ABSTRACT

Yogurt is a healthy food and it has become good candidate for fortification with many healthful ingredients including fruits, herbs, prebiotics, probiotics and omega-3 fatty acids. Whatever the fortification strategies, it is essential that the finished product retain a desirable taste, becomes more nutritious and has long shelf life. The present study investigated the benefits green tea would bring to increase yogurt properties by virtue of the high levels of flavonoids which can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals. The presence of green tea would however affect yogurt bacteria fermentation of milk and subsequently the typical fermentation products responsible for the physicochemical properties (e.g organic acids), nutritional (e.g. digested protein and carbohydrate) and functional (e.g. bioactive peptides, organic acids, viable lactic acid bacteria) values of yogurt. On the other hand changing acidic environment and microbial metabolic activities are expected to exert profound effects on the breakdown of green tea phytochemical contents and subsequently any biological activities associated with these compounds. Milk was incubated (41°C) with starter culture (SC) in the presence of two types of green tea (MGT and JGT) until pH of yogurt reached 4.5. The resulting green-tea-yogurts were evaluated with respect to changes in antioxidant activity, phenolic compounds, the growth of lactic acid bacteria (LAB), acidification, exopolysaccharide (EPS) content, organic acids and rheological characteristics of yogurt during fermentation and storage at 4°C. The total phenolic content (TPC) was higher in MGT than those from JGT leaves infusion. Major phenolic compounds (gallocatechin, epicatechin, epigallocatechin gallate, quercetin-rhamnosylgalactoside, and kaempferol-3-O-rutinoside) in MGT leaves infusion were higher in those JGT leaves infusion. Diphenyl picrylhydrazyl (DPPH, %) and ferric reducing antioxidant power (FRAP, mmol /L) methods were used to evaluate the antioxidant capacity of yogurts. Inhibition of DPPH oxidation was highest for MGTY followed by JGTY and PY whereas FRAP value showed highest ($p>0.05$) ferric reduction power for MGTY followed by JGTY and PY. Viable *S.thermophillus* and *Lactobacillus spp.* counts in yogurt were highest in MGTY followed by JGTY and PY. Proteolysis in green tea yogurts increased with increasing fermentation time resulting in highest OPA

values for MGTY followed by PY at pH 4.5. Viable cells count (VCC) of S. *thermophilus spp.* increased by day 7 of storage with highest number present in MGTY followed by JGTY and PY. *Lactobacilus* spp. VCC decreased gradually for all yogurts during the 28 days of storage. Maximum DPPH inhibition by yogurts was shown on day 7 of storage by MGTY followed by JGTY and PY. The addition of green tea increased the FRAP values in MGTY which was 3 fold higher than JGTY and PY. Four major compounds were detected in green tea yogurts (quercetin-rhamnosyl, gallocatechin, kaempferol-3-rutinoside and epicatechin) with higher amounts present in MGTY than in JGTY but these tend to diminish by 28 days of refrigerated storage. The presence of green tea resulted in higher score recorded for overall appearance, colour, aroma and flavor in MGTY and JGTY compared with PY. However green tea yogurts showed more fluid like characteristics with distinct pseudo plastic properties and lesser ability to resist deformation upon applied shear. The presence of green tea during fermentation of milk increased the radical scavenging activities of yogurt and it stimulated the growth of both *S. thermophillus* and *L. bulgaricus* resulting in increased proteolysis of milk protein, acidification, enzymes activity and organoleptic properties. The catechin-related compounds, despite being not stable during refrigerated storage and had negative effects on rheology, have promising biological effects in increasing the nutritional and functional properties of yogurt.

ABSTRAK

Yogurt adalah makanan yang sihat dan ia menjadi perantara yang baik diperkaya dengan pelbagai ramuan seperti buah-buahan, herba, prebiotik, probiotik dan asid lemak omega. Teramatlah penting, walau apa sahaja strategi pemerkayaan, memastikan produk terbentuk itu menjadi lebih berkhasiat dan tahan lama dalam simpanan. Kajian ini menyelidik manfaat-manfaat yang boleh dibawa teh hijau dalam usaha meningkat kandungan yogurt hasil kebaikan flavonoid tinggi yang boleh melindungi sel-sel dan tisu-tisu dari kerosakan oksidatif akibat radikal-radikal bebas oksigen. Kehadiran teh hijau ini bagaimanapun akan mempengaruhi penapaian susu dan seterusnya produk-produk penapaian lazim bertanggungjawab ke atas ciri-ciri kimia-fizik (contoh: asid-asid organik), nilai-nilai nutrisi (contoh: kehadaman protein dan karbohidrat) dan fungsi (seperti peptida-peptida bioaktif, asid-asid organik, bakteria laktik asid hidup) yogurt.Sebaliknya perubahan sekitaran berasid dan aktiviti-aktiviti metabolik mikrob dijangka memberi kesan-kesan jelas ke atas peleraian kandungan fitokimia teh hijau dan seterusnya aktiviti-aktiviti biologi berkaitan sebatian-sebatian ini. Susu dieram (41^0C) dengan bakteria pemula dalam kehadiran dua jenis teh hijau (MGT dan JGT) sehingga pH yogurt mencecah 4.5. Yogurt-teh hijau terhasil telah dinilai merujuk kepada perubahan dalam aktiviti antioksidan, sebatian-sebatian fenolik, pertumbuhan bakteria asid laktik, pengasidan, kandungan eksopolisakarida, asid-asid organik dan ciri-ciri reologi yogurt semasa penapaian dan penyimpanan pada 4^0C. Jumlah kandungan fenol adalah lebih tinggi dalam MGT berbanding daripad JGT.Sebatian fenolik utama (gallocatechin, epicatechin, epigallocatechin gallate, quercetin-rhamnosylgalactoside, kaempferol-3-O-rutinoside) dalam MGT adalah lebih tinggi daripada yang diperolehi dalam JGT. Kaedah-kaedah difenil pikrilhidrazil (DPPH,%) dan kuasa pengurangan antioksidan ferrik (mmol / L) diguna untuk menilai kapasiti antioksidan yogurt. Perencatan oksidasi DPPH adalah tertinggi untuk yogurt-MGT (MGTY) diikuti yogurt-JGT (JGTY) dan yogurt-biasa (PY) (39.18 ± 0.77, 31.19 ± 0.14 dan 17.43 ± 0.21% masing-masing) manakala nilai-nilai FRAP menunjukkan kuasa penurunan ferrik tertinggi bagi MGTY diikuti JGTY dan PY . *S. thermophillus* dan *Lactobacillus spp*. hidup dalam yogurt adalah tertinggi dalam MGT ($119.1\pm0.98x10^6$ and 15.21 ± 0.70 $x10^8$cfu ml^{-1} masing-masing) diikuti oleh JGT (121.34 ± 1.43 $x10^6$ and 11.06 ± 1.7 $x10^8$ cfu ml^{-1} masing-masing) dan PY (104.65 ± 2.7 $x10^6$ and

6.17±2.5 x10^{8}cfu ml^{-1} masing-masing). Proteolisis dalam yogurt-yogurt teh hijau meningkat seiringan dengan peningkatan masa penapaian menyebabkan kepekatan nilai OPA tertinggi ($p < 0.05$) bagi MGTY diikuti JGT dan PY (22.4 ± 0.5, 18,11 ± 0,23 dan 9,22 ± 1.0 mg / mL masing-masing) di pH 4.5. Kiraan sel hidup (VCC) S. *thermophilus spp.* meningkat menjelang simpanan dingin hari ke-7 dengan bilangan tertinggi dalam MGTY diikuti JGTY dan PY (138.1±0.48, 129.34±0.87 and 110.22±0.99 x 10^{6} cfu ml^{-1} masing-masing).VCC *Lactobacillus spp.* berkurangan secara beransur-ansur untuk semua jenis yogurt sepanjang tempoh 28 hari simpanan dingin. Perencatan maksima DPPH oleh yogurt berlaku dihari ke-7 simpanan bagi MGTY (42.23±1.5%) diikuti JGTY dan PY (37.11± 1.15% and 24.19± 2.01% masing-masing). Tanbahan teh hijau meningkatkan nilai-nilai FRAP bagi MGTY (14.19± 3.67 mmol/L) adalah bersamaan 3 kali ganda dari JGTY (3.79 ± 1.06 mmol/L) berbanding PY (1.25± 0.45 mmol/L). Empat sebatian utama (quercetin-rhamnosyl, gallocatechin, kaempferol-3-rutinoside dan epicatechin) telah dikesan dalam sampel yogurt teh hijau dengan kepekatan tertinggi didapati dalam MGTY berbanding dengan JGTY tetapi sebatian-sebatian ini cenderung menghilang menjelang 28 hari simpanan yogurt. Kehadiran teh hijau dalam yogurt menyebabkan skor lebih tinggi untuk penampilan keseluruhan, warna, aroma dan rasa bagi MGTY dan JGTY berbanding dengan PY. Bagaimanapun yogurt-yogurt teh hijau menunjukkan lebih ciri-ciri kecairan dengan ciri-ciri plastik-pseudo dan kurang keupayaan merintang deformasi apabila dikenakan ricihan. Kehadiran teh hijau semasa penapaian susu meningkatkan aktiviti-aktiviti pengaut radikal yogurt dan merangsang pertumbuhan kedua-dua *S. thermophillus* and *L. bulgaricus* menyebabkan meningkatnya proteolisis protein susu, pengasidan dan ciri-ciri organoleptik. Sebatian-sebatian berkait-katekin, walaupun tidak stabil semasa simpanan dingin dan memberi kesan negatif ke atas reologi, mempunyai potensi kesan-kesan biologi dalam meningkatkan ciri-ciri nutrisi dan fungsi yogurt.

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TABLE OF CONTENET

CHAPTER 3: MATERIALS AND METHODS

CHAPTER 6: EFFECTS OF GREEN TEA (*Camellia sinensis*) ON ANTIOXIDANT PROPERTIES OF YOGURT DURING STORAGE AS EVALUATED BY CHANGES IN POLYPHENOLIC COMPOUNDS CHARACTERISTICS BY LC-MS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION

˚C	Degree Celsius
%	Percentage
µ	Micro
cfu	Colony forming unit
Da	Dalton
dH2O	Distilled water
DPPH	1,1-diphenyl-2-picrylhydrazyl
DRY	radical inhibition
EPS	Exopolysaccharide
etc	et cetera
FRAP	Ferric reducing/antioxidant power
g	Gram
G′	Storage modulus
G□″	Loss modulus
GAE	Gallic acid equivalents
H	Hour
HCL	Hydrochloride acid
Hz	Hertz
HPLC	High performance liquid chromatography
i.e.	For example
JGT	Japanese Green Tea

JGTY	Japanese Green Tea Yogurt
L	Litre
LAB	Lactic Acid Bacteria
Lb	Lactobacillus delbrueckii ssp. Bulgaricus
Lactobacillus spp	L. acidophilus,L. bulgaricus ,L. casei ,L. delbrueckii,L. fermentum,L. plantarum,L. reuteri
LC-MS	Liquid Chromatography-Mass Spectrometry
MGT	Malaysian Green Tea
MGTY	Malaysian Green Tea Yogurt
min	Minute
mL	Millilitre
mmol	Millimol
nm	Nanometer
OPA	O-Phthalaldehyde
pH	Hydrogen ion concentration
rad/s	Radian per second
rpm	Revolutions per minute
ROS	Reactive Oxygen Species
ssp	Subspecies
TA	Titratable acid
TPC	Total Phenolic Content
UV	Ultra violet
VCC	Viable Cell Counts

V	volume (s)
WHC	Water holding capacity
WHO	World Health Organization
Wt	Weight
w/v	Weight per volume

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