

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

MASSEY UNIVERSITY
APPLICATION FOR APPROVAL OF REQUEST TO EMBARGO A THESIS
(Pursuant to AC 98/168 (Revised 2), Approved by Academic Board 16.02.99)

This form is to be used when requesting a Thesis embargo. Once this form has been completed and signed by the Chief Supervisor it needs to be submitted to Wendy Dixon, Administration Manager, Graduate Research School, PN 713, to process for approval.

Name of Candidate: Tejal Nikhil Kolte _____ ID Number: 12162227 _____

Degree: MFoodTech _____ Dept/Institute/School: IFNHH _____

Thesis Title: Development of an acceptable, stable and safe nitrate-rich vegetable juice beverage.

Name of Chief Supervisor: A/Pro Marie Wong _____ Telephone Extn: 41204 _____

As author of the above named thesis, I request that my thesis be embargoed from public access

until (date) 30 August 2016 _____ for the following reasons:

- ☐ Thesis contains commercially sensitive information.
- ☐ Thesis contains information which is personal or private and/or which was given on the basis that it not be disclosed.
- ☐ Immediate disclosure of thesis contents would not allow the author a reasonable opportunity to publish all or part of the thesis.
- ☒ Other (specify): The thesis contains important IP knowledge that the research supervisory team would like to keep confidential at this point in time.
- _____

Please explain here why you think this request is justified:

The team of supervisors involved with this project would like the information and the formulation developed during this project to be kept confidential to allow the team enough time to continue research with it and to develop a commercial product to sell as part of a new Beverage Innovation project at Massey. We would also like to develop the process to produce the product on a commercial scale.

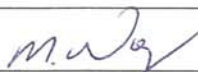
Signed (Candidate):



Date:

30/05/2014

Endorsed (Chief Supervisor):



Date:

30/5/2014

Approved/Not Approved (Representative of VC):



Date:

1/07/2014

Note: Copies of this form, once approved by the representative of the Vice-Chancellor, must be bound into every copy of the thesis.

Development of an acceptable, stable and safe nitrate-rich vegetable juice beverage

A thesis presented in partial fulfilment of the
requirements for the degree of

Master of Food Technology

at Massey University, Albany, New Zealand.



Massey University

Tejal Nikhil Kolte

2014

ABSTRACT

Ingestion of nitrates from a vegetable juice beverage has been reported to improve exercise performance. The research was therefore conducted to produce a vegetable juice beverage with stable nitrate content that could potentially enhance sports activity. In this study, a placebo drink was also produced with low nitrate content and to match the taste and quality parameters of the high nitrate juice beverage.

Juice was extracted from beetroot, pasteurised at $90\pm 1^{\circ}\text{C}$ for 15 s and blended with other ingredients and further tested for pH, titratable acidity, total soluble solids, nitrate and nitrite content and microbial counts. A sensory evaluation trial was conducted on four finalised juice blends along with the commercial product on the market. *Orange flavour low acid* beetroot juice beverage (1572 ± 5 mg nitrate/L) was preferred formulation than the commercial juice beverage, BEET IT.

A shelf life trial, using a full factorial experimental design, was used to determine the effect of temperature ($4\pm 1^{\circ}\text{C}$ and $20\pm 1^{\circ}\text{C}$) and storage conditions (light or dark storage) on *orange flavour low acid* beetroot juice beverage. From the storage trial, the *orange flavour low acid* beetroot juice beverage containing more than 1500 mg nitrate/L, can be stored in transparent bottles and safely consumed after eight weeks storage if stored at $4\pm 1^{\circ}\text{C}$.

The sensory results obtained from performing the triangle test on the *orange flavour low acid* formulation (standard beverage) and placebo drink suggested that only 28 % of the population could identify a difference between the two products. The placebo drink contained 181 ± 4 mg nitrate/L which was nine times less than the nitrate concentration in the standard beverage.

In conclusion, an acceptable high nitrate juice beverage was formulated with a corresponding low nitrate drink placebo drink which could not be differentiated by consumers after sensory testing. It is recommended to develop a commercial manufacturing procedure to produce the nitrate juice beverage from beetroot, beet leaves and celery juices from which larger batches of samples can then be tested for exercise performance.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, A/Prof Marie Wong, Dr Kay Rutherford, Dr John Grigor and Dr Ajmol Ali for their valuable advice, patience, guidance and support throughout this project. I would like to express my deepest appreciation to A/Prof Marie Wong for her academic support that made this thesis possible and for keeping me focused during the study. It has been an honour and a great pleasure to work under her guidance. I would also like to share how grateful I am to Dr Kay Rutherford for helping me out on the HPLC instrument and also allowing me to use one of the lab spaces in the Nutrition Unit building throughout the course of the study. It has been a great pleasure to run sensory trials and work on some statistics under Dr John Grigor.

A huge gratitude goes to Massey University for awarding me the Masterate scholarship for 2013-2014. Their financial support in my research made this possible. Thank you to Andrew Foskett for giving me a chance to demonstrate a physiology anatomy laboratory for one semester.

I would also like to extend my gratitude to:

Helen Mathews, Food Technology laboratory manager for inducting me to the Food labs and also investing time in training me through the laboratory juicer, titratable acidity procedure and UHT plant. Thank you Helen for exposing me to certain lab skills, attention to detail, finding reasons to questions asked and keeping me motivated throughout the year.

Rachael Liu, food technician, for her training to guide me through the microbiology laboratory and helping out with assembling consumer panellists during the sensory trials.

PC Tong, Nutrition Technician, for spending some time on my laboratory induction training and helping me operate the centrifuge.

My suppliers from Freshmax Ltd, Fresh connection Ltd, Bruce the juice for helping me with their fresh produce and the beetroot juice. I would also like to thank Directus Ltd,

Zymus Ltd, RD2 Ltd, Sensient Ltd for helping me with my lab work with some free samples needed for the project work.

I am indeed grateful to my colleagues and the administrative research staff at the Massey University, Albany for their support throughout the project duration. A special thank you to my friend and colleague, Sherina Holland, for her tolerance, assistance during the sensory trial, helpful advice, support and her valuable time in training me through the HPLC instrument.

My biggest 'thank you' to my lovely husband for his patience, tolerance, support and sacrifices throughout my study year. To my family who make life one worth living no matter how stressful it gets, thank you for all your love and support. I would also like to thank my gym instructors, gym buddies and husband to keep me motivated by making me eat healthy, exercise and stay focussed during the course of study.

TABLE OF CONTENTS

Abstract.....	i
Acknowledgements.....	ii
Appendices.....	x
List of Tables.....	xi
List of Figures.....	xiv
List of Abbreviations.....	xvi
CHAPTER 1 INTRODUCTION.....	1
CHAPTER 2 LITERATURE REVIEW.....	4
2.0 Nitrate, nitrite and nitrous oxide.....	4
2.1 Dietary sources of nitrates and nitrites.....	7
2.2	
Beetroot.....	13
2.2.1 Beetroot types and cultivars.....	14
2.2.1.1 Processing beetroots.....	14
2.2.1.2 Table beetroots.....	14
2.2.1.3 Novelty beetroots.....	14
2.2.1.4 Spinach beetroots.....	14
2.2.1.5 Swiss chard.....	14
2.3 Acceptable daily intake (ADI) of dietary nitrates and nitrites.....	15
2.4 Role of nitrates in sports performance and health markets.....	16
2.5 Nitrate health risk.....	27
2.6 Quantification methods to determine nitrates and nitrites.....	29
2.7 Factors affecting stability of nitrates in vegetables.....	34
2.7.1 Processing factors.....	34

2.7.1.1 Washing.....	34
2.7.1.2 Peeling.....	34
2.7.1.3 Cooking.....	35
2.7.1.4 Other methods of processing.....	35
2.7.2 Storage time and temperature.....	36
2.8 Extraction of juice from vegetables.....	40
2.8.1 Stages of commercial juice production.....	41
2.8.1.1 Harvest, transport and storage.....	41
2.8.1.2 Pre-treatments.....	41
2.8.1.3 Juicing stage.....	42
2.8.1.4 Post treatments.....	44
2.8.2 Methodologies for preservation of juice.....	45
2.8.2.1 Pasteurisation-High Temperature Short Time (HTST) processing.....	45
2.9 Juice safety.....	47
2.9.1 Fruits and vegetables.....	47
2.9.2 Microbial control factors.....	48
2.9.2.1 Heat treatments for microorganisms.....	49
2.9.2.2 Effect of freezing on microorganisms.....	50
2.10 Juice flavour perception.....	50
2.11 Nitrate-rich beverages for sports performance on market	52
2.12 Conclusions.....	56
CHAPTER 3 MATERIALS AND METHOD.....	57
3.1 Resources used for juice extraction and testing.....	57
3.2 Raw materials used.....	59
3.3 Quantification of nitrates and nitrites in juice using high pressure liquid chromatography (HPLC).....	59

3.3.1 Instruments and chemicals.....	59
3.3.2 Preparation of mobile phase.....	60
3.3.3 Sample analysis.....	60
3.4 Juice analysis- Quality parameters.....	62
3.4.1 pH.....	62
3.4.2 Total titratable acidity.....	62
3.4.2.1 Preparation of reagents.....	62
3.4.2.2Determination of Titratable acidity.....	63
3.4.3 Total soluble solids.....	64
3.5 Microbiological analysis of juice samples.....	64
3.6 Juice Production.....	65
3.6.1 Determination of nitrate rich vegetables in New Zealand.....	66
3.6.2 To determine the appropriate temperature for nitrate stability for sample storage.....	68
3.7 Extraction of juice from pilot scale quantities.....	68
3.8 Juice blends.....	69
3.8.1 Placebo formulation development.....	70
3.9 Sensory evaluation of juice blends.....	70
3.9.1 Sample preparation.....	71
3.9.2 Testing location.....	71
3.9.3 Panels and participants.....	72
3.9.4 Presentation of samples.....	72
3.9.5 Sensory evaluation form structure.....	72
3.9.6 Discrimination triangle sensory test.....	73
3.9.7 Statistical analysis of sensory data.....	73
3.10 Storage trial conditions and experimental design.....	75

3.10.1 Experimental design for storage trial.....	75
3.10.2 Storage conditions.....	76
3.10.3 Storage time.....	77
CHAPTER 4 JUICE EXTRACTION AND TESTING.....	78
4.1 Extraction of juice from nitrate rich vegetables in New Zealand.....	78
4.1.1 Informal sensory evaluation of juices.....	82
4.2 Impact of storage conditions on nitrate/ nitrite concentrations in vegetable juices..	82
4.3 Pilot plant trial on beetroot, celery and beet leaves juices.....	86
4.3.1 Pasteurisation of vegetable juices.....	88
4.3.2 Microbiological evaluation of vegetable juices.....	88
4.3.3 Quantification of nitrates and nitrites and evaluation of quality parameters of vegetable juices.....	91
CHAPTER 5 JUICE BLENDING & CONSUMER SENSORY EVALUATION.....	93
5.1 Factors to be considered before blending juices.....	93
5.2 Vegetable juice final formulation development.....	95
5.2.1 Vegetable juice blend and acidulant.....	95
5.2.2 Further development of formulation.....	99
5.3 Consumer sensory evaluation of formulated nitrate rich vegetable juices.....	103
5.4 Consumer sensory evaluation results.....	104
5.5 Statistical analysis of results.....	105
5.6 Conclusion.....	110
CHAPTER 6 SHELF LIFE TRIAL.....	112
6.1 Introduction.....	112
6.2 Standard juice beverage properties during storage trial	113
6.2.1 Juice beverage pH.....	113
6.2.2 Juice beverage titratable acidity.....	114

6.2.3 Juice beverage soluble solids content (°Brix).....	116
6.3 Microbiological growth in juice beverage during the storage trial.....	117
6.4 Quantification of nitrates (mg/L) and nitrites (mg/L) during the eight week storage trial.....	119
6.5 Changes in the colour and odour of the juice.....	122
6.6 Conclusion.....	126
CHAPTER 7 PLACEBO DRINK DEVELOPMENT.....	127
7.1 Development of placebo.....	127
7.2 Triangle sensory consumer test.....	130
7.2.1 Consumer triangle test results.....	131
7.2.1.1 Analysis of sensory results.....	131
7.3 Conclusion.....	132
CHAPTER 8 OVERALL DISCUSSION.....	133
8.1 Overall Discussion.....	133
8.2 Pilot scale production of juice.....	134
8.3 Sensory evaluation of juices.....	135
8.4 Juice in retail environment.....	136
8.4.1 Co-relation between pH, nitrate ad nitrite concentrations (mg/L) and microbial counts at 4±1°C and 20±1°C.....	136
8.5 Placebo formulation and consumer triangle sensory test.....	138
8.6 Consumption of nitrate rich beverage and safety.....	139
8.7 Nitrate rich products in the market besides juice.....	139
8.8 Cost of producing the final product formulation.....	141
CHAPTER 9 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH.....	143
9.1 Conclusions.....	143
9.2 Recommendations.....	144

REFERENCES.....146

APPENDICES.....175

APPENDICES

Appendix A: Specification sheets of ingredients.....	175
Appendix B: Questionnaires for two sensory trials.....	183
Appendix C: Forms used during sensory evaluation (participant consent form, participant information form and ethics committee approval form).....	190
Appendix D: Randomisation charts used for sensory trials.....	194
Appendix E: P values	197
Appendix F: Total Coliforms (cfu/ml) lab report from Assure Quality.....	198
Appendix G: Blending protocol using MINITAB.....	199
Appendix H: P values derived from Tukey's Test.....	202
Appendix I: Normality plots for five formulations (<i>apple flavour low acid</i> , <i>apple flavour high acid</i> , <i>orange flavour low acid</i> , <i>orange flavour high acid</i> and <i>BEET IT</i>) for four attributes (overall product liking, acidity liking, sweetness liking and flavour liking).....	203
Appendix J: Critical Values for Ryan-Joiner Test for Normality	204
Appendix K: Minimum number of correct responses needed to conclude that a perceptible difference exists based on a triangle test.....	205

LIST OF TABLES

Table 2.1: Classification of vegetables in Italy according to nitrate content (mg/kg).....	9
Table 2.2: Concentrations of nitrate and nitrite in different vegetables from the ‘Total Diet survey’ conducted in New Zealand (mg/kg fresh weight of sample; as sodium salt).....	10
Table 2.3: Comparison of NZ nitrate concentration in vegetables with the international data (mg/kg fresh weight basis).....	12
Table 2.4: Studies of nitrate supplementation in trained and untrained populations....	19
Table 2.5: High Pressure Liquid Chromatography (HPLC) methods for nitrate and nitrite determination.....	31
Table 2.6: Intrinsic and Extrinsic microbial growth factors.....	48
Table 2.7: Competitive nitrate rich beverages.....	53
Table 3.1: Resources used for juice production.....	57
Table 3.2: Resources used for juice testing.....	58
Table 3.3: Ingredients sourced for blending and placebo development	59
Table 4.1: Juice yield (mL juice/ g wet weight of vegetables) of top nitrate vegetables of New Zealand	78
Table 4.2: Nitrate and nitrite concentrations in extracted vegetable (beetroot, beet leaves, celery stalk, celery leaves, lettuce (iceberg) and spinach) raw and heated (90°±1°C) juices with p values.....	81
Table 4.3: Changes in nitrate (mg/L) concentration in beetroot juice stored at three different temperatures over time.....	83
Table 4.4: Changes in nitrite (mg/L) concentration in beetroot juice at three different temperatures over time.....	85

Table 4.5: The pH, titratable acidity and % soluble solids of raw beetroot juice over two weeks (Storage temperature = $-20\pm 1^{\circ}\text{C}$, Testing temperature = $25\pm 1^{\circ}\text{C}$).....	85
Table 4.6: Juice yield (mL juice/ g wet weight of vegetables) of beetroot, beet leaves and celery	86
Table 4.7: Microbiological test results for beetroot, beet leaves and celery juices before and after pasteurisation.....	89
Table 4.8: Nitrates (mg/L), Nitrite (mg/L), pH, titratable acidity and $^{\circ}\text{Brix}$ results of beetroot, beet leaves and celery juice from the pilot plant trial.....	91
Table 5.1: $^{\circ}\text{Brix}$ /Acid ratios of some commercial vegetable and fruit juices.....	94
Table 5.2: Formulation blends calculated to achieve nitrate concentration $\geq 1.5\text{g/L}$ and $10\text{-}11^{\circ}\text{Brix}$	97
Table 5.3: Overall product liking scores for vegetable juice formulations	98
Table 5.4: Formulation blending development II from MINITAB based on nitrate $\geq 1.5\text{g/L}$ and $10\text{-}11^{\circ}\text{Brix}$	101
Table 5.5: Overall product liking scores for batch II vegetable juice formulations.....	102
Table 5.6: Final formulations for consumer sensory evaluation.....	102
Table 5.7: Summary of quality parameters, nitrates (mg/L) and nitrites (mg/L) and microbial counts (TPCs and Y &M) of final formulation before sensory evaluation.....	103
Table 5.8: Mean scores of 'liking' for five formulations used for consumer sensory evaluation.	104
Table 5.9: Test statistic values of attributes for formulations.....	106
Table 5.10: ANOVA results for overall product liking	107
Table 5.11: ANOVA results for acidity, sweetness and flavour liking.....	108

Table 6.1: Microbiological test results for juice beverages stored at $20\pm1^{\circ}\text{C}$ and $4\pm1^{\circ}\text{C}$ under light and dark conditions.....	118
Table 7.1: Placebo formulations with their respective nitrate content (mg/L), total soluble solids ($^{\circ}\text{Brix}$) and titratable acidity g/100 ml (as citric acid) results.....	129
Table 8.1: Cost of ingredients for <i>orange flavour low acid</i> formulation.....	142

LIST OF FIGURES

Figure 2.1: The pathways of NO generation in humans	6
Figure 2.2: Formation of NO from nitrate-nitrite- NO pathway.....	7
Figure 2.3: HPLC chromatogram with nitrate and nitrite peaks of organic non-heading Chinese cabbage sample (B) and standard solution (A).....	30
Figure 2.4: Effects of ambient ($22\pm 1^{\circ}\text{C}$) storage on nitrate (upper) and nitrite (lower) concentrations of four species of vegetables over 1 week. A, spinach; B, crown daisy; C, organic Chinese spinach; D, organic non-heading Chinese cabbage.....	38
Figure 3.1: HPLC chromatograms of raw beetroot juice sample (B) and standard solution (A) containing 500 $\mu\text{g/ml}$ of sodium nitrate and sodium nitrite using the HPLC analytical method under condition for a mobile phase solution of 0.01 M octylammonium orthophosphate and adjusted to a pH value of 3.5.....	61
Figure 3.2: Process flow diagram for juice production.....	67
Figure 3.3: Process flow diagram post juice extraction.....	69
Figure 3.4: Storage trial full balanced factorial experimental design for refrigerated ($4^{\circ}\pm 1^{\circ}\text{C}$) and ambient ($20^{\circ}\pm 1^{\circ}\text{C}$) storage time in light and dark conditions.....	76
Figure 4.1: (A) Left photo: Beetroots purchased from supermarket (B) Right photo: Beetroots purchased from Freshmax NZ Ltd.....	87
Figure 4.2: (A) Left photo: Beet leaves/stalks purchased from supermarket (B) Right photo: Beet leaves purchased from Freshmax NZ Ltd.....	88
Figure 5.1: PCA bi-plot (top) and score-plot (bottom) for overall product liking for five sample formulations.....	109
Figure 6.1: pH values of juice beverage stored at $4\pm 1^{\circ}\text{C}$ and $20\pm 1^{\circ}\text{C}$ under light and dark conditions over an eight week storage period.....	113

Figure 6.2: Titratable acidity g/100 ml (as citric acid) of juice beverage at 4±1°C and 20±1°C under light and dark conditions over an eight week storage period.....	115
Figure 6.3: Soluble solids (°Brix) of juice beverage at 4±1°C and 20±1°C under light and dark conditions over an eight week storage period.....	116
Figure 6.4: Nitrate content (mg/L) of juice beverage at 4±1°C and 20±1°C under light and dark conditions over an eight week storage period.....	120
Figure 6.5: Nitrite content (mg/L) of juice beverage at 4±1°C and 20±1°C under light and dark conditions over an eight week storage period.....	121
Figure 6.6: Photos of beetroot juice beverages stored at 4±1°C and 20±1°C under light and dark conditions over a period of eight weeks.....	123
Figure 6.7: Shelf life trial summary of trends.....	125
Figure 7.1: Pie chart illustrating the results from triangle consumer sensory test.....	131
Figure 8.1: Nitrate rich products available in the market for exercise performance. Top left: ZipVit® gel, Top right: Pure clean beet juice powder, Bottom left: GoPlus® nitrate gel and bottom right: BEET IT® sports bar.....	141

LIST OF ABBREVIATIONS

°B/A	°Brix/Acid
ADI	Acceptable Daily Intake
H _a	Alternative Hypothesis
BW	Body weight
Ca ²⁺	Calcium
CIP	Cleaning In Place
CFU	Colony-Forming Unit
D	Decimal reduction time
DF	Degree of Freedom
EC	European Commission
EU	European Union
E.G.	Example
FAO	Food and Agriculture Organisation
FSANZ	Food Safety Australia New Zealand
GDP	Gross Domestic Product
HDPE	High Density Polyethylene
HPLC	High Pressure Liquid Chromatography
HTST	High Temperature Short Time
JEFCA	Joint Expert Committee of the Food and Agriculture
L	Lethal rate
F	Lethality
VO ₂	Maximal Oxygen Uptake
W _{max}	Maximal power
min(s)	Minutes
MAP	Modified Atmosphere Packaging
NF	Nano Filtration
NZ	New Zealand
NO ₃	Nitrate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NO ₂	Nitrite
N/A	Not applicable
H ₀	Null Hypothesis
%	Percentage
PET	Polyethylene Terephthalate
PCA	Principal component analysis
PEF	Pulsed Electric Field
RCF	Relative Centrifugal Force
RO	Reverse Osmosis
SCF	Scientific Committee On Food
TPC	Total Plate Count
TTE	Transthoracic Echocardiogram

UHT	Ultra High Temperature
UK	United Kingdom
WHO	World Health Organisation
Y & M	Yeasts And Mould