Study on the natto extract polysaccharide as a new ice cream stabilizer

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Natto has been shown to have many benefits to human, and its polysaccharide containing mucus produced by the Bacillus subtilis might be a suitable stabilizer for ice cream. The purpose of this study was to investigate the effect of natto mucus extract (NME) as ice cream stabilizer on the quality of ice cream. The ice cream mixture includes: milk, cream, sugar, milk powder, and stabilizer (0.15% of CMC or 0.25%, 0.5%, 0.75%, 1.0% of NME). The finish ice cream samples were tested for their first drop time, melting rate, mass retention, viscosity, the color, overrun rate, anti-oxidation and the sensory evaluation. Viscosity significantly increased (P < 0.05) from 270 to 473 cps as NME addition increased from 0.5% to 1.0%, while mass retention was better at 0.5%, and 0.75% NME than other stabilizer groups (P < 0.05). However, the melting rate increase (P < 0.05) when the NME reached 1.00%. The results of sensory test participants were not favor for those ice creams containing NME more than 0.50%. Based on these results, adding 0.50% NME seemed to perform better in both melting rate and sensory test, and therefore, might be an excellent substitute replacing CMC as ice cream stabilizer.

Keywords: Natto extract, ice cream, stabilizer, anti-thaw, Bacillus subtilis

INTRODUCTION

In Taiwan, four billion NT dollars (about a hundred and forty million USD) worth of ice creams are consumed annually. Ice cream is a highly complex frozen food mainly composed of proteins, fats, sugar, air, water and stabilizers, and is built with micro ice crystals and emulsified fat globules coated air bubbles as the main structure within a continuous phase of solution known as serum which contains sugars, proteins, and stabilizers (Goff, 1997). Previously, researches on ice cream mostly focused on fat particle size and how it is made (Koxholt et al., 2001; Tosaki et al., 2009). The stability of ice cream especially types of stabilizer and the functional properties have gradually become more significant in recent years (Cakmakci et al., 2015; Erkaya et al., 2012). These researches mainly addressed the properties of anti-thawing test, expansion rate, consumer’s preference and ice crystal growth.

Among those properties studied, the time it takes for a solid ice cream to melt usually is the consumers most likely to notice (Goff, 1997). Sofjan and Hartel, 2004 indicated that, the rate of thawing is largely affected by the level of ice cream overrun. Muse and Hartel, (2004) suggested that, as affected by stabilizer, the network of fat globules and coated air bubbles also affect the rate of melting. In recent years, studies on natural substance as ice cream stabilizers have increased significantly that aimed to replace chemically synthesized or chemically modified stabilizers. The water-soluble polysaccharide and cell wall extracted from Okra (Abelmoschus esculentus L.) improved ice cream physical quality and slow down ice recrystallization (Yuennan et al., 2014). Basil seed gum (Ocimum basilicum L.) by itself or in combination with other stabilizers, could be used in ice cream formulation (Bahram-Parvar et al. 2012). The Gundelia (Gundelia tournefortii L.) dried leaves and milk had effects on ice cream quality and sensorial characteristics as well. (Cakmakci and Dagdemir 2013). The addition dried Cape gooseberry (Physalis peruviana L.) as ingredients improved melting time, modified minerals concentration and increased viscosity without significantly altered overrun (Ekaya et al. 2012). Those nature stabilizers are mostly the extraction of polysaccharides or colloidal substances from plants, and those extracts may also provide functional properties to

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ice cream.

Natto (Satto or natto), a soy bean product, is fermented with *Bacillus subtilis var. natto*, a bacteria first purified from natto by Professor Shin Sawamura at the University of Tokyo and named it (Sawamura, 1906). After fermentation, *B. subtilis* and soy bean mix will produce viscous filaments (Tanimoto et al., 2001) and trademark strong odor and taste (Tsuji, 1982). Chemically, *B. natto* fermented soybeans mucus has been identified containing nattokinase which is a fibrinolytic enzyme stable in gastrointestinal tract (GIT) with medical applications (Dabbagh et al., 2014); and polyglutamic acid (poly-γ-glutamic acid, γPGA) which keep nattokinase functional (Tanimoto et al., 2001). γPGA has also known to improve the survival of probiotic in GIT (Bhat et al., 2015) and uses in food processing such as thickener for juice, anti-aging health food and anti-freeze (Shih and Van, 2001).

*B. subtilis* has been found containing biofilm surface layer protein A (BslA) which was also confirmed to contain hydrophobin (Hobley et al., 2013). Hydrophobins have been used in many food industries, such as emulsifiers, foam stabilizers and surfactants (Bromley et al., 2015), and BslA has also been proved to have both hydrophilic and hydrophobic property, which leads to the possibility of been an emulsifier (Bromley and MacPhee, 2017).

The purpose of this study was to investigate the effect of adding natto mucus extract (NME) as ice cream stabilizer on the quality of ice cream.

**MATERIALS AND METHODS**

**Natto polysaccharide extraction**

Natto polysaccharide extraction (NME) used in this study was prepared using commercial natto product (Stubborn Daddy Natto, SHIKA Corporation, Japan), purchase from local market, were mixed and stirred in 65 °C distilled water until the silk completely dissolved in water and then filtered with gauze. The liquid was centrifuged at 10000g for 30 min; the supernatant was lyophilized and stored in -20 °C until analysis.

**Ice cream preparation**

The ice cream recipe included: fresh milk 64% (v/v); Reisui fresh milk, Uni-President Enterprises Corporation, Tainan City, Taiwan), fresh cream 30% (v/v; Président Whipping Cream, Président, Laval, France), skimmed milk powder 10% (w/v; Quaker high calcium non-fat milk powder, Quaker, Chicago, Illinois, US), sugar 6% (w/v). Different stabilizers that were added into the mixture includes: natto polysaccharide extract (NME) 0.25% (NME0.25), 0.50% (MNE0.50), 0.75% (MNE0.75) or 1.0% (NME1.00); carboxymethyl cellulose 0.15% (CMC; Ashland, US) as control group and without any stabilizer as a blank group (BLK).

The different ice cream mixtures were pasteurized at 85 °C for 25 sec and aging at 4 °C for 24 h. Aged ice cream mix stirred 15 min using a batch ice cream maker (MODEL ICE-21TW, Conair Corporation, Stamford, Connecticut, USA) to form frost cream, and then froze and hardened at -20 °C in small aliquot containers.

**Anti-thaw test**

One hundred gram (100g) of ice cream, after 24 hours frozen and hardened, was removed from refrigerator and tested immediately. The frozen ice cream sample was placed on a stainless steel wire mesh #10 mesh (BUNSEKIFURUI; Gilson, USA) at room temperature (22 ± 2 °C). Recorded items included: time of the first drop of sample placed on the screen after being removed from the refrigerator (first drop time); the drips of ice cream were collected and weighed every 5 min for the duration of 70 min, the statistical regression was use to evaluate the rate of thawing (melting rate); and finally, the weight of ice cream remaining on the screen after 70 min was used to estimate sample retention (mass retention). Each sample was repeated three times.

**The rate of overrun**

Overrun refers to the amount of air incorporated into the ice cream. Similar to Goff and Hartel (2013), the same volume of ice cream mix before stirred and frozen ice cream were weight, and the overrun was estimated using the following equation:

\[
\text{Overrun} = \frac{\text{ice cream mix (g)} - \text{frozen ice cream (g)}}{\text{frozen ice cream (g)}}
\]

**Viscosity and color analysis**

After 24 h aging, the ice cream mix was evaluated for its viscosity (RVA-Ezi, Newport Scientific, Jessup, Maryland, USA) with 30 mL ice cream mix at 25 °C and 350 rpm. Similar to Corradini et al. (2014) described, the International Commission on Illumination (CIE) L*a*b* system was used to evaluate ice cream color characteristic. 10mL of ice cream mix was loaded into a quartz vial and the reflected light was calculated using a colorimeter (COLOR-JA555, Luyifo Technology, New Taipei City, Taiwan). Sample viscosity and color were analyzed in triplicate.

**Anti-oxidation test**

The antioxidants are capable of removing free radicals, and chelating metal ions. Their anti-oxidation feature can be evaluated with DPPH radical scavenging test, and/or chelating of ferrous ions. All samples were also done in triplicate during anti-oxidation test.

**DPPH antioxidant assay**

Based on the method of Sharma and Bhat (2009) with minor modification: 1 mM DPPH alcohol solution was
prepared by mixing the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH; Sigma-Aldrich, St. Louis, Missouri (MO), USA) with 95% ethanol. 400 μL sample mixed 800 μL DPPH alcohol solution and shaked evenly before kept mixture in the dark for 30 min. under weak light, Sample solutions were loaded into a quartz vial, and their 517 nm absorbance were determined with a spectrophotometer (DU 730, Beckman Coulte, Brea, California CA, USA). The lower the absorbance value indicated the better DPPH free radicals scavenging ability. The calculation equation shown as following:

\[
I\% = \left(1 - \frac{A_{517\text{nm sample}}}{A_{517\text{nm blank}}}\right) \times 100%
\]  

(2)

Where I%: DPPH radical scavenging rate; \(A_{517\text{nm blank}}\): Absorbance at 517 nm of 1 mM DPPH along stand for 30 min; and \(A_{517\text{nm sample}}\): Absorbance at 517 nm of DPPH and sample mixture stand for 30 min in dark.

Chelate effect of ferrous ions

As Ye et al. (2013) described with little modification: 2 mM FeCl₂ solution and 5 mM Ferrozine solution dilute were prepared by dissolving FeCl₂ (Sigma-Aldrich, St. Louis, MO, USA) and Ferrozine (Sigma-Aldrich, St. Louis, MPO, USA) in distilled water respectively. 25 μL of FeCl₂ solution, 800 μL of 95% ethanol and 250 μL of sample were mixed and allowed to stand for 30 sec; and then added 50 μL Ferrozine solution and let mixture stand for 10 min. Absorbance at 562 nm of mixture was measured using a spectrophotometer (DU 730, Beckman Coulte, Brea, CA, USA). The higher the absorbance value, the stronger the ability of the sample to chelate ferrous. Fe²⁺ chelating rate (FCR) calculated as follow:

\[
FRC\% = \left(1 - \frac{A_{562\text{nm sample}}}{A_{562\text{nm blank}}}\right) \times 100\%
\]  

(3)

Where FRC%: Fe²⁺ chelating rate; \(A_{562\text{nm blank}}\): Absorbance at 562 nm of FeCl₂ and Ferrozine solution mixed with distilled water instead of ice cream sample; and \(A_{562\text{nm sample}}\): Absorbance at 562 nm of FeCl₂ and Ferrozine solution mixed with ice cream sample.

Sensory Testing

36 participants who like ice cream were selected and 30 min training section was given to all participants on ice cream sensory test grading prior to first ice cream sample was served. Subjects were asked not to consume any irritating food at least 2 hours before sensory evaluation. Between samples, participants were provided white toast and warm water to a clear their oral residuals, and there was 15 min interval before the next sample of ice cream was served. Sensory test of all samples were repeated twice for each participant at two different times, and the average of each sample was used for the statistical analysis. On the scale of 1 to 9 (1 for disliked the most, and 9 for like the most), the participants were asked to grade each sample on six characteristics including: sweetness, aftertaste, smoothness, viscosity, flavor, and overall preference.

Statistical analysis

Data from experiment were analyzed using SAS version 9.4 and the results were described as mean ± SD. Data analysis variables was performed using PROC ANOVA and Duncan’s new multiple range test to compare the effect of different stabilizers. When \(p < 0.05\) the differences were considered as significant at 95% confidence level.

RESULT AND DISCUSSION

Ice cream anti-thaw test

As shown in Figure 1(A), there were no significant differences detected among BLK, CMC0.15 and NME0.25 treatments, but when ice cream containing 0.5% and above NME, the first drip time was significantly increased near two folds (\(P < 0.05\)). The results of melting rate (Figure 1 (B)) and mass retention (Figure 1(C)) showed that the CMC0.15 and NME0.25 did not differ from BLK in thawing performance, and 0.50 and 0.75% of NME significantly prolonged the time of ice cream melting (\(P < 0.05\)); interestingly, however, high level of NME (1.00%) unexpectedly reduced such improvement (\(p < 0.05\), although still is better than BLK, CMC0.15 and NME0.25 (\(p < 0.05\)). Those data indicated that 0.50% and 0.75% of NME had better ice cream anti-thaw performance.

In the past, the additions of stabilizers were intent to increase mass retention (Bahramparvar and Mazaheri, 2011). It is inconvenient and annoying for the consumers having a fresh ice cream but dripping over their hand and clothes soon after purchase. As a result, the first drip time of ice cream was always an important part of improving ice cream quality (Goff and Hartel, 2013). In this study, it was found that the proper level of NME (0.50% and 0.75%) did improve the mass retention, but the excess level of NME (1.00%) resulted in a loss of ice cream mass retention. This unexpected result might need further investigation, and we suspected that component(s) from natto extract might affect the network structure of fat globules and coated air bubbles.

Studies have suggested that there are two factors could affect melting rate: first, the type and amount of stabilizer could affect the rate of thawing (Bahram-Parvar and Mazaheri-Tehrani, 2011; Goff and Hartel, 2013). Second, the level of overrun, and the lower level of expansion the faster the ice cream melt (Muse and Hartel, 2004; Sofjan and Hartel, 2004).

The ice cream overruns, viscosity and color (hunter l, a, b) analysis

The overall overruns of the ice cream samples seemed
low (≤ 30%), which might due to the efficiency of ice cream maker incorporating air among stabilizers added (Table 1.), NME0.50 and NME0.70 treatments had greater overruns, and NME0.75 was the highest among all (p < 0.05), except that its value was statistically similar to BLK (p > 0.05). Excess NME (1.00%) seemed to had a negative effect on overrun compare to NME0.50 and NME0.75 (p < 0.05). CMC0.15 and NME0.25, statistically similar, had lower overruns compare to other additives (p < 0.05). As Warren and Hartel (2018) suggested that ice cream with a lower overrun might lead to a higher drip-through rate. Similarly the overrun results of this study seemed to support the melting rate shown in Figure 1 that NME0.50 and NME0.75 had greater overruns and lower melting rate; and excess NME had negative effect on both properties.
Compared to BLK and CMC0.15, the addition of natto extract increased the viscosity of ice creams (Table 1). Although NME0.25 did not differ from BLK and CMC0.15 (p > 0.05), when the NME level were above 0.50%, the viscosity of ice cream samples continuously and significantly increase (p < 0.05). It has been studied that natto extracts and/or B. subtilis natto fermented products contained polymers such as: poly-γ-glutamic acid (Tanimoto et al., 2001); heparin oligosaccharides (Zhanget al., 2015) and fructooligosaccharides (Bersanetiet al., 2016). Those and other polymers might play a role in ice cream viscosity.

The results of color analysis Hunter L, a*, b* analysis were showed in Table 1. L* value indicated the brightness (0 to 100) of samples and with the NME level increase, the brightness of ice cream decreased (p < 0.05); a* negative value decreased when NME level rose (p < 0.05); and b* value increased when NMEs were added (p < 0.05) in comparison to BLK and CMC0.15 treatments. Although no significant difference could be detected by naked eye (p > 0.05), the colorimeter suggested that ice cream with NME seemed to be greenish and yellowish. And the reason might be that the color of natto itself is yellow.

### Anti-oxidation test

Compared to BLK, the ice creams with NMEs were found increase DPPH radical scavenging activity except for NME0.25 and when NME level was 0.50% and above, the capability was significant (p < 0.05). In contrast to DPPH radical scavenging activity, the ice creams with NMEs were all found significantly increase their ability to chelate the ferrous (p < 0.05); however, the capability became significant higher for NME0.25 in comparison to NME1.00 (p ≤ 0.05) (Table 2). Ping et al. (2012) suggested a significant increase in DPPH radical scavenging rate using different strains of Bacillus natto fermented products compared to non-fermented soybean product.

Natto itself has been shown to have a good antioxidant capability (Lee et al., 2015; Xu et al., 2015) but has not yet appeared in food supplements. Studies on the products developed from B. subtilis natto in other fermented foods were also found potential of anti-oxidation function (Guo et al., 2015) or reduction of circulation lipid level in human (Lin et al., 2017). Kitamura et al. (2010) used B. natto to fermented milk and, after freezing, found out that there was still 49-92% of the viable B. subtilis natto and 62-98% of the natto kinase activity. Based on the studies described above, it is hoped that in the future it could be developed a functional natto ice cream.

### NME ice creams sensory testing

In the product sensory results (Table 3), the taste of sweetness decline when NME over 0.50%. Participants pointed out that when NME was 0.50% or above the bitter

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Overrun(%)</th>
<th>Viscosity (cp)</th>
<th>Color L*</th>
<th>Color a*</th>
<th>Color b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLK</td>
<td>25.29 ± 4.12 ab</td>
<td>42 ± 5.30 d</td>
<td>82.06 ± 0.31 abc</td>
<td>-4.08 ± 0.43 a</td>
<td>10.75 ± 0.32 f</td>
</tr>
<tr>
<td>CMC</td>
<td>17.39 ± 4.10 d</td>
<td>73 ± 8.10 d</td>
<td>82.31 ± 0.43 ab</td>
<td>-3.41 ± 0.22 ab</td>
<td>11.27 ± 0.37 d</td>
</tr>
<tr>
<td>NME0.25</td>
<td>15.94 ± 3.83 d</td>
<td>89 ± 5.97 d</td>
<td>82.45 ± 0.74 a</td>
<td>-3.16 ± 0.32 c</td>
<td>12.35 ± 0.29 c</td>
</tr>
<tr>
<td>NME0.50</td>
<td>23.04 ± 3.66 b</td>
<td>270 ± 13.69 f</td>
<td>82.12 ± 0.21 b</td>
<td>-2.92 ± 0.18 d</td>
<td>13.12 ± 0.24 b</td>
</tr>
<tr>
<td>NME0.75</td>
<td>30.17 ± 4.20 a</td>
<td>427 ± 21.88 e</td>
<td>81.44 ± 0.84 c</td>
<td>-2.83 ± 0.09 e</td>
<td>12.23 ± 0.21 d</td>
</tr>
<tr>
<td>NME1.00</td>
<td>19.01 ± 3.21 c</td>
<td>473 ± 28.38 a</td>
<td>80.26 ± 1.09 d</td>
<td>-2.75 ± 0.11 f</td>
<td>13.69 ± 0.19 a</td>
</tr>
</tbody>
</table>

Table 1. Comparison of overrun, viscosity and color (mean ± SD, n=3) of ice cream.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH radical scavenging rate (%)</th>
<th>Chelate of ferrous (FRC%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLK</td>
<td>34.12±1.6392 ab</td>
<td>82.06±1.039 f</td>
</tr>
<tr>
<td>CMC0.15</td>
<td>19.25±1.4142 c</td>
<td>43.58±0.4622 b</td>
</tr>
<tr>
<td>NME0.25</td>
<td>41.71±1.7518 b</td>
<td>43.03±1.0958 e</td>
</tr>
<tr>
<td>NME0.50</td>
<td>53.07±0.8805 a</td>
<td>53.06±1.9421 f</td>
</tr>
<tr>
<td>NME0.75</td>
<td>53.41±1.4506 b</td>
<td>32.85±1.6318 d</td>
</tr>
<tr>
<td>NME1.00</td>
<td>53.07±0.8805 a</td>
<td>427 ± 21.88 e</td>
</tr>
</tbody>
</table>

Table 2. Comparison of anti-oxidation (mean ± SD, n=3) of ice cream.

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*Means within a column possessing a different superscript are significantly different (p < 0.05). According to Duncan's new multiple range test.

**Treatments**: BLK: Blank group without any stabilizer; CMC0.15: Carboxymethyl cellulose at 0.15% and NME0.25, NME0.50, NME0.75 and NME1.00: Natto polysaccharide extract at 0.25, 0.50, 0.75 and 1.00%, respectively.

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Impact of Natto polysaccharides on ice cream properties and sensory acceptability.
taste will appear, and thus the ice cream tasted less sweet. This bitterness might be caused by the residual of natto unique flavor(s). However, no significant differences were detected ($p > 0.05$) among ice cream samples for aftertaste and flavor. The smoothness was lower for CMC0.15 in comparison to NMEs over 0.50% treatments ($p > 0.05$), id est, more favorable by participants when the NME content was over 0.50%. The CMC0.15 viscosity was lower than the rest of treatments ($p \leq 0.05$) and the viscosity of NMEs over 0.50% were higher than BLK, CMC0.15 and NME0.25 ($p \leq 0.05$) (Table 3). Some participants described that when added more than 0.50% of NME, the ice creams would become sticky and appear like a Dondurma (Turkish ice cream) instead of regular easy ice cream. On overall preference, there were no significant difference detected ($P < 0.05$) among the BLK, CMC0.15, NME0.25 and NME0.50, being statistically similar to that of NME0.50 but higher than that for NME0.75 and NME1.00 ($p \leq 0.05$). Goff and Sahagian (1996) described that an excess stabilizer would cause undesirable problems including excessive viscosity and melting characteristics in ice cream, and the current study was all in agreement with their finding.

Ice cream can produce various textures by combining different ingredients and their ratio during freezing and stirring process (Marshall et al 2012). The quality of ice cream largely depends on the stability of fat (Muse and Hartel, 2004). To increase ice cream anti-thawing capacity, overrun rate, and consumer acceptance, commercial ice creams often add various stabilizers and/or emulsifiers. Those stabilizers, including: gelatin, sodium alginate, or plant gums, provide smooth and uniform product by reducing ice crystal forming; contribute mixture viscosity to suspend the flavor particles without covering them; maintain ice cream structure during the periods of temperature fluctuation; and help to incorporate air to improve overrun (Bahram-Parvar and Mazaheri-Tehrani., 2011; Goff and Hartel, 2013).

Foaming feature, incorporate air into ice cream mixture, is known to be associated with milk proteins; even though, the main milk protein casein may likely function as emulsion due to its amphiphilic characteristic (Zhang and Goff, 2004). On the other hand, fat particles, endogenous or supplementary, are known to interfere foaming processing (Dickinson, 1992). When fat network and air cells were held together, however, the stabilization of fat networks plays an important role in stabilizing ice cream micro-structure (Amador et al., 2017). Stabilizers/emulsions in ice cream play a key on the stabilization of networks (Cropper et al., 2013), and thus become central component for ice cream quality and its taste.

In recent years, the food safety concern has raised up among consumers in Taiwan, due to many worrisome events such as: the plasticizer incident in 2011 and the tainted oil scandal in 2013. Nature and safe foods have become priority concern for consumers, and the consumers nowadays also care about lifestyle and healthy food. As a result, ice creams with nature and functional stabilizers may have a great potential in consumers market. Natto, a traditional Japanese $B. subtilis$ var. natto fermented soy bean product, has been known to have several health benefits such as reducing GIT discomfort, regulating blood pressure, and improving bone health (Xu, 2005). Iwai et al. (2002) suggested that anti-oxidative property of natto improved lipid metabolism; Fujii et al, (1995) demonstrated that nattokinase might have an effect on thrombosis; and Chen et al. (2010) identified that water-soluble soy bean polysaccharides could become a suitable dietary fiber. Those researches highlighted the important functional characteristic of natto and it is hoped that this study provides that NME can be used as an alternative natural substitute for ice cream stabilizer.

**CONCLUSION**

In the anti-thaw experiment, ice cream with 0.50% and 0.75% NME were better. In sensory test, the participants preferred the viscous ice cream but overall preference was declined when NME content was 0.50% and above. Adding NMEs into ice cream has improved antioxidant effect, and effect was most noticeable at 0.50% and 0.75% NMEs. With results above, it is concluded that 0.50% NME is an ideal level as ice cream stabilizer.

**ACKNOWLEDGEMENT**

The authors are thankful to Dr. T. Y. Lin who generously

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Table 3. Comparison of sensory testing (mean ± SD) of ice cream

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sweetness</th>
<th>Aftertaste</th>
<th>Smoothness</th>
<th>Viscosity</th>
<th>Flavor</th>
<th>Overall Preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>6.5 ± 1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9 ± 0.83</td>
<td>4.88 ± 0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.38 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.25 ± 1.21</td>
<td>7.25 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMC0.15</td>
<td>7.1 ± 2.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 0.91</td>
<td>4.38 ± 0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.75 ± 1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.75 ± 0.96</td>
<td>7.13 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NME0.25</td>
<td>6.0 ± 1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4 ± 0.86</td>
<td>6.63 ± 1.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.88 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00 ± 0.44</td>
<td>6.38 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NME0.50</td>
<td>5.8 ± 1.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.0 ± 0.49</td>
<td>7.00 ± 1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.13 ± 0.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.38 ± 0.39</td>
<td>5.63 ± 1.42&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>NME0.75</td>
<td>5.8 ± 1.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.1 ± 0.58</td>
<td>6.88 ± 1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.63 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.50 ± 0.57</td>
<td>5.13 ± 1.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NME1.00</td>
<td>5.1 ± 2.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8 ± 0.42</td>
<td>7.01 ± 1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.88 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.38 ± 0.83</td>
<td>5.00 ± 1.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>bMeans within a column possessing a different superscript are significantly different ($p < 0.05$) according to Duncan's new multiple range test.

Treatments: BLK: Blank group without any stabilizer; CMC0.15: Carboxymethyl cellulose at 0.15% and NME0.25, NME0.50, NME0.75 and NME1.00: Natto polysaccharide extract at 0.25, 0.50, 0.75 and 1.00%, respectively.
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REFERENCES


