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Effect of Nisin on Microbial, Physical and Sensory Qualities of Micro-Filtered Coconut Water (*Cocos Nucifera L.*) During Refrigerated Storage

NUTSUDA SUMONSIRI

Department of Agro-Industrial, Food and Environmental Technology, King Mongkut's University of Technology North Bangkok (KMUTNB), Bangsue, Bangkok 10800 Thailand.

Abstract

The efficacy of nisin (25 - 75 ppm) and effect of storage time on quality attributes of micro-filtered coconut water during refrigerated storage (4 °C) for 8 days was investigated in terms of total viable counts, color, turbidity and overall sensory acceptance. All treatments significantly retarded the bacterial growth in coconut water during storage when compared to the control sample ($p < 0.05$). The samples treated with 50 and 75 ppm nisin had significantly lower aerobic microbial counts than the control ($p < 0.05$) without affecting color, turbidity and sensory acceptability. The treated samples also had the significantly higher scores in overall acceptance than the control sample after 7 days of storage. 50 ppm Nisin was suggested to be applied in micro-filtered coconut water without effects on color, turbidity, and sensory acceptability by a reduction in changes of the microbial growth during the refrigerated storage.

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Keywords

Bacteriocin;
Coconut Water;
Microfiltration; Nisin;
Quality; Storage.

Introduction

Coconut water is clear liquid taken out from immature green coconuts (*Cocos nucifera* L.). It is a very popular drink, which is consumed worldwide as a refreshing drink due to its therapeutic and nutritional properties.[1] It has also been used for treatment of oral dehydration, diarrhea, gastroenteritis, cholera, and other infectious diseases that cause dehydration, especially of children in underdeveloped countries.[2] The chemical composition of coconut water consists of water (96.11 %), sugars (including glucose, fructose and sucrose, 2.7 %), proteins (0.25 %), lipids (0.51 %), and ash (0.43 %). There are plenty of minerals, such as potassium, calcium, magnesium, and manganese, but low in sodium. The number of chemical components varies with the cultivar and maturation stage of coconut.[3]

Since coconut water is rich in nutrients, it deteriorates easily once exposed to air. In commercial processing of coconut water, ultra-high temperature technology is usually used to prolong its shelf-life. However, this

CONTACT Nutsuda Sumonsiri nutsuda.s@sci.kmutnb.ac.th Department of Agro-Industrial, Food and Environmental Technology, King Mongkut's University of Technology North Bangkok (KMUTNB), Bangsue, Bangkok 10800 Thailand.

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thermal processing causes loss of fresh flavor and some nutrients, leading to the limited marketability of canned coconut water.[4] Non-thermal processing, such as microfiltration, is an alternative way to preserve freshness, aroma, taste and nutrient contents of coconut water and other fruit juices.[2,5,6,7]

Nisin is a polypeptide which can be used as a preservative in food products against Gram-positive bacteria, including *Clostridium botulinum*, *Bacillus sporothermodurans*, and *B. cereus*, as well as some bacterial spores.[8,9,10] It can be produced by *Lactococcus lactis* subspecies *lactis*. Nowadays, nisin is allowed in variety of food products in many countries.[11] In Mexico and Peru, nisin is allowed in any food products. In the United States, it is accepted as a GRAS (generally recognized as safe) agent and allowed to be used to inhibit *Clostridium botulinum* spore outgrowth and toxin formation in pasteurized cheese spreads with meats, vegetables, and/or fruits.[12] Moreover, this peptide can be found in the list of European food additives (No. E234).[13] For its application in beverages, salad dressings, dairy products, and vegetables.[14] In Russia, nisin (up to 100 ppm) is allowed in vegetables such as raw, peeled, minimally processed, or semi - preserved cauliflower, green peas, and potatoes. In Slovak Republic, nisin (up to 12.5 ppm) has been approved in pickled and sterilized vegetables, and some ready - to - eat meals.[15] In India, nisin with a permitted concentration at 125 ppm is approved in coconut water.[8] Recently, the research on application of nisin in a blend juice containing carrot juice (20%), tomato juice (20%), and beet root juice (60%) has revealed that nisin (20 ppm) can help stabilize the thermal treated juice packed in polyethylene standy pouches over storage of 90 days at room temperature (28°C).[16] Another study also showed a positive effect of nisin on microbial inactivation in fruit juices, such as cashew, peach, passion fruit, soursop, mango, orange, cupuassu, and guava.[17] Many studies showed the combination of thermal processing and nisin to preserve tropical fruit juices; however, there are still limited numbers of studies on non-thermal processing and nisin in coconut water.

The aim of this research was to study the influence of nisin on quality characteristics and overall acceptability of micro - filtrated coconut water during refrigerated storage (4 °C).

Materials and Methods

Materials

Green coconuts (with maturity levels of approximately 5 to 7 months), were purchased from a local market in Nonthaburi, Thailand, immediately stored in a refrigerator (4 °C), and used in sample preparation within 24 h after purchasing or within 72 h after harvesting.

Table 1: Lightness of control and treated sample with nisin (25-75 ppm) during storage at 4°C (n=3)

Nisin (ppm)	Days of Storage			
	0^{ns}	7^{ns}	14^{ns}	21
0 (Control)	$^{C}20.94 \pm 0.66$	$^{B}25.01 \pm 1.44$	$^{B}27.17 \pm 1.69$	$^{A}30.54^{a} \pm 0.15$
25	$^{D}20.94 \pm 0.66$	$^{C}24.00 \pm 0.37$	$^{B}28.14 \pm 0.14$	$^{A}29.20^{b} \pm 0.02$
50	$^{D}20.94 \pm 0.66$	$^{C}24.26 \pm 0.14$	$^{B}27.40 \pm 1.77$	$^{A}29.77^{ab} \pm 0.13$
75	$^{D}20.94 \pm 0.66$	$^{C}24.23 \pm 0.47$	$^{B}28.01 \pm 1.59$	$^{A}30.92^{a} \pm 1.26$

Uppercase characters (A, B, C, D) indicate significant difference among samples with different storage days at the same concentration of nisin ($p<0.05$).

Lowercase characters (a, b) indicate significant difference among samples with different concentration of nisin at the same storage day ($p<0.05$).

ns indicates that samples with different concentration of nisin at the same storage day was not significantly different ($p\geq0.05$).

Sample Preparation

Coconuts were initially rinsed in tap water to remove soil, and then immersed in calcium hypochlorite solution (200 mg / L) for 15 min to reduce initial number of microorganism on the surface of coconuts.[18] Afterwards, they were rinsed with distilled water to remove residual chlorine and then allowed to air - dry. Following Good Manufacturing Practices, the coconuts were cut with a sanitized stainless - steel knife and coconut water was thoroughly mixed using a glass stirring rod in a sterile container. Refined sucrose (Mitr Phol Sugar Co., Ltd., Bangkok,

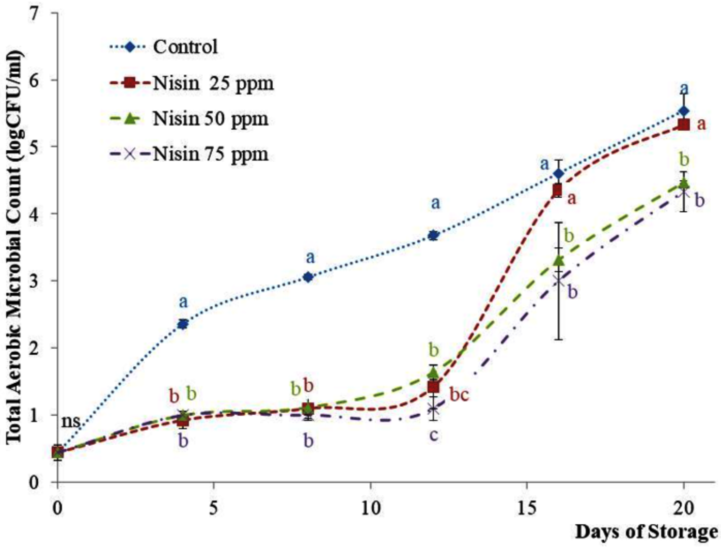

Fig. 1: Total viable counts of coconut water (control and samples with nisin at different concentration) during refrigerated storage. Significant difference among different treatments at the same day of storage was indicated by different lowercase characters (a, b, c) (p<0.05) (error bars: standard deviations, n=3).

Table 2: Chroma of control and treated sample with nisin (25-75 ppm) during storage at 4°C (n=3)

Nisin (ppm)	Days of Storage			
	0^{ns}	7^{ns}	14	21
0 (Control)	$^{A}3.09 \pm 0.07$	$^{A}3.21 \pm 0.02$	$^{A}3.15^{a} \pm 0.15$	$^{B}1.39^{b} \pm 0.05$
25	$^{A}3.09 \pm 0.07$	$^{A}3.18 \pm 0.07$	$^{A}3.16^{a} \pm 0.03$	$^{B}1.16^{c} \pm 0.02$
50	$^{A}3.09 \pm 0.07$	$^{A}3.17 \pm 0.04$	$^{A}3.13^{a} \pm 0.03$	$^{B}1.85^{a} \pm 0.10$
75	$^{A}3.09 \pm 0.07$	$^{A}3.19 \pm 0.11$	$^{B}2.40^{b} \pm 0.10$	$^{C}1.13^{c} \pm 0.08$

Uppercase characters (A, B, C) indicate significant difference among samples with different storage days at the same concentration of nisin (p<0.05).

Lowercase characters (a, b, c) indicate significant difference among samples with different concentration of nisin at the same storage day (p<0.05).

ns indicates that samples with different concentration of nisin at the same storage day was not significantly different (p≥0.05).

Thailand) and citric acid (Ajax Finechem Pty., Ltd., New Zealand) were used to standardize coconut water to 7˚Brix and pH of 4.3 - 4.5 respectively.

To prepare the control sample, coconut water was filtered by filter cloth to remove suspended solids. The microfiltration was carried our using sterile Whatman Polyethersulfone Puradisc syringe filter (0.2 µm pore size, 25 mm diameter) (Sigma-Aldrich Pte. Ltd., Singapore) within the same day of extraction.

For preparing nisin - treated samples, food-grade nisin (Shandong Freda Biotechnology Co., Ltd., China) was mixed with coconut water at 25, 50, and 75 ppm for 5 min before the filtration. Other procedures were similar to those steps for the control sample.

All samples were kept in sterile glass bottles and stored in a refrigerator (4 °C). The process of sample preparation was independently repeated on 3 different days.

Microbiological Analysis

The pour-plate method[19] was used to determine total viable counts (TVC), yeast and molds, and coliforms of every sample at 0, 4, 8, 12, 16, and 20 days of

Table 3: Hue angle (˚) of control and treated sample with nisin (25-75 ppm) during storage at 4°C (n=3)

Nisin (ppm)	Days of Storage			
	0^{ns}	7^{ns}	14	21
0 (Control)	$^{C}256.93 \pm 0.67$	$^{B}259.41 \pm 0.30$	$^{A}260.87^{a} \pm 0.46$	$^{D}245.70^{b} \pm 0.87$
25	$^{B}256.93 \pm 0.67$	$^{AB}259.23 \pm 0.13$	$^{A}260.97^{a} \pm 0.64$	$^{C}253.60^{a} \pm 2.99$
50	$^{B}256.93 \pm 0.67$	$^{A}259.08 \pm 0.65$	$^{C}255.06^{b} \pm 1.03$	$^{D}237.27^{c} \pm 0.39$
75	$^{B}256.93 \pm 0.67$	$^{A}259.41 \pm 0.34$	$^{B}256.17^{b} \pm 0.84$	$^{C}235.30^{c} \pm 2.18$

Uppercase characters (A, B, C, D) indicate significant difference among samples with different storage days at the same concentration of nisin ($p<0.05$).
Lowercase characters (a, b, c) indicate significant difference among samples with different concentration of nisin at the same storage day ($p<0.05$).
ns indicates that samples with different concentration of nisin at the same storage day was not significantly different ($p\geq0.05$).

Table 4: Turbidity (NTU) of control and treated sample with nisin (25-75 ppm) during storage at 4°C (n=3)

Nisin (ppm)	Days of Storage			
	0^{ns}	7^{ns}	14^{ns}	21^{ns}
0 (Control)	$^{C}34.17 \pm 0.85$	$^{B}39.99 \pm 0.26$	$^{B}41.81 \pm 2.07$	$^{A}52.00 \pm 1.64$
25	$^{C}34.17 \pm 0.85$	$^{B}39.80 \pm 2.18$	$^{B}41.03 \pm 1.46$	$^{A}51.53 \pm 0.50$
50	$^{C}34.17 \pm 0.85$	^{B}C39.17 ± 2.25	$^{AB}41.93 \pm 3.28$	$^{A}47.63 \pm 6.29$
75	$^{C}34.17 \pm 0.85$	$^{BC}38.67 \pm 0.60$	$^{B}40.40 \pm 1.18$	$^{A}45.67 \pm 4.53$

Uppercase characters (A, B, C) indicate significant difference among samples with different storage days at the same concentration of nisin ($p<0.05$).
ns indicates that samples with different concentration of nisin at the same storage day was not significantly different ($p\geq0.05$).

storage. 1.0 mL of sample from an appropriate serial dilution, which had been prepared by using sterile peptone water (Merck, KGaA, Germany), was pipetted into duplicate sterile Petri dishes. Each Petri dish containing sample was added with Plate Count Agar (PCA) for TVC, acidified potato dextrose agar (PDA) for yeast and molds, and violet red bile agar (VRB) for coliforms before incubating in an incubator at 37 ± 2 °C (48 h) for TVC, 25 ± 1 °C (4 - 5 d) for yeast and molds, and 32 ± 1°C (24 h) for coliforms. Colonies were counted after the incubation and total viable counts were expressed using log CFU/mL.

Color and Turbidity

Color values of samples were measured with a colorimeter (Color Quest 45/0, Hunter Associates Laboratory, Inc., Reston, VA) after 0, 7, 14, and 21 days of storage. Before the color determination, a standard white and black reflector plate had been used for calibrating the instrument. Three samples with four readings (by changing a position of sample) were measured for each treatment at room temperature. CIE L*C*h* color space was used to express the color values, including lightness (L*), chroma (C*) and hue angle (h*).

Turbidity of samples was determined by using TU001 Turbidimeter (Qingdao Tlead International Co., Ltd., China), and recorded as nano turbidity units (NTU).

Sensory Evaluation

The selected treatment after comparing with the control in terms of microbial growth, color changes, and turbidity was determined for overall acceptance among 30 untrained panelists, who had been screened from undergraduate students in *Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Thailand*. The samples at 0 and 8 days of refrigerated storage were presented to the panelists in random order. Random 3-digit codes were used for labelling each sample. All panelists were asked to evaluate samples for overall acceptance with a 9-point hedonic scale (1: dislike extremely, 2: dislike very much, 3: dislike moderately, 4: dislike slightly, 5: neither like nor dislike, 6: like slightly, 7: like moderately, 8: like very much, 9: like extremely).

Statistical Analysis

Every analysis was conducted in triplicate, except that analysis of microbial growth, which was

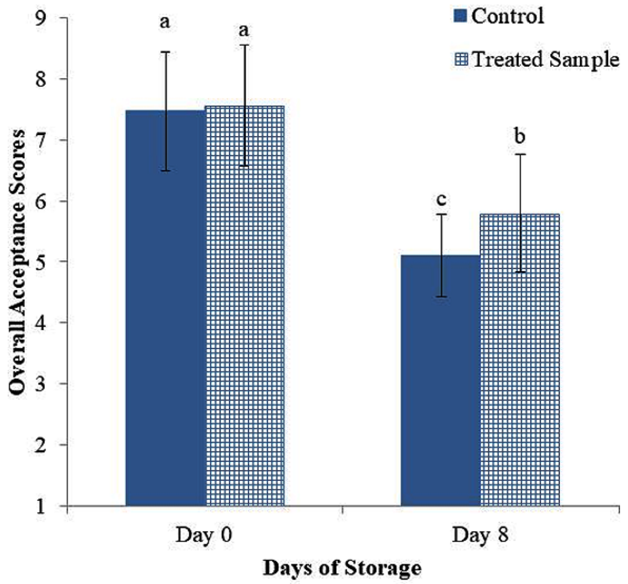

Fig. 2: Acceptance scores of control and micro-filtered coconut water with 50 ppm nisin at the first and eighth days of storage according to a 9-scale hedonic procedure. Different lowercase characters (a, b, c) indicate a significant difference among samples ($p<0.05$) (n=30, error bars: standard deviations).

conducted in duplicate. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) by IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY) were used to analyze the data to indicate significant differences among samples at $p < 0.05$.

Results and Discussion

Microbiological Analysis

Figure 1 presented TVC of control and nisin-treated samples during the refrigerated storage. The TVC in the control increased with prolonging the storage time ($p < 0.05$) while this microbial parameter in the treated samples was increased after 10 days of storage. Yeast and mold counts were lower than 1.0 log CFU/mL while total coliforms were not detected in all samples during storage (data not shown). In the previous research, the coconut water contained 1.08×10^1 CFU/mL total microbes after microfiltration (1,000 nm pore size), ultrafiltration (50 nm pore size), and UV treatment.[20]

All treatments significantly retarded the bacterial growth in coconut water during storage when compared to the control sample ($p<0.05$). The coconut water treated with 50 and 75 ppm nisin had the lowest TVC with less than 4.5 logCFU/mL until the last day of storage. In the recent research, the combination of malic acid (1500 ppm) and nisin (75 ppm) provided the lowest D55 (decimal reduction times at 55˚C) of *Escherichia coli* O157:H7 in young coconut liquid endosperm.[21] A synergistic effect of high hydrostatic pressure (HHP) with homogenization and nisin (100 IU/mL) is also found in the inactivation of microbiota which has naturally occurred in cucumber juice.[22] Moreover, nisin (5000 IU/mL) could improve microbial safety against *B. cereus*, *Alicyclobacillus acidoterrestris*, *Listeria monocytogenes*, and *Staphylococcus aureus* in soursop, peach, cashew, and mango juices during storage at room temperature (30 ± 2˚C) and 4 ˚C.[23] Samples treated with nisin had slower growth of bacteria since nisin can form pores on cytoplasmic membrane, especially in Gram-positive bacteria. These pores can interrupt a proton motive force, as well as pH equilibrium, which lead to an ion leakage, ATP hydrolysis, and finally death of cell. Nisin can also bind with lipid II, which is a peptidoglycan precursor, leading to an inhibition of cell wall biosynthesis.[24,25,26]

Color and Turbidity

The lightness (L*), chroma (C*), hue angle (h*), and turbidity in Tables 1 - 4 respectively revealed that the color attributes and turbidity of micro-filtrated coconut water were not significantly affected by addition of nisin during storage ($p \geq 0.05$). No significant differences in L* were observed in samples with or without nisin during 14 days of storage. In addition, no differences were observed in the chroma and hue angle for samples with or without nisin during 6 days of storage ($p \geq 0.05$). Moreover, there was no significant difference in turbidity in all samples during 20 days of storage. Bacteriocins are well known to cause no changes in physico-chemical characteristics of foods.[26] In the previous study, there was no difference in browning index for cashew, soursop, juices with and without nisin at the same day of storage at room or refrigerated temperatures.[17] The color changes between control and nisin treated sugarcane juice (140 ppm) were not significantly different during 20 days of refrigerated storage.[27]

Sensory Evaluation

The levels of overall acceptance between the control and coconut water treated with nisin at the first and eighth days of refrigerated storage were evaluated by untrained panelists (n = 30). At the first day of storage, the control and treated samples obtained insignificantly different scores in overall acceptance ($p \geq 0.05$) (Figure 2). Both control and treated sample obtained overall acceptance scores above '7' or 'moderately like'. In the application of nisin (140 ppm) in sugarcane juice,[27] nisin treated sample obtains slightly lower overall acceptability score than the control sample; however, the overall acceptability score of the treated sample is still at '7' from a 9 - point hedonic scale.

At the 8th day of storage, the overall acceptance scores of both nisin-treated and control samples were significantly decreased; however, the nisin-treated sample had significantly higher score than the control at the same storage period. The results assured that nisin did not have significant effect on overall acceptance in sensory evaluation at the beginning of storage and it also kept micro-filtered coconut water in the better condition during storage when compared to the sample without nisin added.

Bacteriocins are well known to cause no changes in organoleptic characteristics of foods since it does not affect the physico-chemical properties of foods.[26]

Conclusion

Nisin significantly retarded the bacterial growth in coconut water during storage when compared to the control sample ($p < 0.05$). The samples treated with 50 and 75 ppm nisin had significantly lower aerobic microbial counts than the control ($p < 0.05$) without affecting color, turbidity and sensory acceptability. The treated samples also had the significantly higher scores in overall acceptance than the control sample after 7 days of storage. Nisin at 50 ppm was suggested to be applied in micro-filtered coconut water without effects on color, turbidity, and sensory acceptability by a reduction in changes of microbial growth during the refrigerated storage.

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