BLACK TEA WATER KEFIR BEVERAGE

A Thesis submitted in partial fulfilment of the requirement for the degree of
Master of Food Technology

Massey University
Albany, New Zealand

Maria Vina Kania Subardjo
2017

ABSTRACT

Fermented foods and beverages play an important role in the human diet as they provide essential nutrients as well as contribute towards prevention of diseases. Lactic acid bacteria and yeasts are a major group of microorganisms associated with fermented products. Some of the microorganisms, known as probiotics, confer health properties to human health. Thus, many different types of fermented foods and beverages containing probiotics are produced around the world to support wellness and health. In recent years, there has been increased interest in the development of fermented functional plant-based foods and beverages due to a surge in scientific research of the products. Further, there is evidence that probiotic microorganisms can grow well in plant-based substrates.

Water kefir is a sparkling fermented beverage with an acidic, sweet, slightly alcoholic taste, and a yeasty flavour. Water kefir fermentation can be achieved by the inoculation of water kefir grains as a starter culture into a solution containing sugar. Kefir grains consist of a symbiotic starter culture of lactic acid bacteria (LAB) and yeasts contained in a polysaccharide matrix. Microorganisms present in kefir grains are recognized as probiotics. The majority of previous studies have focused on the isolation and identification of water kefir cultures responsible for fermentation. There is, therefore, scanty information on the fermentation of plant-based water kefir beverages. The main objective of this study was to develop fermented black tea beverage using water kefir grains as a starter culture.

Fermentation of black tea infusions as single and mixed substrate with carrot juice using water kefir grains were investigated. Microflora of water kefir grains used consisted of symbiotic starter culture of lactic acid bacteria (*Lactococcus* spp. and *Lactobacillus* spp.) and a yeast (*Saccharomyces cerevisiae*). The study was conducted in three main phases. The first phase investigated the effect of sucrose concentration (5% and 10%) and fermentation temperature (25°C and 30°C) in black tea water kefir fermentation for 72 h. Meanwhile, the effect of added carrot juice (5%, 10%, and 15%) on kefir beverage during secondary fermentation (24 h) at 25°C was investigated in the second phase. The stability of the final black tea water kefir beverage formulation during storage (4°C) for four weeks was investigated in phase three. Samples of black tea water kefir beverages were subjected to various analyses during fermentation and storage (4°C) for 4 weeks: titratable acidity, total soluble solids (°Brix), colour, viable cell counts of constituent starter culture, sensory evaluation, sugars, organic acids, antioxidants, and pH was also measured.

Results showed that fermentation temperature, sugar concentration, and carrot juice concentration contributed to the physico-chemical and microbiological characteristic as well as sensory properties of the product. In phases one and two, pH and total soluble solids (°Brix) decreased, while titratable acidity and cell counts of LAB and yeasts increased during fermentation of the products. LAB and yeasts were able to grow in black tea and addition of carrot juice into the beverages slightly increased their growth. The best fermentation conditions based on physico-chemical and sensory properties were kefir beverage containing sugar (10%) and carrot juice (10%) fermented at 25°C for 96 h. In phase three, the growth and survival of *Lactococcus* spp. and *Lactobacillus* spp. were low during storage of the product (4°C) while *Saccharomyces cerevisiae* maintained high cell numbers (7.03±0.07 log cfu/ml) at the end of storage (28 days). Results showed the possibility to produce low sugar water kefir beverage containing 0.08±0.01% (w/v) sucrose, 1.55±0.04% (w/v) glucose, and 2.93±0.20% (w/v) fructose. The fermented kefir beverage also contained 0.202±0.02% (w/v) lactic acid, 0.114±0.03% (w/v) acetic acid and some antioxidants (gallic acid, ECG, EGC, EGCG, theobromine and caffeine) which may be beneficial to human health. There was significant difference ($p<0.05$) in the colour (L*, a*, b*) of the fermented beverages during storage (4°C).

Black tea water kefir beverage containing 10% sugar and 10% carrot juice fermented at 25°C for 96 h was well-liked by consumer sensory panellists.

ACKNOWLEDGEMENTS

It has been a great experience for me to complete my Master of Food Technology degree at Massey University. I am grateful and thank God Jesus that I could finish my postgraduate on time. I acknowledge the invaluable contribution of those who made this thesis possible. This project was made possible by the support given by Massey University who provided financial support, and other people.

I would like to thank:

Dr. Tony Mutukumira, my supervisor, who believed in me and gave me unlimited support, training, guidance, and encouragement during my study. It has been an honour to work with him and, I appreciate all his invaluable contributions and ideas as well as the opportunity to help me conduct the research;

Associate Prof. Marie Wong for HPLC training and her invaluable knowledge in the analysis of sugars and organic acids;

Dr Sung Je Lee for his expertise in the characterisation of food and teaching me the measurement of colour by spectrophotometry;

Dr. Daniel Walsh for his expertise and advice in statistical analysis;

Dr. Evelyn Sattleger and Associate Prof. Kay Rutherfurd for their guidance and passion in the subject matter;

Rachel Liu, PC Tong, and Kenneth Teh for training in microbiology and analytical techniques in chemistry as well as general assistance in the laboratory; and,

Last but not least, I would like to thank my parents, my boyfriend, Georgius Prihantoro, and friends for their endless support and kindness.

LIST OF ABBREVIATIONS

a*	=	redness-greenness
AAB	=	Acetic acid bacteria
ACK	=	Acetate kinase
Acs	=	Acetyl-coenzyme A synthetase
Adh	=	Alcohol dehydrogenase
ADP	=	Adenosine diphosphate
Ald	=	Acetaldehyde dehydrogenase
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemist
ATP	=	Adenosine triphosphate
B	=	Bifidobacterium
b*	=	yellowness-blueness
BOP	=	Broken orange pekoe
BT	=	Black tea
CFU	=	Colony forming per Unit
CTC	=	Crush-Tear-Curl
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
EC	=	Epicatechin
ECG	=	Epicatechin gallate
ECP	=	Endless chain pressure
EGC	=	Epigallocatechin
EGCG	=	Epigallocatechin gallate
EMP	=	Emden-Meyerhoff-Parnas
EPS	=	Exopolysaccharides
F	=	Fanning
FADH	=	Flavin adenine dinucleotide
FAO	=	Food and Agriculture Organization
FBD	=	Fluid bed dried
FSANZ	=	Food Standards Australia New Zealand
g	=	Gram
GABA	=	Gamma-Amino Butyric Acid

GAP	=	Glyceraldehyde-3P
GRAS	=	Generally Recognized As Safe
GC	=	Gas chromatography
GI	=	Gastrointestinal
H	=	Hour
HIV	=	Human Immunodeficiency Virus
HPLC	=	High performance liquid chromatography
L	=	Litre
L*	=	Lightness
LAB	=	Lactic acid bacteria
Lb.	=	Lactobacillus
Lc.	=	Leuconostoc
LDH	=	Lactate dehydrogenase
Min	=	Minute
ml	=	milliliter
mg	=	milligram
mm	=	millimeter
NaCl	=	Sodium chloride
NAD	=	Nicotinamide adenine dinucleotide
NADH	=	Nicotinamide adenine dinucleotide hydride
OP	=	Orange pekoe
ORS	=	Oral rehydration solution
OXPHOS	=	Oxidative phosphorylation
PCR	=	Polymerase chain reaction
Pdc	=	Pyruvate decarboxylase
Pdh	=	Pyruvate-dehydrogenase complex
PFL	=	Pyruvate formate-lyase
PKP	=	Phospho-ketolase pathway
PTA	=	Phospho-trans-acetylase
SD	=	Standard deviation
spp	=	species (plural)
TSS	=	Total soluble solids
T.A.	=	Titratable Acidity
TCA	=	Tricarboxylic acid

TF	=	theaflavins
TFA	=	trifluoroacetic acid
TR	=	thearubigins
MRS	=	de Man, Rogosa and Sharpe
VOC	=	Volatile Organic Compounds
v/v	=	volume per volume
WHO	=	World Health Organization
w/v	=	weight per volume
YGC	=	Yeast Glucose Chloramphenicol

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES