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EFFECT OF NISIN ON QUALITIES OF MILK PUDDING WITH FRUIT COCKTAIL DURING REFRIGERATED STORAGE

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ABSTRACT

The objective of this research was to investigate the efficacy of nisin (1.25-5.00 μg/g) on quality attributes of milk pudding with fruit cocktail, a gelled dairy dessert made of sweetened milk mixed with agar and topped with fruit cocktail, during storage at 4°C. The samples were analyzed for changes in microbial counts, syneresis, and texture profile analysis during storage, as well as chemical compositions and sensory acceptability at the beginning of storage. All samples containing nisin had significantly lower aerobic microbial counts when compared to that of the control ($p<0.05$). The sample with 5.00 μg/g had the fewer changes in syneresis, hardness, and chewiness when compared to those of the control during storage without affecting the springiness, cohesiveness, chemical compositions and sensory acceptability, including appearance, texture, flavor, and overall acceptance at the beginning of storage. Furthermore, the panelists could not discriminate the treated sample from the control in the duo-trio difference test.

1. Introduction

Dairy products contain a lot of essential nutrients such as calcium, essential amino acids, fatty acids, vitamins, and minerals. A recent study has shown that pudding and ice cream are the most frequently consumed dairy products at an average of 4.9 times/week (Colić Barić, 2001). Ready-to-eat creamy or gelled milk desserts with long shelf life have become popular in Europe and their popularity is growing in the USA. Milk pudding is one of commercially produced dairy desserts made of sweetened milk and starch or other hydrocolloids, such as agar, carrageenan, and low-methoxyl pectin (Hansen, 1993). According to Schmutz et al. (2007), milk pudding has a short shelf life of 5-6 days at 1-4°C.

Nisin is a low-molecular-weight polypeptide (3.3 kDa) produced by *Lactococcus lactis* subspecies *lactis*. It is effective against Gram-positive bacteria, including *Bacillus cereus*, *Bacillus sporothermodurans*, and *Clostridium botulinum* (Thomas and Delves-Broughton, 2005; Carballo et al., 2012), as well as some bacterial spores (Ray, 1992). In the United States, nisin has been approved as generally recognized as safe (GRAS) for inhibiting the outgrowth of *Clostridium botulinum* spores and toxin formation in pasteurized cheese spreads and pasteurized cheese spread with fruits, vegetables, or meats (US Food and Drug

Administration, 2015). In Abu Dhabi, Bahrain, and Dubai, nisin has been approved in pasteurized flavored milk with no limit level. In China, nisin is allowed in dairy products at permitted level of 12.5 μg/g food. In EU and Czech Republic, nisin is allowed in puddings at permitted level of 3 μg/g food (Thomas et al., 2000). In Argentina, Australia, France, Netherlands, USA, and Russia, nisin is permitted in processed cheese. In Mexico and Peru, nisin is also approved as a permitted additive in any food (Cleveland et al., 2001). According to Thomas and Delves-Broughton (2005), typical addition levels of nisin in dairy products and pasteurized chilled dairy desserts are 0.25-1.25 and 1.88-5.0 μg/g respectively. Recently, Martinez et al. (2016) have found that free and encapsulated (gum Arabic as a carrier agent) commercial nisin (Nisaplin®) were very effective against outgrowth and spore germination of *Bacillus cereus* in skimmed and whole milk during 21 days of storage at 6±1°C. Other *Bacillus* strains, such as *Bacillus pumilus* and *B. licheniformis*, were also inhibited by low concentrations of nisin in heat-treated cream (90°C for 15 min) during storage at 8°C (Nissen et al., 2001). Moreover, Wirjantoro et al. (2001) have reported that addition of nisin can prolong the shelf life of reduced heat-treated milk (117°C for 2 s) compared with UHT milk (processed at 142°C for 2 s) during storage at 10, 20, and 30°C.

The objective of this study was to investigate the efficacy of nisin on quality attributes of milk pudding with fruit cocktail during storage at 4°C.

2. Materials and methods

2.1. Materials

The following commercial food-grade ingredients and formulation were used for preparing milk pudding with fruit cocktail in this study: 49.87%(w/w) pasteurized fresh milk (CP-Meiji Co., Ltd., Saraburi, Thailand), 0.22%(w/w) agar (Phattanasin Enterprise Part., Ltd., Bangkok, Thailand), 26.60%(w/w) drinking water (Nestlé (Thai) Co., Ltd., Bangkok, Thailand), 2.49%(w/w) sucrose (Mitr Phol Sugar Co., Ltd., Bangkok, Thailand), 4.16%(w/w) non-dairy creamer (Nestlé (Thai) Co., Ltd., Bangkok, Thailand), and 16.66%(w/w) canned fruit cocktail in heavy syrup (Malee Sampraan Pub. Co., Ltd., Nakornpathom, Thailand).

2.2. Sample preparation

To prepare a control, agar was slowly dispersed in drinking water, which had been heated to 90°C and held for 1 min, and the mixture was continuously stirred until dissolved. Sucrose, milk (heated to 60±2°C and held for 10 min before used), and non-dairy creamer were slowly added to the agar solution. The sample was continuously stirred until dissolved and then cooled down to 40±2°C. The pudding was distributed in polyethylene hinged-lid containers (30 g each) and allowed to set at 4±1°C. The fruit cocktail was taken out of the heavy syrup and added on the top of the set pudding.

The procedures for preparing treated samples were similar to those for the control, except food-grade nisin (Shandong Freda Biotechnology Co., Ltd., China), which had been mixed into agar solution at three difference concentrations of 1.25, 2.50, 5.00 μg/g before sucrose, milk, and non-dairy creamer were added.

All samples were stored at 4°C for further analysis. The preparation process was independently repeated on 3 separate days as replication.

2.3. Microbiological analysis

Total viable counts (TVC) were determined by the pour-plate method (AOAC, 2000) at 0, 3, 6, 9, 12, 15, 18, and 21 days of storage. 10 g of each sample was aseptically weighted and homogenized with 90 ml of sterile peptone water (Merck, KGaA, Germany) for 1 min using a stomacher (Funke-Gerber, Germany) at a speed of 230 rpm. The homogenized samples were serially diluted (1:10) in sterile peptone water. 1 ml of sample from serial dilutions was added to each duplicate sterile Petri dish. Plate Count Agar (PCA) was added to each Petri dish

and samples were then incubated at 37±2 °C for 48 h. After incubation, colonies were counted and microbiological data was expressed as logarithms of number of colony-forming units (logCFU/g).

2.4. Syneresis

The determination of syneresis was carried out at 1, 8, 15, and 21 days of storage as described by Supavititpatana et al. (2008).

2.5. Texture profile analysis

Texture profile analysis (TPA) was measured by using a texture analyzer TA.XT2i (Stable Micro Systems Ltd., UK) as described by Liu et al. (2013) at 1, 8, 15, and 21 days of storage. The fruit cocktail was taken out from the top of each sample and liquid from syneresis was separated before analysis. The measurements were carried out at 10±1°C on three replicates. The following texture parameters of the samples were reported: hardness, springiness, cohesiveness, and chewiness.

2.6. Chemical analysis

Chemical analysis of the control and the best treatment (from microbiological analysis, syneresis and TPA) at the beginning of storage was performed following AOAC (2012) including moisture, lipid, protein, fat, ash, and carbohydrate contents.

2.7. Sensory evaluation

Two sets of sensory evaluation were performed to determine the difference between control and the best treated sample (from microbiological analysis, syneresis and TPA), as well as their acceptability, among 30 untrained panelists screening from juniors and seniors in the Department of Agro-Industrial, Food, and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Thailand. In the first evaluation, duo-trio difference test was performed to indicate the difference between control and the treated sample at the beginning of storage. The panelists were initially asked to evaluate and remember the control, labeled with "S" as a standard. Then, they were given a pair of samples (the control and treated samples labeled with random 3-digit codes and presented in random order) and asked to indicate which the standard was. The number of panelists who indicated the standard correctly was reported.

In the second evaluation, the control and the treated sample at the beginning of storage were evaluated for acceptance (appearance, texture, flavor, and overall acceptance) using a nine-point hedonic scale, including 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely. All samples in both experiments were labeled with random 3-digit codes and presented in random order.

2.8. Statistical analysis

All analyses were run in triplicate, except microbiological analyses, which were run in duplicate. Data from microbiological analysis, syneresis, TPA, chemical analysis, and acceptance test were analyzed by analysis of variance (ANOVA) using IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY). Duncan's multiple range test (DMRT) was used to determine significant differences among means. Data from the duo-trio test were analyzed by comparing the number of correct responses to the critical number by Meilgaard et al. (2007). Significance was defined at $p<0.05$.

3. Results and discussions

3.1 Microbiological analysis

Total viable counts of control and treated samples with different concentrations of nisin during storage at 4°C are presented in Fig. 1. The initial number of bacteria in all samples was less than 1.00 logCFU/g which indicated appropriate sanitation used in this study. Putthongsiri et al. (2011) also reported that there was less than 1.00 logCFU/g of initial total viable counts in milk pudding with and

without chitosan added in the recent study. Total viable counts increased with storage time in every sample ($p<0.05$) (Fig. 1). Compared with the control, all treatments significantly inhibited the growth of bacteria in milk pudding with fruit cocktail during storage ($p<0.05$). The sample treated with 5.00 µg/g nisin had the lowest total viable counts and maintained less than 4.00 logCFU/g until the end of study.

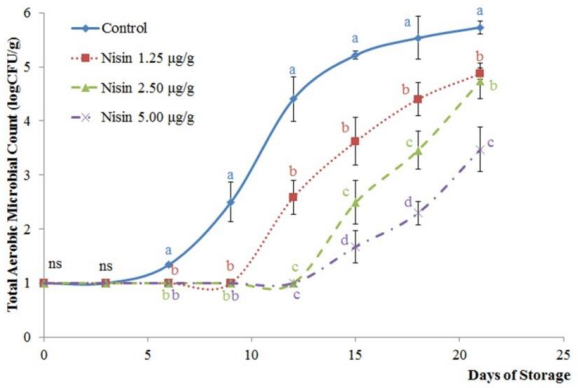

Figure 1. Total viable counts of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b, c, d) indicate significant difference at the same day of storage at $p<0.05$. ns indicates no significant difference between samples at the same day of storage ($p\geq0.05$) (n=3, error bars: standard deviations).

Samples containing nisin showed slower growth of bacteria since nisin is effective against Gram-positive bacteria by forming pores at cytoplasmic membrane. These pores disrupt a proton motive force and pH equilibrium leading to leakage of ions, hydrolysis of ATP, and eventually cell death. Nisin can also inhibit cell wall biosynthesis by binding lipid II, a peptidoglycan precursor (Bauer and Dicks, 2005; de Arauz et al., 2009; Deegan et al., 2006).

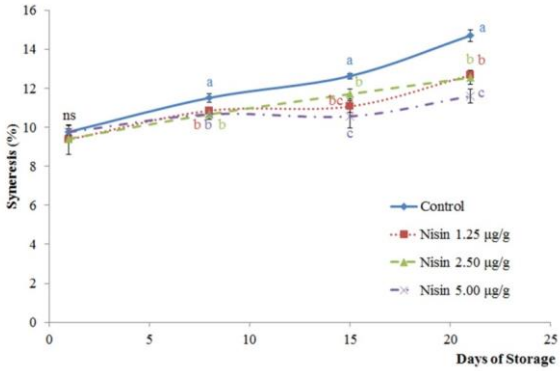

Figure 2. Syneresis of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b, c) indicate significant difference between samples at the same day of storage at $p<0.05$. ns indicates no significant difference at the same day of storage ($p\geq0.05$) (n=3, error bars: standard deviations).

Recently, Ibrahim and Elbarbary (2012) have reported that bacteriocin from *Lactobacillus acidophilus* strain showed effective antimicrobial activity and extended shelf-life of pasteurized milk to 12 days during refrigerated storage. Nissen et al. (2001) also found inhibition of nisin against some bacteria, including 6 strains of *Bacillus*, in heat-treated cream (90°C for 15 min) during storage at 8°C. Arques et al. (2008) suggested that nisin combined with reuterin and lactoperoxidase system at low concentrations could improve microbial safety against *Listeria monocytogenes* and *Staphylococcus aureus* in "cuajada" (curdled milk), a semisolid dairy product manufactured in Spain, during storage at temperature abuse of 10°C.

3.2 Syneresis

Syneresis was expressed as percentage of water separated from the formed gel of pudding. The syneresis naturally occurs since gel water cannot be held in the gel structure during storage. Food containing higher concentration of gel, higher water holding capacity of gel, and higher gel strength tends to have lower rate of syneresis (Benerjee and Bhattacharya, 2011; Matsuhashi, 1990). Syneresis increased with storage time in every

sample ($p<0.05$) (Figure 2). Gels with low concentration of agar (1% or lower) can separate large amount of water over time (Matsuhashi, 1990). Abo-el-Fetoh (2010) also reported the increase of syneresis of pudding and white sauce produced from different starches, such as tiger nuts, sweet potato, and taro, during 5 days of storage at -10°C, 4°C, and room temperature. Samples containing nisin had significantly slower rate of syneresis during storage when compared with the control ($p<0.05$). The sample treated with 5.00 µg/g nisin had the lowest syneresis, especially at the end of storage. These results correlated with total viable counts presented in Fig. 1. As microbial counts increased, the gel structure might lose its strength, leading the separation of water during storage.

3.3 Texture profile analysis

Texture is another important factor that affects acceptability of consumers. Hardness and chewiness of control and treated samples with different concentrations of nisin during storage at 4°C are presented in Figure 3. TPA hardness is defined as the force needed for attaining a given deformation and chewiness is defined as the energy needed for masticating solid food to a state of readiness for swallowing (Bourne, 2002). In every sample, hardness and chewiness remained constant at the beginning of storage and increased at the end of the storage. The higher hardness and chewiness with time might occur due to the volumetric shrinkage of the pudding gel, influenced by syneresis (Matsuhashi, 1990). At the end of storage, the control sample had the highest hardness and chewiness while the sample with 5.00 µg/g nisin had the lowest hardness and chewiness ($p<0.05$). These results also correlated with total viable counts and syneresis presented in Fig. 1 and 2 respectively.

Springiness and cohesiveness of all samples did not show significant difference between samples throughout the storage ($p\geq0.05$) (data not shown).

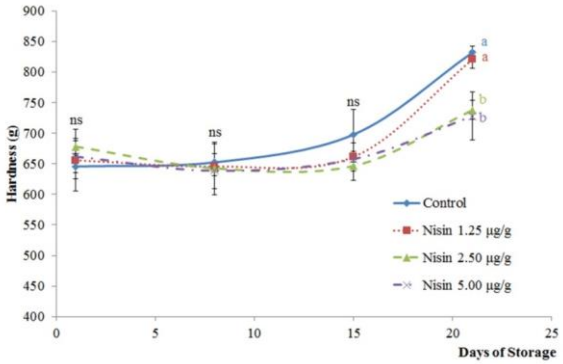

Figure 3. Hardness of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b) indicate significant difference between samples at the same day of storage at *p<0.05*. ns indicates no significant difference at the same day of storage *(p≥0.05)* (n=3, error bars: standard deviations).

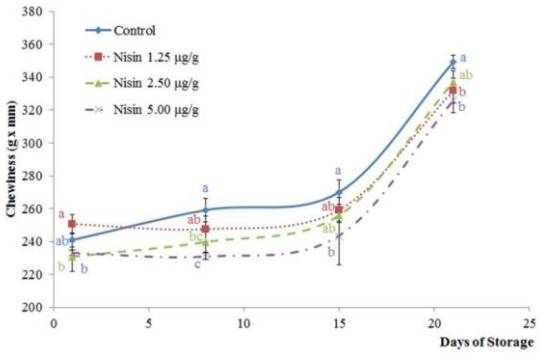

Figure 4. Chewiness of control and treated samples with different concentrations of nisin during storage at 4°C. Different letters (a, b, c) indicate significant difference between samples at the same day of storage at $p<0.05$ (n=3, error bars: standard deviations).

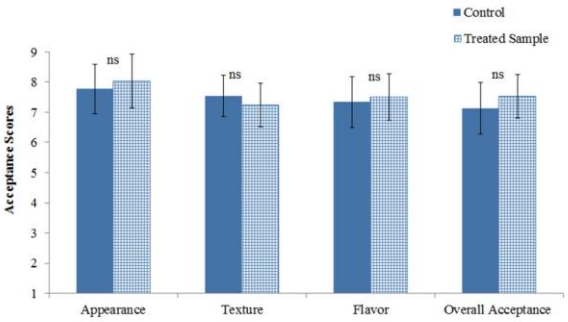

Figure 5. Acceptance scores of control and treated sample with 5.00 μg/g nisin at the beginning of storage (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). ns indicates no significant difference between samples at the same attribute (*p≥0.05*) (n=30, error bars: standard deviations).

3.4. Chemical analysis

Since the sample containing 5.00 μg/g nisin showed the best results from microbiological analysis, syneresis, and TPA, it was selected for chemical analysis and sensory evaluation to compare with the control sample. Chemical compositions of control and treated sample with 5.00 μg/g nisin during storage at 4°C are presented in Table 1. There was no significant difference between control and treated sample in every chemical composition at the first day of storage (p≥0.05). Similarly, Benech et al. (2002) reported no significant difference between nisin-free cheddar cheese and *in situ* nisin-containing cheddar cheese on moisture content, total proteins and fat content after production.

3.5. Sensory evaluation

In the duo-trio test, the control was first provided to each untrained panelist as a standard. Then, both control and treated sample with 5.00 μg/g nisin were served and the panelists needed to indicate which the standard was. According to the table of critical number of correct responses in a duo - trio or one - sided directional difference test (Meilgaard et al., 2007), the minimum number of corrected responses required for significant difference at α-level of 0.05 (n=30) is 20. However, the number of corrected responses from this test was 16 (53%), indicating that the panelists could not discriminate the treated sample from the control sample. Similarly, the control and treated samples were not significantly different in microbiological analysis, syneresis, TPA, and chemical analysis at the beginning of storage (p≥0.05).

Table 1 Chemical compositions of control and treated sample with 5.00 μg/g nisin at the first day of storage (n=3).

Chemical composition (%w/w)	Control	Sample with 5.00 μg/g nisin
Moisture ^{ns}	83.22 ± 0.16	83.62 ± 0.56
Carbohydrate ^{ns}	11.44 ± 0.17	11.06 ± 0.55
Fat ^{ns}	3.16 ± 0.05	3.23 ± 0.07
Protein ^{ns}	1.66 ± 0.04	1.53 ± 0.17

ns indicates no significant difference (*p≥0.05*).

The acceptance test was conducted to evaluate the level of acceptability on the treated sample among untrained panelists (n=30), compared with the control sample. Scores from the acceptance test are presented in Fig. 5. There was no significant difference between the control and the treated sample in appearance, texture, flavor, and overall acceptance at the beginning of storage (p≥0.05). Both samples obtained scores at approximately '7' or 'like moderately'. These results could ensure that nisin did not affect the sensory qualities of the samples. In the application of nisin in buffalo meat sausage, Sureshkumar et al. (2010) also found that nisin did not affect the sensory qualities of samples, including appearance, flavor, juiciness, and overall acceptability at the beginning of storage. Moreover, Mahalingaiah et al. (2014) reported no significant difference between "kunda" (a sweet product prepared from milk and added sugar) with and without nisin added in sensory scores, including flavor, texture, and overall acceptance, at the beginning of storage (p≥0.05).

4. Conclusions

The recommended concentration of nisin in milk pudding with fruit cocktail was 5.00 μg/g since it efficiently reduced the changes in microbial growth, syneresis, hardness, and

chewiness of the sample while provided no effect on springiness, cohesiveness, chemical compositions and sensory acceptability.

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