7 cm·h\(^{-1}\) (34.60%). The gels prepared in milk at pH 6.6 subjected to immersion freezing in glycol and in liquid nitrogen had the lowest free thawing drip loss. For a sample with a 2.2% \( \kappa \)-carrageenan content subjected to immersion freezing in liquid nitrogen, no free thawing drip loss was observed. The results are confirmed in a number of studies [4, 15, 24], in which thawing drip loss as a general indicator of gel quality after thawing depended on the freezing rate and its relationship with the nature and size of ice crystals being formed. Furthermore, in product with a high water content, such as gels, cracks and structural damage in surface product layers may form at very high rates (above 10 cm·h\(^{-1}\)), which may lead to increased thawing drip loss. According to the guidelines of the International Institute of Refrigeration [16], a freezing rate of 5.0 cm·h\(^{-1}\) is sufficient to maintain appropriate gel structure.

A significant effect was found (\( p < 0.05 \)) of the \( \kappa \)-carrageenan content, freezing method and media at various pH values on the occurrence of thawing drip loss. The higher the carrageenan content, the lower thawing drip loss in the gel. Milk-based gels with pH 6.6 were found to be the most favourable systems in terms of pH changes. The relations between changes of thawing drip loss versus carrageenan content and freezing method were expressed by linear relationships with high correlations (Table 2). Determination
coefficients were between 0.61 and 0.96. Schmidt & Smith [26] defined the ability of polysaccharides, including carrageenan, to form more viscous solutions in milk than in water as milk reactivity. Similar interactions between carrageenan and milk and its effect on the rheological properties of carrageenan gels were also confirmed by other authors [7, 14, 21, 28, 31].

Hardness of all the formulated gels (the unfrozen samples and those thawed after earlier freezing at varying freezing rates) was examined on the grounds of the results of compression tests. The higher compression force applied, the higher gel hardness was displayed (Table 3).

The carrageenan content was found to determine the hardness of resulting gels in a statistically significant manner (p<0.05). Milk-based gels with a 2.2% carrageenan content had the maximum compression force (44.47 N). Water-based and juice-based gels had much lower compression force values with the same carrageenan content (18.31 N and 17.97 N, respectively). The available results of other authors [12, 21, 27, 29] confirm that the force needed to disrupt a carrageenan gel is a function of carrageenan concentration in a solution. Indeed, gel samples frozen at various rates had significantly (p<0.05) lower compression force values than those not subjected to freezing. Gels frozen in air at a rate of 0.6 cm·h⁻¹ had the lowest compression force values in a range of 0.59 N to 4.90 N (medium: water, pH 7.0). Reduced compression force values, and thus the

### Table 1. Dependence of drip loss ($L_D$) on freezing method and carrageenan addition

| Addition of carrageenan (%) | Freezing in air | Freezing in glycol | Freezing in liquid nitrogen |
|-----------------------------|-----------------|-------------------|-----------------------------|
| **Carrageenan – water**     |                 |                   |                             |
| 1.0                         | 50.10±0.79      | 3.70±0.45         | 34.60±1.53                  |
| 1.3                         | 45.07±0.32      | 1.40±0.00         | 21.37±0.92                  |
| 1.6                         | 41.03±0.66      | 0.97±0.28         | 12.43±0.47                  |
| 2.0                         | 35.90±1.32      | 0.97±0.20         | 11.27±0.85                  |
| 2.2                         | 33.37±1.59      | 0.40±0.00         | 6.77±0.83                   |
| p                           | p = 0.00        | p = 0.0004        | p = 0.00                    |
| **Carrageenan – juice**     |                 |                   |                             |
| 1.0                         | 7.10±0.26       | 2.83±0.40         | 2.47±0.15                   |
| 1.3                         | 4.67±0.64       | 2.40±0.17         | 1.00±0.17                   |
| 1.6                         | 3.17±0.15       | 1.97±0.15         | 0.50±0.17                   |
| 2.0                         | 1.83±0.11       | 1.37±0.05         | 0.37±0.05                   |
| 2.2                         | 0.77±0.05       | 0.50±0.17         | 0.27±0.23                   |
| p                           | p = 0.00        | p = 0.73          | p = 0.0008                  |
| **Carrageenan – milk**      |                 |                   |                             |
| 1.0                         | 3.00±0.26       | 0.83±0.09         | 0.33±0.05                   |
| 1.3                         | 2.07±0.11       | 0.50±0.10         | 0.27±0.23                   |
| 1.6                         | 1.07±0.05       | 0.30±0.00         | 0.13±0.23                   |
| 2.0                         | 0.87±0.20       | 0.33±0.03         | 0.10±0.17                   |
| 2.2                         | 0.40±0.00       | 0.23±0.12         | 0.00±0.00                   |
| p                           | p = 0.59        | p = 0.00          | p = 0.00002                 |

abc Mean values designated with different letters are statistically different in the columns (p ≤ 0.05).

### Table 2. Relations between the drip loss ($L_D$) (%) and the content of carrageenan (x) (%) depending on freezing method

| Environmentgels | Freezing method     | Carrageenan – water | R²        | Carrageenan – juice | R²        | Carrageenan – milk  | R²        |
|-----------------|---------------------|---------------------|-----------|---------------------|-----------|---------------------|-----------|
| **Freezing in air** |                     |                     | $L_D = 13.74x – 63.34$ | -0.96    | $L_D = 4.98x – 11.57$ | -0.96    | $L_D = 2.05x – 4.80$ | -0.90    |
| **Freezing in glycol** |                 |                     | $L_D = 2.23x – 5.12$ | -0.71    | $L_D = 1.84x – 4.79$ | -0.90    | $L_D = 0.42x – 1.13$ | -0.61    |
| **Freezing in liquid nitrogen** |              |                     | $L_D = 21.01x – 51.33$ | -0.86    | $L_D = 1.63x – 3.55$ | -0.74    | $L_D = 0.26x – 0.59$ | -0.64    |
Table 3. Compression force (N) in relation to gel freezing method

| Addition of carrageenan (%) | Control unfrozen | Freezing in air | Freezing in glycol | Freezing in liquid nitrogen |
|-----------------------------|------------------|-----------------|-------------------|-----------------------------|
| Carrageenan – water         |                  |                 |                   |                             |
| 1.0                         | 1.74±0.30        | 0.59±0.03       | 1.41±0.09         | 1.11±0.04                   |
| 1.3                         | 5.21±0.21        | 0.74±0.06       | 6.19±0.19         | 1.84±0.05                   |
| 1.6                         | 7.51±0.61        | 1.34±0.03       | 7.35±0.27         | 2.12±0.16                   |
| 2.0                         | 12.72±0.06       | 3.61±0.42       | 12.79±0.53        | 3.93±0.16                   |
| 2.2                         | 18.31±0.33       | 4.90±0.17       | 15.15±0.52        | 6.56±0.56                   |
| p                           | p = 0.0005       | p = 0.206       | p = 0.0001        | p = 0.01                    |
| Carrageenan – juice         |                  |                 |                   |                             |
| 1.0                         | 5.10±0.53        | 3.43±0.30       | 4.68±0.47         | 2.33±0.06                   |
| 1.3                         | 6.14±0.62        | 5.23±0.28       | 6.61±0.13         | 3.83±0.05                   |
| 1.6                         | 8.48±0.31        | 7.73±0.50       | 8.59±0.12         | 6.38±0.15                   |
| 2.0                         | 14.69±0.44       | 10.57±0.45      | 11.72±0.67        | 10.49±0.49                  |
| 2.2                         | 17.97±0.74       | 13.54±0.38      | 13.95±0.55        | 12.74±0.43                  |
| p                           | p = 0.00         | p = 0.00        | p = 0.00          | p = 0.00015                 |
| Carrageenan – milk          |                  |                 |                   |                             |
| 1.0                         | 12.60±0.36       | 5.97±0.15       | 10.70±0.85        | 7.63±0.15                   |
| 1.3                         | 16.67±0.50       | 7.07±0.25       | 11.57±0.20        | 10.17±0.25                  |
| 1.6                         | 22.37±0.86       | 9.27±0.20       | 14.67±0.60        | 13.13±0.11                  |
| 2.0                         | 35.83±1.83       | 16.07±0.10      | 21.27±0.45        | 20.13±0.25                  |
| 2.2                         | 44.47±0.58       | 20.30±0.26      | 24.10±0.10        | 22.83±0.11                  |
| p                           | p = 0.00         | p = 0.00        | p = 0.00          | p = 0.00                    |

abc Mean values designated with different letters are statistically different in the columns (p ≤ 0.05).

The hardness of frozen gels (compared to non-frozen ones) are related to changes due to the formation of crystalline ice structures. This may lead to changes in original properties of the gels [18, 22].

The relationships between the maximum compression force versus various carrageenan contents and freezing methods for carrageenan gels prepared in the media with various pH values are expressed by regression equations with high determination coefficients (Table 4).

A statistically significant (p<0.05) increase in penetration force with increasing carrageenan contents in samples irrespective of the medium in which they were prepared was found in the assessment of gel hardness, based on the maximum penetration force (Table 5). At the maximum carrageenan content (2.2%), the hardest gels were obtained in the carrageenan/juice (1.69 N) and carrageenan/milk (1.77 N) system. The freezing process had a significant effect (p<0.05) on penetration forces. Frozen gels had lower maximum penetration force values compared to the non-frozen ones.

The correlation between the carrageenan content and penetration force values for the gels tested depending on the freezing method is described using line equations (Table 6). The higher carrageenan content in a sample, the higher hardness of a gel (higher penetration force value). However, the dynamics of the changes varied for water, juice or milk based gels.

Table 4. Relations between the force compression of gels (Fc) (N) and the content of carrageenan (x) (%) depending on freezing method

| Environment gels | Carrageenan-water R² | Carrageenan - juice R² | Carrageenan - milk R² |
|------------------|-----------------------|------------------------|-----------------------|
| Control unfrozen | F_c = 12.97x – 11.91  | 0.96                   | F_c = 11.07x – 7.45   | 0.93                  |
| Freezing in air  | F_c = 3.71x – 3.77    | 0.89                   | F_c = 8.18x – 5.15    | 0.97                  |
| Freezing in glycol | F_c = 10.97x – 9.21 | 0.97                   | F_c = 7.60x – 3.20    | 0.98                  |
| Freezing in liquid nitrogen | F_c = 4.11x – 3.55 | 0.84                   | F_c = 8.86x – 7.21    | 0.98                  |
CONCLUSIONS

To conclude, it is noted that carrageenan gels are systems which form defined structures which undergo various changes during freezing. Various pH values and increased carrageenan contents had no significant effect on the gel freezing rate. Immersion freezing with liquid nitrogen ensured the highest freezing rate (35.7 cm·h⁻¹). Air-blast freezing (rate: 0.6 cm·h⁻¹) led to the greatest damage of gel structure, resulting in the largest thawing drip loss.

Gels prepared in milk at pH 6.6 and in juice at pH 3.7 with a 2.2% carrageenan content subjected to immersion freezing in glycol and in liquid nitrogen had the lowest thawing drip loss. Such factors as carrageenan content, medium type and pH (juice: 3.7; milk: 6.6; water: 7.0) and freezing conditions, had a significant (p<0.05) effect on the hardness of carrageenan gels in terms of compression forces and penetration. Frozen gels revealed a significant decrease in hardness, that is, lower compression forces and penetration, compared to non-frozen samples.

Table 5. Penetration force (N) in relation to gel freezing method

| Addition of carrageenan (%) | Control unfrozen | Freezing in air | Freezing in glycol | Freezing in liquid nitrogen |
|-----------------------------|------------------|----------------|-------------------|---------------------------|
| Carrageenan – water         |                  |                |                   |                           |
| 1.0                         | 0.05±0.00        | 0.02±0.002     | 0.04±0.006        | 0.030±0.003              |
| 1.3                         | 0.14±0.01        | 0.04±0.006     | 0.07±0.005        | 0.050±0.000              |
| 1.6                         | 0.30±0.01        | 0.06±0.005     | 0.12±0.010        | 0.083±0.000              |
| 2.0                         | 0.63±0.01        | 0.09±0.007     | 0.21±0.002        | 0.087±0.010              |
| 2.2                         | 1.03±0.04        | 0.10±0.004     | 0.35±0.010        | 0.120±0.010              |
| p                           | p = 0.00001      | p = 0.00001    | p = 0.00001       | p = 0.0102               |
| Carrageenan – juice         |                  |                |                   |                           |
| 1.0                         | 0.5±0.01         | 0.18±0.01      | 0.18±0.005        | 0.24±0.005               |
| 1.3                         | 0.78±0.01        | 0.31±0.01      | 0.28±0.005        | 0.65±0.003               |
| 1.6                         | 1.05±0.08        | 0.46±0.05      | 0.41±0.008        | 0.96±0.006               |
| 2.0                         | 1.51±0.04        | 0.63±0.01      | 0.62±0.006        | 1.41±0.04                |
| 2.2                         | 1.69±0.01        | 0.78±0.03      | 0.76±0.006        | 1.63±0.03                |
| p                           | p = 0.00001      | p = 0.00001    | p = 0.00001       | p = 0.00001              |
| Carrageenan – milk          |                  |                |                   |                           |
| 1.0                         | 0.7±0.01         | 0.17±0.05      | 0.20±0.006        | 0.34±0.02                |
| 1.3                         | 1.0±0.04         | 0.38±0.01      | 0.39±0.01         | 0.51±0.006               |
| 1.6                         | 1.27±0.01        | 0.52±0.01      | 0.61±0.01         | 0.72±0.02                |
| 2.0                         | 1.53±0.06        | 0.84±0.01      | 0.98±0.00         | 1.04±0.02                |
| 2.2                         | 1.77±0.02        | 1.10±0.07      | 1.24±0.01         | 1.22±0.02                |
| p                           | p = 0.0012       | p = 0.00001    | p = 0.00001       | p = 0.00001              |

abc Mean values designated with different letters are statistically different in the columns (p ≤ 0.05).

Table 6. Relations between the force penetration of gel (Fp) (N) and the content of gelatin (x) (%) depending on freezing method

| Environment gels | Carrageenan - water | R² | Carrageenan - juice | R² | Carrageenan - milk | R² |
|-----------------|---------------------|----|---------------------|----|-------------------|----|
| Control unfrozen| Fp = 0.78x – 0.84   | 0.91| Fp = 1.01x – 0.53   | 0.99| Fp = 0.87x – 0.16 | 0.99|
| Freezing in air | Fp = 0.07x – 0.05   | 0.99| Fp = 0.49x – 0.32   | 0.99| Fp = 0.74x – 0.59 | 0.97|
| Freezing in glycol| Fp = 0.24x – 0.24 | 0.91| Fp = 0.48x – 0.32   | 0.98| Fp = 0.86x – 0.71 | 0.98|
| Freezing in liquid nitrogen| Fp = 0.07x – 0.03 | 0.89| Fp = 1.15x – 0.88   | 0.99| Fp = 0.73x – 0.42 | 0.99|
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