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ARTICLE

Effect of fermented vinegar on the reduction in trimethylamine in konjac glucomannan gel

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Abstract *Konjac glucomannan* (KGM) is one of the non-digestible dietary polysaccharides. Hydration of KGM at elevated temperature in the presence of calcium hydroxide affects the aggregation of KGM, making a konjac glucomannan gel formation that has a fish-like off odor. Trimethylamine (TMA) in konjac glucomannan gel has been investigated by solid-phase microextraction and analyzed by GC–MS. A fish-like off odor generated in the process of gelation has been remarkably reduced by treatment of the gel formation with fermented vinegar in which acetic acid concentration-treated group was over 1% (v/v), soaking time, for 10 min. Together with the instrumental analysis, a fish-like off odor generated from the same treatment was also analyzed by sensory evaluation. Acetic acid treatment at a concentration of over 1% (v/v) resulted in significant decrease in the fish-like off odor ($p < 0.05$) supporting the action mechanism of acetic acid as a proton donor for the reaction with TMA. Correlation between TMA content by GC–MS analysis and fish-like off odor by sensory evaluation was statistically significant by ANOVA, Duncan's multiple range test, operated by SPSS program ($R^2 = 0.9848$, $p < 0.05$).

Keywords Fermented vinegar · Fish-like off odor · *Konjac glucomannan* · Trimethylamine

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Introduction

Konjac glucomannan (KGM), a glucomannan isolated from the tuber of *Amorphophallus konjac C. Koch*, is soluble in water, and the resultant sol is readily gelatinized following treatment with alkali [1–3]. KGM is mainly a straight-chain polymer and consists of D-mannose and D-glucose in a ratio of 1.6:1. KGM consists of indigestible dietary fiber, is present in low-calorie food, and has a role in weight control. A review of studies reports that KGM has also anti-obesity activity, anti-hyperglycemic and hypercholesterolemia activities, a laxative effect, and prebiotic activity as a food ingredient [4].

However, konjac glucomannan gel is limited in common uses without further treatment to alleviate its original off odor generated during the gelation process with alkali, in that commercial konjac and *konnyaku* (gelation of konjac) products usually have a fish-like off odor, one of the compounds of which is known as trimethylamine (TMA) [5]. Although konjac glucomannan gel has many advantages, the fish-like off odor is the major factor limiting its application in food processing.

Many trials are found to reduce the fish-like off odor in konjac glucomannan gel. *Konnyaku* TMA is reduced by treatment with boiling water [5]. Deodorization of purified fish oil from squids has been reported by treatment with organic acids [6].

Additionally, a previous report indicated that TMA in mackerel oil can be eliminated following treatment with citron [7]. TMA compound has been reported to be able to react with acetic acid to result in the formation of deprotonated acetic acid and protonated TMA, suggesting that acetic acid could play a role as a proton donor for the reaction with TMA [8].

The trimethylamine of odor causing substances occurring in konjac glucomannan gel was reduced by vinegar (acetic acid), and the purpose is to analyze the change in TMA content. The results suggest that acetic acid could function as a proton donor with the formation of deprotonated acetic acid and protonated TMA that makes TMA into non-volatile salts [9].

The quantitative methods of analysis of TMA using solid-phase microextraction (SPME) have been reported [10–12], in which Carboxen/Polydimethylsiloxane (CAR/PDMS) for the analysis of dimethylamine and trimethylamine has been used as adsorption fiber [12–16].

The purpose of this study is to find out a new method to eliminate the volatiles in which the critical compound is TMA in konjac glucomannan gel formation, demonstrating the possible action mechanism of acetic acid as a proton donor to react with TMA. In the present study, TMA content using SPME associated GC–MS was measured as an instrumental analysis, together with the evaluation of sensory characteristics by the trained panels, ultimately proving the statistical correlation of the parameters.

Materials and methods

Reagents and chemicals

Konjac flour (Hanwoore, China) and Calcium hydroxide (ES food, Korea) were used in this study, and fermented vinegar was supplied by Daesang Co. in Korea. CAR/PDMS, SPME fiber, with a needle size of 24 ga, was purchased from Supelco in USA. TMA hydrochloride (98%) and n-Octanol as an internal standard were acquired from Sigma-Aldrich in USA.

Preparation of sample

Konjac flour was added to heated water (65–70 °C), making a 4% (w/v) concentration, stirring a mechanical stirrer at a speed of 500 rpm for 3 min, by making a konjac glucomannan gel formation, followed by cooling off at 45 °C. It was added to a 5% calcium hydroxide solution (w/v) and mixed to strengthen the gel formation and to induce the self-aggregation of glucomannan molecules. Konjac glucomannan gel was molded, aged and stored at 4 °C in a refrigerator until use [17, 18].

For the treatment of konjac glucomannan gel to alleviate its fish-like off odor, the sample was cut into small cubes (2.0 × 2.0 × 2.0 cm) and soaked in fermented vinegar with the concentrated of 1–4% acetic acid (v/v) for 10 min. After soaking, a sample was washed in distilled water for 10 min, put into 20 mL vials with the internal standard solution sealed with PTFE (Polytetrafluoroethylene)—coated silicone rubber septa and extracted by SPME [19].

HS-SPME (Headspace solid-phase microextraction) procedure

The SPME apparatus consisted of SPME fiber and SPME holder. CAR/PDMS, SPME fiber, with a needle size of 24 ga was conditioned for 30 min at 300 °C. A sample was put into a 20 mL glass vial sealed with PTFE-coated silicone rubber septa and allowed to reach equilibrium in a 40 °C water bath for 30 min. SPME fiber was adsorbed onto the fiber for 20 min at 40 °C. After adsorption, the fiber was desorbed in the injection port of the GC for 5 min.

GC–MS analysis

A gas chromatography detector (Agilent type 6890N) coupled with a mass spectrometric detector (MS) (Agilent 5973N) was used during the analysis. The separation was carried out on HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) [20–22]. The flow rate of the helium carrier gas was 1.0 mL/min, and injector temperature was 230 °C in splitless mode. The column temperature program was set as follows: maintained at 80 °C for 5 min and then increased at a rate of 10 °C/min and held at 230 °C for 4 min. After analysis, post-run time was 5 min at 280 °C [11, 14, 23, 24]. The mass spectrometer was used in Electron Impact (EI) ionization mode at 70 eV using scan mode. Identification of TMA in konjac glucomannan gel was carried out by spectra comparison with the National Institute Standards and the Technology (NIST) Mass Spectral Library. In addition, the mass spectrometer was operated in selected ion mode (SIM) to quantify specific compounds with increased sensitivity relative to scan mode. For the quantification of TMA, ions 42, 58, and 59 were monitored with quantification based on the response of ion 58; ions 41, 56, 70, and 84 were used to identify the internal standard (n-Octanol), and the response of ion 56 was used to determine the response factor [25]. Standard solution was prepared by dissolving TMA hydrochloride, the purity of which was 98%, in 1000 mL of deionized water. A calibration curve was obtained at the range of the standard concentration, 0.22–55 ppm.

Sensory evaluation

The sensory evaluation of konjac glucomannan gel samples was performed by the 15 trained panels and measured by 9-point scale, the attributes of which was the overall

preference of fragrant and the intensity of fish-like off odor and sour odor [26].

The sample was cut into 1 × 2 × 2 cm cubes and sealed in a cutting and sealing in a aseptic bag to prevent the loss of fragrant. The performance of the sensory evaluation with the trained panels was repeated 3 times.

Statistical analysis

The statistical of the results obtained was analyzed by ANOVA, Duncan's multiple range test, operated by SPSS (statistical package for social science) program [27]. Pearson's correlation analysis was performed to find out the statistical relationship between the GC–MS and sensory evaluation results. The statistical significance of differences between mean values of those was interpreted at the significant level, $p < 0.05$ [28].

Results

Identification of TMA in konjac glucomannan gel

Among the peaks representing volatiles found in gas chromatographic scan mode separation, TMA was identified at a particular target peak in the SIM data. The retention time of TMA standard was estimated to be 1.62 min at the experimental conditions described in the analytical method, and the same compound in konjac glucomannan gel was identified as TMA according to the NIST Mass Spectral Library (data not shown).

Optimization of TMA adsorption time in SPME extraction

To extract TMA out of konjac glucomannan gel effectively, the adsorption time was set up at 5, 10, 20, and 30 min, respectively, and TMA content was measured by GC–MS. Referring to the experimental data, the adsorption time of 20 min was shown to be the highest TMA contents, 3.31 ± 0.34 ppm (Table 1).

Table 1 Optimization of adsorption time of TMA in SPME

Adsorption time (min)	TMA content (ppm)
5	2.37 ± 0.18^{a}
10	2.99 ± 0.19^{bc}
20	3.31 ± 0.34^{c}
30	2.78 ± 0.27^{ab}

[a,b,c] Mean with different letters are significantly different according to Duncan's multiple range test ($p < 0.05$)

Effect of acetic acid soaking concentration on TMA content

To measure TMA content of the treated konjac glucomannan gel according to the soaking condition of different acetic acid concentration-treated group originated from fermented vinegar, the concentration of acetic acid was set up at 0, 1, 2, 3, and 4% (v/v), respectively, where 0% acetic acid concentration-treated group means no addition of acetic acid into the distilled water, where the soaking time of konjac glucomannan gel into the acetic acid solution was set to 10 min following 10-min washing with distilled water. Non-treated and 0% of acetic acid concentration-treated group showed TMA content of 3.18 ± 0.62 and 3.27 ± 0.32 ppm, respectively, which there were no significant changes between the two groups. However, TMA content of 1, 2, 3, and 4% (v/v) of acetic acid concentration-treated group were measured to be 1.59 ± 0.48, 0.98 ± 0.43, 1.1 ± 0.55, and 1.16 ± 0.69 ppm, respectively. Table 2 shows that TMA content at more than 1% (v/v) of acetic acid concentration-treated group was significantly decreased relative to the non-treated and 0% of acetic acid concentration-treated group according to Duncan's multiple range test ($p < 0.05$).

Effect of acetic acid soaking time on trimethylamine (TMA) content

TMA content according to soaking konjac glucomannan gel into acetic acid solution (1%, v/v) was estimated, in which soaking time was set to 10, 20, and 30 min, respectively compared to the non-treated group. Non-treated group showed TMA content of 4.54 ± 0.99 ppm, whereas TMA content of 10-, 20-, and 30-min-treated groups were estimated to be 0.98 ± 0.25, 1.34 ± 0.32, and 1.49 ± 0.67 ppm, respectively (Table 3). TMA content at more than 10-min-treated groups was statistically decreased significantly relative to the non-treated group according to Duncan's multiple range test ($p < 0.05$).

Table 2 Effect of acetic acid soaking concentration on TMA content

Acetic acid concentration-treated group (%)	TMA content (ppm)
Non-treated	3.18 ± 0.62^{a}
0	3.27 ± 0.32^{a}
1	1.59 ± 0.48^{b}
2	0.98 ± 0.43^{c}
3	1.1 ± 0.55^{c}
4	1.16 ± 0.69^{c}

[a,b,c] Mean with different letters are significantly different according to Duncan's multiple range test ($p < 0.05$)

Table 3 Effect of acetic acid soaking time on trimethylamine(TMA) content

Soaking time (min)	TMA content (ppm)
Non-treated	4.54 ± 0.99^{a}
10	0.98 ± 0.25^{b}
20	1.34 ± 0.32^{b}
30	1.49 ± 0.67^{b}

[a,b] Mean with different letters are significantly different according to Duncan's multiple range test ($p < 0.05$)

Sensory evaluation and statistical correlation analysis

Table 4 shows the results of the 9-point scale sensory analysis about the acetic acid-treated konjac glucomannan gel compared to the non-treated group, performed by 15 trained panels about the attributes for the intensity of fish-like off odor and sour odor and for the preference of the fragrant. The sensory score of the intensity of the fish-like off odor decreased significantly with the concentration of acetic acid compared to the non-treated group and 0% acetic acid-treated group ($p < 0.05$), where no significant differences in fish-like off odor among 1–4% (v/v) acetic acid concentration-treated group was observed. The intensity of sour odor was evaluated to be increased with the concentration of acetic acid. Overall preference of fragrant considering fish-like off odor together with sour odor was 5.75 ± 0.66 in the 1% (v/v) acetic acid group, which was shown to be the highest among the conditions tested. The Pearson's correlation analysis between TMA content analyzed by gas chromatographic mass spectrometer and fish-like off odor evaluated by the sensory analysis showed the correlation coefficient ($R^2 = 0.9848$, $p < 0.05$) (Fig. 1).

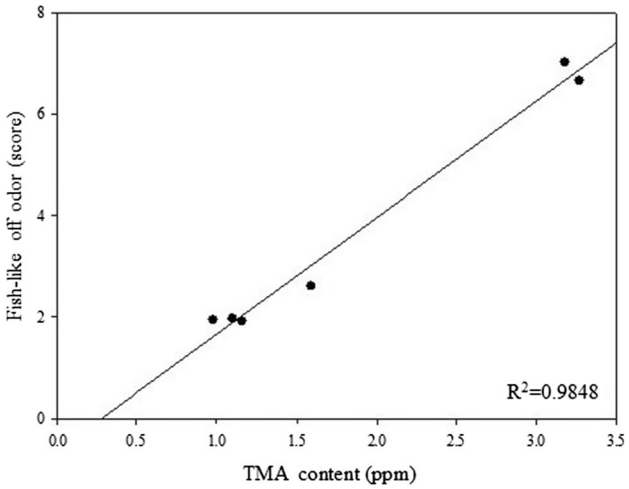

Fig. 1 Pearson's correlation between TMA content by GC–MS and intensity of fish-like off odor by sensory evaluation

Discussion

The purpose of this study is to find out a new method to eliminate the volatiles in which the critical compound is TMA in konjac glucomannan gel formation [5, 29]. Among various trials to reduce the fish-like off odor, treatment of konjac glucomannan gel with acetic acid originated from fermented vinegar was found to be the novel method to do it based on the analysis of TMA by gas chromatographic mass spectroscopy [6, 7]. TMA content was significantly reduced at all concentrations of acetic acid except for 0% (v/v) of acetic acid concentration-treated group, suggesting that the decreased TMA content should be caused by the chemical change of TMA rather than dilution of TMA with distilled water, supported by the fact that 0% acetic acid treatment group showed statistically not significant TMA content compared with the non-treated konjac glucomannan gel. This implies the evidence for the proposed mechanism by which TMA content was reduced by acetic acid may be attributed to acetic acid functioning as a proton donor which makes TMA into non-volatile salts [8, 9].

Sensory evaluation of fish-like off odor statistically correlated with TMA content by the analysis of Pearson's coefficient, analyzed by ANOVA, Duncan's multiple range test, demonstrating that TMA could play a critical role resulting in the fish-like off odor among volatiles [26–28].

Table 4 Sensory evaluation score of odor intensity of KGM gel

Acetic acid concentration-treated group (%)	Intensity of fish-like off odor (score)	Intensity of sour odor (score)	Preference of fragrant (score)
Non-treated	7.03 ± 0.49^{a}	1.53 ± 0.1^{a}	2.81 ± 0.44^{ab}
0	6.67 ± 1.32^{a}	1.81 ± 0.45^{a}	2.67 ± 0.88^{a}
1	2.61 ± 0.51^{b}	3.08 ± 0.69^{b}	5.75 ± 0.66^{c}
2	1.94 ± 0.2^{b}	4.81 ± 1.05^{c}	4.56 ± 0.79^{b}
3	1.97 ± 0.2^{b}	5.5 ± 0.3^{c}	4.28 ± 0.27^{b}
4	1.92 ± 0.2^{b}	6.86 ± 0.14^{c}	3.42 ± 0.25^{ab}

[a,b,c] Mean with different letters are significantly different according to Duncan's multiple range test ($p < 0.05$)

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