

## ABSTRACT

The effects of herbs on the changes in probiotic growth and metabolism in yoghurt during storage were studied. *Angelica sinensis*, *Codonopsis pilosula*, *Illicium verum*, *Lycium barbarum*, *Momordica grosvenori* and *Psidium guajava* water-extracts were co-incubated with pasteurized full cream milk containing *Lactobacillus acidophilus*, *Bifidobacterium*, *Streptococcus thermophilus* and *L.casei* as starter culture. The addition of herbal water extracts stimulated *Lactobacillus* sp. growth in *P. guajava*-yoghurt ( $126.3\pm26.3 \times 10^6$  CFU ml $^{-1}$ ) only on day 0 and in *L. barbarum*-yoghurt from 0 to 3 days ( $109.3\pm21.5$  to  $112.7\pm28.8 \times 10^6$  CFU ml $^{-1}$  respectively) of refrigerated storage. The growth of *S. thermophilus* were recorded higher than plain yoghurt for *A. sinensis*-yoghurt ( $53.0\pm9.00 \times 10^8$  CFU ml $^{-1}$ ), *C. pilosula*-yoghurt ( $37.8\pm22.19 \times 10^8$  CFU ml $^{-1}$ ) and *L. barbarum*-yoghurts (day 0 and day 6;  $97.0\pm19 \times 10^8$  CFU ml $^{-1}$  and  $42.9\pm4.8 \times 10^8$  CFU ml $^{-1}$  respectively). Total titratable acid (TTA) of *A. sinensis*-yoghurt ( $1.02 \pm 0.01$ ) was highest followed by *M. grosvenori*-, *L. barbarum*-, *I. verum*-, *C. pilosula*-, control- and *P. guajava*-yoghurts. All herbal-yoghurts had lower pH than plain-yoghurt during storage when they were incubated for 18 hours whereas *P. guajava*- and *L. barbarum*-yoghurts showed higher pH than control during storage for yoghurts when incubation was terminated at pH 4.5. The presence of *A. sinensis*, *I. verum* and *L. barbarum* caused an increase in peptide concentration in yoghurts especially on day 7 ( $28.6\pm0.4$ ,  $30.58\pm2.2$  and  $32.0\pm2.0$  mg/ml respectively) vs. control ( $25.42\pm1.1$  mg/ml). *A. sinensis* also significantly enhanced the total free amino acids ( $0.31\pm0.03$  to  $0.43\pm0.12$  mM) in water soluble nitrogen (WSN). IC<sub>50</sub> for evaluating anti- ACE activity of all yoghurts decreased with time to the minimum value by day 7 of storage followed by gradual increase in IC<sub>50</sub> values during the remaining period of storage. All herbal-yoghurts, except *C. pilosula*-yoghurt, showed lower IC<sub>50</sub> values than plain-yoghurt on day 21 of

storage. Specific activity was also lower on day 7 for all treatments but *P. guajava*- and *C. pilosula*-yoghurts extract had less effect to reduce enzyme activity on day 0. Densitometric integrated intensity from SDS-PAGE pattern of yoghurt proteins degradation showed that the first week had the greatest  $\alpha$ -casein and  $\beta$ -casein values followed by continuous declined in proteolysis as the storage time progressed. In conclusion adding selected medicinal herbs into yoghurt had the potential to enhance yoghurt fermentation, milk protein proteolysis and reduction in ACE-1 activity *in vitro*. These herbs may be used as functional additives for the enhancement of yoghurt nutritional and potential therapeutical values.

## ABSTRAK

Kesan herba-herba ke atas perubahan pertumbuhan dan metabolisme probiotik dalam yoghurt semasa penstoran telah dikaji. Ekstrak air *Angelica sinensis*, *Codonopsis pilosula*, *Illicium verum*, *Lycium barbarum*, *Momordica grosvenori* dan *Psidium guajava* telah diinkubasi bersama susu penuh krim terpasteur yang mengandungi *Lactobacillus acidophilus*, *Bifidobacterium*, *Streptococcus thermophilus* dan *L.casei* yang berfungsi sebagai kultur pemula. Penambahan ekstrak air herba-herba merangsang pertumbuhan *Lactobacillus sp.* di dalam yoghurt-*P. guajava* ( $126.3 \pm 26.3 \times 10^6$  CFU ml<sup>-1</sup>) hanya di hari 0 dan dalam yoghurt-*L. barbarum* dari hari 0 ke hari ke 3 ( $109.3 \pm 21.5$  to  $112.7 \pm 28.8 \times 10^6$  CFU ml<sup>-1</sup> masing-masing) penstoran dingin. Pertumbuhan *S. thermophilus* direkod lebih tinggi dari yoghurt control bagi yoghurt-*A. sinensis* ( $53.0 \pm 9.00 \times 10^8$  CFU ml<sup>-1</sup>), yoghurt-*C. pilosula* ( $37.8 \pm 22.19 \times 10^8$  CFU ml<sup>-1</sup>) dan yoghurt *L. barbarum* (hari 0 dan hari ke-6,  $97.0 \pm 19 \times 10^8$  CFU ml<sup>-1</sup> dan  $42.9 \pm 4.8 \times 10^8$  CFU ml<sup>-1</sup> masing-masing). Jumlah asid tertitrat (TTA) *A. sinensis*-yoghurt ( $1.02 \pm 0.01$ ) adalah tertinggi diikuti yoghurt-yoghurt *M. grosvenori*, *L. barbarum*, *I. verum*, *C. pilosula*, kontrol dan *P. guajava*. Kesemua yoghurt-yoghurt herba ber-pH lebih rendah dari yoghurt kontrol semasa penstoran bilamana inkubasi dilakukan selama 18 jam manakala yoghurt-*P. guajava* dan yoghurt-*L. barbarum* menunjukkan pH lebih tinggi dari kontrol semasa penstoran untuk yoghurt yang ditamatkan inkubasinya ketika pH = 4.5. Kehadiran *A. sinensis*, *I. verum* dan *L. barbarum* menyebabkan peningkatan kepekatan peptida dalam yoghurt terutamanya pada hari ke-7 ( $28.6 \pm 0.4$ ,  $30.58 \pm 2.2$  dan  $32.0 \pm 2.0$  mg/ml masing-masing) berbanding kontrol ( $25.42 \pm 1.1$  mg/ml). *A. sinensis* turut mempertingkatkan jumlah asid amino bebas ( $0.31 \pm 0.03$  to  $0.43 \pm 0.12$  mM) di dalam nitrogen larut “water” (WSN). IC<sub>50</sub> untuk activity ACE-1 bagi kesemua yoghurt berkurangan dengan masa ke tahap terendah menjelang hari ke-7 penstoran dingin

diikuti dengan peningkatan berdikit-dikit nilai IC<sub>50</sub> semasa baki tempoh penstoran. Kesemua yoghurt-yoghurt herba, kecuali yoghurt-*C. pilosula*, menunjukkan nilai IC<sub>50</sub> lebih rendah dari yoghurt-kontrol pada hari ke-21 penstoran. Aktiviti khusus adalah lebih rendah pada hari ke-7 bagi kesemua rawatan tetapi ekstrak yoghurt-*P. guajava* dan yoghurt-*C. pilosula* mempunyai kurang kesan merencatkan aktiviti enzim pada hari 0 penstoran. Intensiti kesepadan densitometrik dari pola degradasi protein yoghurt SDS-PAGE menunjukkan minggu pertama penstoran yoghurt mempunyai nilai-nilai tertinggi kasein- $\alpha$  dan kasein- $\beta$  diikuti dengan penurunan berterusan proteolisis bilamana tempoh penstoran meningkat. Kesimpulannya, penambahan herba-herba ubatan terpilih ke dalam yoghurt mempunyai potensi meningkatkan fermentasi yoghurt, proteolisis protein dan mengurangkan aktiviti *in vitro* ACE-1. Herba-herba yang dikaji ini boleh digunakan sebagai aditif berfungsi bagi meningkatkan nilai-nilai nutrisi dan terapi yoghurt.