# THE EFFECT OF HONEY ON BACTERIAL GROWTH, PROTEIN DEGRADATION, AMINO ACIDS CONTENTS AND VOLATILE COMPOUNDS OF MILKS AT STORAGE

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## ABSTRACT

Pasteurized milks spoiled at refrigerated storage due to growth of psychrotrophic bacteria. Honey which contain antibacterial and aromatic compounds may be used as supplement to inhibit psychrotrophic bacteria' activities. To know nutritional and flavor compounds of milks with and without honey, effect of honey on bacterial growth0protein degradation, amino acids and volatile compounds of stored milks were detected. Bacterial growth, protein degradation, amino acids and flavor compounds were detected by total plate counts, formol titration, HPLC and GCMS, respectively. The results show that bacterial growth and protein degradations in honey milks were lower than that without honey. Bacterial growth ($5.2x10^3$ - $9.3x10^6$ cfu/mL ) and protein degradations (2.37-2.59% ) in honey whole milks were lower than that ($6.2x10^4$ - $6.5x10^7$ cfu/mL)( 2.54-2.88%) in skim milks, respectively ($P<0.05$). At 10 days after use by date, changing between amino acids' contents in whole milks with and without honey were more significant than that of skim milks ($P<0.05$);. and volatile compounds' percentages in honey whole milks were higher than that without honey, while that in honey skim milks vice versa. Honey caused decreasing bacterial growth and protein degradation, changing amino acids' contents and producing volatile compounds of stored milks, and honey whole milks were better than honey skim milks.

**Keywords**: pasteurized milks, skim, honey, protein, amino acids, volatile compounds

## ABSTRAK

Susu pasteurisasi rusak pada saat penyimpanan suhu dingin, dikarenakan adanya pertumbuhan bakteri psikhotroph. Madu yang mengandung senyawa antibakteri dan senyawa aromatik, dapat digunakan sebagai suplemen untuk menghambat aktivitas bakteri psikhotroph. Untuk mengetahui komponen nutrisi dan flavor pada susu dengan dan tanpa madu, pengaruh madu terhadap pertumbuhan bakteri, degradasi protein, kandungan asam amino dan komponen flavor pada susu selama penyimpanan dideteksi. Pertumbuhan bakteri, degradasi protein, kandungan asam amino dan komponen flavor, masing-masing dideteksi menggunakan penghitungan total bakteri, titrasi formol, HPLC dan GCMS. Hasil penelitian menunjukkan bahwa pertumbuhan bakteri dan degradasi protein susu madu selama penyimpanan lebih rendah dibandingkan yang tanpa madu. Pertumbuhan bakteri ($5.2x10^3$ - $9.3x10^6$ cfu/mL) dan degradasi protein (2.37-2.59%) susu madu berlemak, masing-masing lebih rendah dibandingkan pada susu madu skim ($6.2x10^4$-$6.5x10^7$ cfu/mL) dan (2.54-2.88%) ($P<0.05$). Pada 10 hari setelah kadaluarsa, perubahan diantara kandungan asam amino susu berlemak selama penyimpanan dengan dan tanpa madu lebih berbeda nyata dibandingkan perubahannya di antara susu skim dengan dan tanpa madu ($P<0.05$); dan persentase komponen flavor susu madu berlemak lebih tinggi dibandingkan yang tanpa madu, sedangkan pada susu madu skim kebalikannya. Madu menyebabkan penurunan pertumbuhan bakteri dan degradasi protein, perubahan kandungan asam amino dan pembentukan komponen flavor pada susu selama penyimpanan, dan susu madu berlemak lebih baik dibandingkan susu madu skim.

**Kata kunci**: susu pasteurisasi, skim, madu, protein, asam amino, komponen flavor

## INTRODUCTION

Pasteurised milks spoiled after use by date, at refrigerated temperatures due to the activities of psychrotrophic bacteria, especially *Pseu–domonas* spp. The main species of *Pseudomonas* spp. spoiled pasteurized milks was *Pseudomonas fluorescens*. (Chandler *et al.*, 1990; Deeth *et al.*, 2002). It has been reported that pasteurized milks temperature 72°C 15' to 88°C 15' in carton package stored at 4.5 and 7$^0$C spoiled due to the activities of psychrotrophic bacteria, especially *Pseudomonas* sp., and the average shelf life of milks stored at 4.5 and 7$^0$C were around 7 days in non tropical area (Bishop and White.1986). However, Heo (1989) examined commercial milk samples temperature Sumbawa island in Indonesia which contain antibacterial and aromatic compounds were produced from materials which contain antibacterial and aromatic compounds, and the materials were collected from some supermarkets in Bogor-Indonesia. All honeys were soluted in hot water (10 gram in 100 mL hot water) and the liquid honey was then filtered to produce liquid materials as juice supplements.

### *Juice supplements of honey*

Fifty grams honey was diluted by adding 500 mL boiled waters in hot, as juice. The juices of honey produced were filtered by stainless steel filter Ø (diameter) 1mm and the cleared juices of honey were kept in refrigerated temperatures up to ready to be used as supplements.

### *Pasteurised milks with addition of honey*

Aliquots (100 mL) of commercial pasteu–rized milks were transferred aseptically from the cartons into 200 mL sterile bottles. A liquid honey was added into separate 200-mL aliquots of the batch pasteurized milks. The samples with addition of honey, together with milks without honey (control), were incubated at 4°C for up to 15 days (5 days before use by date, at use by date, 5 days after use by date, and 10 days after use by date). Bacterial growth, protein degradation, amino acids contents and volatile compounds of control and samples with addition of honey were analysed and the results presented are mean values for the three replicates.

### *Total aerobic bacterial counts*

Ten-fold serial dilutions of milks with and without addition of honey were made and spread plate counts performed according to Australian Standard AS 1766.1.4 using Nutrient Agar. The plates were incubated for 2-3 days at 30$^0$C.
Detection of protein degradation

Protein degradation of milks with and without addition of honey at all times of storage were detected by using formol titration (Kimg, 1978) with modification. The amount of 10 mL milks were added 20 mL aquadest, 0.4 mL saturated di-potassium oxalate monohydrate ($K_2C_2O_4.H_2O$), and 1 mL phenolphtalein 1%. These solutions were then kept for 2 minutes, and these solutions were titrated with NaOH 0.1 M up to the change of colour to be pink. Furthermore, 2 ml formaldehyde 40% were poured into samples in erlenmeyer. The sample solution was again titrated with NaOH 0.1M up to the change of colour to be pink. Calculation of protein degradation, that is: percentages (%) of milk protein = 1.83 x the amounts of NaOH 0.1 M for titration.

### *Detection of amino acid compounds*

Detection of amino acid contents in milks with and without addition of honey at storage in use by date and 10 days after use by date used HPLC (Pryde and Gilbert, 1980) with modification. The amino acid contents of milks with and without addition of honey were detected at the times of storage. Samples of milks were hydrolyzed to breakdown milks' protein by using 6 M HCL for 12-18 hours in vacuum gauge.The reason why to do this hydrolyzation was to measure total amino acids which composed milks' proteins. To release compounds interfered complex reaction between PITC with amino acids, methanol and trietylamine were added as drying solution. The result formed between PITC with amino acids was complex compounds detected by fluorescens detector. Sample solutions for amino acids' analyses were injected to HPLC.

### *Detection of volatile compounds*

Detection of volatile compounds in milks with and without addition of honey at 10 days after the use by date used GCMS (Wood and

Aston, 1994) with modification. Condition of GC-MS for milks' samples was collum: DB-17; length: 30 mm; diameter: 0.25 mm. Program temperature: 40°C/5'/10°C per minute/150°C/2'/10°Cper minute/175°C, with temperature of injector: 200°C, temperature of interface: 200°C, pressure: 60 KPa, and split: 11. Type of GCMS: GC-17A; MS QP-500. The amount of sample injected: 5 ìL. Sample preparation was conducted by homogenizing samples of milks with and without honey, the homogenized milks' samples were then filtered by using paper Whatman 42 and the filtrate was filtered by milliphore Ô 0.45 ìm. The filtrate produced was injected to GCMS.

### *Statistical analysis*

All treatments of milks' samples with addition of honey were statistically analysed by ANOVA with Factorial Complete Randomized Design (Snedecor and Cochran, 1989) using General Linear Model with three replications.

## RESULTS

The bacterial growth of skim and whole milks with addition of honey at the times of storage was different with that without honey. The bacterial growth in whole milks at storage ($5.2 \times 10^3$–$9.3 \times 10^6$ cfu/mL) were lower than that in skim milks ($6.2 \times 10^4$–$6.5 \times 10^7$) ($P < 0.05$). Furthermore, the bacterial growth in honey milks at storage were lower than that without honey, and the bacterial growth in whole honey milks ($2.0 \times 10^1$–$5.3 \times 10^3$) were lower than that in skim honey milks ($7.0 \times 10^1$–$9.8 \times 10^3$ cfu/mL) ($P < 0.05$). The bacterial growth of skim and whole milks at times of storage were shown in Table 1.

The percentages on protein degradation of skim and whole milks with addition of honey at the times of storage were different with that without honey. The protein degradations in whole milks at storage (2.64-2.98%) were lower than that in skim milks (2.97-3.22%) ($P<0.05$). Furthermore, the protein degradations in honey milks at storage were lower than that without honey, and the degradation in whole honey milks (2.37–2.59%) were lower than that in skim honey milks (2.54–2.88%) ($P < 0.05$). The percentages of protein degradation of skim and whole milks at times of storage were shown in Table 2.

From seventeen amino acids, all amino acids contents of whole milks with honey weren't different with that without honey at storage in use by date, in similarity, sixteen amino acids of skim milks with honey at the same storage weren't different with that without honey, however, one amino acid of leucine in skim milks with honey (310.5 mg/mL) were higher than that without honey (218.5 mg/mL) ($P<0.05$). The more leucine in skim milks with honey than that without honey may be because the leucine of honey skim milks consist of the leucine of milks itself and the leucine which came from honey. The amino acid contents of skim and whole milksoat storage in use by date (mg/100 mL) were shown in Table 3.

**Table 1.** The bacterial growth of skim and whole milks with and without addition of honey at times of storage (cfu/mL)

| No. | Times of storage | Whole milk | Skim milk |
|---|---|---|---|
| A | With addition of honey | | |
| 1 | 5 days before use by date | $2.0 \times 10^1$ (a) | $7.0 \times 10^1$ (b) |
| 2 | At the use by date | $1.2 \times 10^2$ (c) | $5.6 \times 10^2$ (d) |
| 3 | 5 days after use by date | $6.2 \times 10^2$ (d) | $8.4 \times 10^2$ (e) |
| 4 | 10 days after use by date | $5.3 \times 10^3$ (f) | $9.8 \times 10^3$ (g) |
| B | Without addition of honey | | |
| 1 | 5 days before use by date | $5.2 \times 10^3$ (f) | $6.2 \times 10^4$ (h) |
| 2 | At the use by date | $7.5 \times 10^4$ (i) | $8.6 \times 10^5$ (j) |
| 3 | 5 days after use by date | $8.5 \times 10^5$ (j) | $6.1 \times 10^6$ (k) |
| 4 | 10 days after use by date | $9.3 \times 10^6$ (l) | $6.5 \times 10^7$ (m) |

Note: different letters show significantly different ($P<0.05$)

**Table 2**. The percentages of protein degradation of skim and whole milks with and without addition of honey at times of storage (%)

| No. | Times of storage | Whole milk | Skim milk |
|---|---|---|---|
| A | with addition of honey | | |
| 1 | 5 days before use by date | 2.37 a | 2.54 b |
| 2 | at the use by date | 2.39 a | 2.69 e |
| 3 | 5 days after use by date | 2.58 c | 2.79 f |
| 4 | 10 days after use by date | 2.59 c | 2.88 g |
| B | without addition of honey | | |
| 1 | 5 days before use by date | 2.64 d | 2.97 i |
| 2 | at the use by date | 2.68 e | 3.18 j |
| 3 | 5 days after use by date | 2.91 h | 3.20 j |
| 4 | 10 days after use by date | 2.98 l | 3.22 k |

Note: different letters show significantly different (P<0.05)

**Table 3**. The contents of amino acids of skim and whole milks at storage in use by date (mg/100 mL)

| No. | Amino acids | Whole milk | Whole honey milks | Skim milk | Skim honey milks |
|---|---|---|---|---|---|
| 1 | Aspartic acid | 0.230 ef | 0.230 ef | 0.239 e | 0.238 e |
| 2 | Glutamic acid | 0.560 a | 0.560 a | 0.586 a | 0.575 a |
| 3 | Serine | 0.150 h-m | 0.150 h-m | 0.153 h-l | 0.151 h-m |
| 4 | Glycine | 0.049 y | 0.046 y | 0.049 y | 0.047 y |
| 5 | Histidine | 0.102 p-t | 0.100 q-t | 0.104 p-t | 0.103 p-t |
| 6 | Arginine | 0.120 o-t | 0.118 n-s | 0.111 o-t | 0.110 o-t |
| 7 | Threonine | 0.115 n-t | 0.116 n-t | 0.121 n-q | 0.120 n-r |
| 8 | Alanine | 0.099 r-u | 0.098 r-v | 0.097 s-v | 0.095 t-w |
| 9 | Proline | 0.335 bc | 0.335 bc | 0.025 b-d | 0.350 b |
| 10 | Tyrosine | 0.124 m-p | 0.125 l-p | 0.135 i-o | 0.130 k-o |
| 11 | Valine | 0.164 g-i | 0.163 g-j | 0.173 g-h | 0.175 g-h |
| 12 | Methionine | 0.081 v-x | 0.082 v-x | 0.079 wx | 0.077 x |
| 13 | Cysteine | 0.028 z | 0.028 z | 0.024 za' | 0.022 a' |
| 14 | Isoleucine | 0.160 h-k | 0.161 g-j | 0.162 gh | 0.181 gh |
| 15 | Leucine | 0.305 bd | 0.300 bd | 0.218 f-g* | 0.310 b-d* |
| 16 | Phenylalanine | 0.134 i-o | 0.132 j-o | 0.140.5 i-n | 0.140 i-n |
| 17 | Lysine | 0.280 c-e | 0.262 d-e | 0.271 d-e | 0.267 d-e |

Note: The different one of the letters shows significantly difference(P<0.05)
*. significantly different

The 4 of the 9 amino acids' contents of arginine (0.199 mg/100mL), proline (0.313 mg/100mL), valine (0.109 mg/100mL) and leucine (0.700 mg/100mL) in whole honey milks at 10 days after use by date were higher than that of arginine (0.112 mg/100mL), proline (0.171 mg/100mL), valine (0.038 mg/100mL) and leucine (0.463 mg/100mL) in whole milks without honey, respectively. On the contrary, the 5 of the 9 amino acids' contents of glycine (0.042 mg/100mL), threonine (0.045 mg/100mL), tyrosine (0.047 mg/100mL), isoleucine (0.140 mg/100mL) and lysine (0.023 mg/100mL) in whole honey milks were lower than that of glycine (0.068 mg/100mL), threonine (0.245 mg/100mL), tyrosine (0.077 mg/100mL), isoleucine (0.406 mg/100mL) and lysine (0.123 mg/100mL) in milks without honey, respectively (P<0.05).

Furthermore, the 2 amino acids' contents of tyrosine (0.051 mg/100mL) and lysine (0.072 mg/100mL) in skim honey milks at the same storage were lower than that of tyrosine (0.120 mg/100mL) and lysine (0.133 mg/100mL) in skim milks without honey, respectively (P<0.05). The amino acid contents of skim and whole milks with and without honey at storage 10 days after the use by date (mg/100 mL) were shown in Table 4.

The total percentages of volatile compounds detected in skim and whole milks with honey at storage 10 days after use by date were different with that without honey, respectively. The total percentages of volatile compounds detected in whole honey milks (84.62%) were higher than that without honey (78.83%), on the contrary, the percentages of volatile compounds detected in skim honey milks (84.53%) were lower than that without honey (92.96%). In whole milks with and without honey, percentage of furfuryl alcohol in whole honey milks (7.44%) was higher than that without honey, on the contrary, percentage of acetic acid in the same milks (41.44%) were lower than that without honey. Furthermore, volatile compounds of 2-benzyloxyethylamine (17.63%), 2-heptanol (3.07%), benzoic acid (3.41%) and N-pentanal (8.97%) were produced in whole honey milks, on the contrary in these milks, there were no volatile compounds of 2-Butanone, fluoroethylene, isopropyl alcohol and 1,2-pentanol in which these compounds occurred in whole milks without honey.

On the other hand, in skim milks with and without honey, the percentages of furfuryl alcohol (8.45%) and 2,3 Butanediol (6.54%), respectively, were higher than that without honey, on the contrary, the percentages of acetic acid (21.41%), 2-Propanone (3.10%), and Butanal (2.24%) in the same milks were lower than that without honey. Furthermore, the volatile compounds of 2-Furanmethanol (3.33%), 4H-Pyran-4-one (11.37%), 2-Butanone (2.37%), Butanal (8.90%) and 1,2,3-Propanetriol (3.30%) were produced in skim honey milks, on the contrary, in these milks, there were no volatile compounds of n-Pentanal, Fluoroethylene and Isopropyl alcohol in which these compounds occurred in skim milks without honey. The percentages of volatile compounds of skim and whole milks with and without honey at storage 10 days after use by date were shown in Table 5.

**Table 4**. The contents of amino acids of skim and whole milks at 10 days after the use by date (mg/100 mL)

| No. | Amino acids | Whole Milk | Whole honey milk | Skim Milk | Skim honeymilk |
|---|---|---|---|---|---|
| 1 | Aspartic acid | 0.465 c-e | 0.393 c-f | 0.354 c-g | 0.402 c-f |
| 2 | Glutamic acid | 1.069 a | 0.986 a | 0.943 a | 0.879 a |
| 3 | Serine | 0.341 c-g | 0.282 e-j | 0.293 e-I | 0.262 e-k |
| 4 | Glycine | 0.068 r-x* | 0.042 yz* | 0.069 r-x | 0.069 r-x |
| 5 | Histidine | 0.114 o-s | 0.068 r-x | 0.100 p-u | 0.074 r-x |
| 6 | Arginine | 0.112 p-u** | 0.199 g-o** | 0.202 g-o | 0.149 j-p |
| 7 | Threonine | 0.245 e-m* | 0.045 w-y* | 0.054 u-y | 0.044 w-y |
| 8 | Alanine | 0.414 c-f | 0.397 c-f | 0.311 e-I | 0.425 c-f |
| 9 | Proline | 0.171 I-p** | 0.313 d-h** | 0.280 e-j | 0.257 e-l |
| 10 | Tyrosine | 0.077 q-v* | 0.047 w-y* | 0.120 o-s* | 0.051 v-y* |
| 11 | Valine | 0.038 x-z** | 0.109 o-t** | 0.146 k-p | 0.094 p-u |
| 12 | Methionine | 0.050 v-y | 0.051 v-y | 0.064 s-x | 0.044 w-y |
| 13 | Cysteine | 0.118 o-t | 0.060 t-y | 0.064 s-x | 0.045 w-y |
| 14 | Isoleucine | 0.406 c-f* | 0.140 k-p* | 0.161 i-p | 0.137 l-q |
| 15 | Leucine | 0.463 c-e** | 0.700 a-b** | 0.588 a-d | 0.608 a-c |
| 16 | Phenylalanine | 0.224 f-n | 0.250 e-m | 0.264 e-k | 0.242 e-m |
| 17 | Lysine | 0.123 n-r* | 0.023 z* | 0.133 m-p* | 0.072 u-y* |

Note : The different one of the letters shows significantly different(P<0.05)
* : significantly different (P<0.05)
** : the increase amino acid was significantly different (P<0.05)

In general, bacterial growth and protein degradation of skim and whole honey milks at times of storage 5 days before up to 10 days after use by date, were lower than that without honey ($P<0.05$). The bacterial growth and protein degradation of whole honey milks were lower than that skim honey milks ($P<0.05$). Further–more, there were the higher number of amino acids of whole milks which changed its contents, before and after adding honey, at storage 10 days after use by date, than that of skim milks. Moreover, honey may result in producing volatile compounds of skim and whole milks at storage 10 days after use by date. It can be concluded that based on decreasing bacterial growth and protein degradation, the number amino acids which changed its contents, and the volatile compounds produced at storage 10 days after use by date, whole honey milks were better than skim honey milks. The reason why whole honey milks were better than skim honey milks at the storage, may be because the higher lipid contents of whole honey milks may result in the more protection in attacking psychrotrophic bacteria in whole honey milks than that in skim honey milks. As a result, the decrease bacterial growth and protein degradation in whole honey milks at storage were lower than that in skim honey milks, and the number amino acids which changed its contents, and volatile compounds produced were higher in whole honey milks at storage than that in skim honey milks.

**Table 5.** The percentages of volatile compounds of skim milks with and without the addition of honey at 10 days after use by date (%)

| No | Compounds | Whole milks (%) | Whole honey milks (%) | Skim milks (%) | Skim honey milks (%) |
|---|---|---|---|---|---|
| 1 | 2-Benzyloxyethylamine | 0.00 | 17.63* | 0.00 | 0.00 |
| 2 | Acetic acid | 41.44 | 37.32 | 34.00 | 21.41 |
| 3 | Furfuryl alcohol | 3.88 | 7.44 | 6.65 | 8.45 |
| 4 | 2-Heptanol | 0.00 | 3.07* | 0.00 | 0.00 |
| 5 | 2 (3H)-Furanone | 2.13 | 2.12 | 2.31 | 2.10 |
| 6 | n-Pentanal | 4.62 | 4.66 | 6.01 | 0.00 |
| 7 | Benzoic acid | 0.00 | 3.41* | 0.00 | 0.00 |
| 8 | N-Pentanal | 0.00 | 8.97* | 0.00 | 0.00 |
| 9 | Formic acid | 0.00 | 0.00 | 0.00 | 8.90* |
| 10 | 2,3 Butanediol | 0.00 | 0.00 | 2.04 | 6.54 |
| 11 | 2-Furanmethanol | 0.00 | 0.00 | 0.00 | 3.33* |
| 12 | 2-Propanone | 0.00 | 0.00 | 7.50 | 3.10 |
| 13 | Butanal | 0.00 | 0.00 | 6.65 | 2.24 |
| 14 | Maltol | 0.00 | 0.00 | 2.36 | 2.52 |
| 15 | 4H-Pyran-4-one | 0.00 | 0.00 | 0.00 | 11.37* |
| 16 | 2-Butanone | 2.60 | 0.00 | 0.00 | 2.37* |
| 17 | Butanal | 0.00 | 0.00 | 0.00 | 8.90* |
| 18 | 1,2,3-Propanetriol | 0.00 | 0.00 | 0.00 | 3.30* |
| 19 | Fluoroethylene | 5.09 | 0.00 | 17.65 | 0.00 |
| 20 | Isopropyl alcohol | 14.53 | 0.00 | 7.79 | 0.00 |
| 21 | 1,2-Pentanol | 4.54 | 0.00 | 0.00 | 0.00 |
| | Total percentages | 78.83 | 84.62 | 92.96 | 84.53 |

*. volatile compounds' produced in honey milks

## DISCUSSION

The lower bacterial growth and percentages of protein degradation in skim and whole milks with honey than that without honey, at the times of storage 5 days before up to 10 days after use by date, may be because the activities of antibacterial compounds in honey may resulted in the inhibition of psychrotrophic bacterial growth in the stored milks, and at the same times, there were the decrease of the protease activities in protein degradation of skim and whole honey milks, on the contrary, there were no activities of the antibacterial compounds in skim and whole milks. Furthermore, the lower bacterial growth and percentages of protein degradation in whole honey milks than that of in skim honey milks may be because there were the lipid contents in whole honey milks which had more protection in attacking psychrotrophic bacteria and protein degradation than that in skim honey milks. It has been reported that antibacterial compounds of "inhibine" and specific honey aromatic compounds (Bogdanox, 1997; Lusby *et al.* 2005) may inhibit the bacterial growth of psychrotrophic bacteria. The inhibition of the bacteria may result in the decrease in protein degradation of milks at storage. Bacterial growth and protease activities of whole milks at storage were lower than that of skim milks (Janzen *et al.*, 1982; Deeth *et al.*, 2000). The lower protease activities in whole milks than that in skim milks at storage may result in the lower protein degradation in whole milks than that in skim milks at storage (Griffith, 1989; Sorhaug and Stepaniak, 1997)

At storage in use by date, there were generally no differences between amino acid contents of both whole milks with and without addition of honey and that of skim milks. No differences in amino acid contents of pasteurized milks between that with and without addition of honey at use by date may be because at storage, there were no change in total amino acids of milks with and without honey. Beside, the bacterial growth of psychotrophic bacteria of the milks at use by date was starting, so, there were the low activities of psychrotrophic bacteria in protein degradation. Moreover, the protein degradation in the milks for storage at use by date may not result in the change of the contents of amino acids. As a result, there were no differences in amino acids contents between skim and whole milks with and without addition of honey at the use by date. It has been reported that psychrotrophic bacteria grew at refrigerated temperatures and these bacteria spoiled pasteurized milks at storage, mainly in degradation of milks protein (Chandler *et al.*, 1990; Reinheimer *et al.*, 1993).

At storage 10 days after use by date, the higher contents of some amino acids in whole milks with honey than that without honey may be because there were antibacterial compounds in honey which may inhibit the growth of psychrotrophic bacteria in milks at storage to reduce nutritional degradation, and at the same times, there were amino acids' compounds from honey which may contributed in increasing amino acids' contents in whole honey milks. It has been reported that there were various types of honey which contain the differences in the contents of amino acids Gonzalez Paramas *et al.*, 2006) which may contributed in increasing the contents of amino acids in whole honey milks. On the contrary, the lower contents of some amino acids in whole milks with honey than that without honey may be because there were the growths of psychrotrophic bacteria in milks at storage to degrade protein compounds which may affect in decreasing amino acids' contents in whole honey milks. Furthermore, the lower contents of some amino acids in skim milks with honey than that without honey may be because no fat contents in skim milks which may resulted in decreasing of protection from psychrotrophic bacterial attack may affect in the decrease of the contents of amino acids in skim honey milks. It has been reported that the growth of psychrotrophic bacteria in milks at storage may resulted in the change of nutritional compounds of milks (Craven and Macauley, 1992; Allen *et al.*, 1989; Pieper and Timms, 1987).

In relation to amino acids of skim and whole milks at storage 10 days after use by date, the more significant in the change in the amino acids' contents between seventeen amino acids in whole milks at storage with and without honey than that in skim milks may be because there were the more protection in attacking the protease activities of psychrotrophic bacteria in whole honey milks which contain more lipid than that in skim honey milks. It has been reported that the contents of lipids in whole milks were higher than that of skim milks (Chandler *et al.*, 1990). Furthermore, the antibacterial compounds of "inhibine" and specific aromatic compounds in honey (Adcok, 1962; Mundo *et al.*, 2004) may resulted in the more effective antibacterial activities in inhibiting protease activities of psychrotrophic bacteria of whole milks at storage, than that of skim milks. The protease activities of whole milks in protein degradation were lower than that in skim milks at storage (Deeth *et al.*, 2002; Janzen *et al.*, 1982).

On the other point of view, at storage 10 days after use by date, the higher percentages of volatile compounds produced in whole milks with honey than that without honey may be because there were antibacterial compounds in honey which may inhibited the growth of psychrotrophic bacteria in milks at storage to reduce nutritional degradation, and at the same times, there were aromatic compounds from honey in milks which may contributed in synthesizing volatile compounds in whole honey milks. Furthermore, the lower percentages of volatile compounds produced in skim milks with honey than that without honey may be because there were no fat contents in skim milks which may resulted in decreasing of protection from psychrotrophic bacterial attack, as a results, percentages of volatile compounds produced in skim honey milks were lower than that in skim milks without honey. It has been reported that honey contain specific honey aromatic compounds (Radovic *et al.*, 2001) which may contributed in producing volatile compounds of skim honey milks.

In general, it can be concluded that honey supplements which contain antibacterial compounds of "inhibine" and specific honey aromatic compounds (Lusby *et al.* 2005; Mundo *et al.*, 2004) may resulted in decreasing bacterial growth and protein degradation, changing amino acids contents, and producing volatile compounds in skim and whole milks at storage for 10 days after use by date; and based on the different in these nutritional and volatile compounds, whole honey milks were better than skim honey milks.

## CONCLUSIONS

The bacterial growth and percentages of protein degradation in skim and whole milks with honey were lower than that without honey at the times of storage, and the bacterial growth and percentages of protein degradation in whole honey milks were lower than that in honey skim milks ($P<0.05$). At 10 days after use by date, the change in the amino acids' contents between seventeen amino acids in whole milks at storage with and without honey were more significant than that in skim milks ($P<0.05$). Furthermore, at 10 days after use by date, the percentages of volatile compounds produced in whole milks with honey were higher than that without honey, while that produced in skim milks vice verca. Honey supplements may resulted in decreasing bacterial growth and protein degradation, changing amino acids contents, producing volatile compounds in milks at storage, and whole honey milks were better than skim honey milks.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge to: (1) JSPS-RONPAKU for providing the scholarship, (2) Biodiversity Research Project, Research Center for Biology, Indonesian Institute of Sciences for partly the fund of this research, and (3) Ms Wati & Ms. Khamdanah for their technical assistances in analysis.

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