CASH: CORRELATING ANALYTICAL, SENSORY, AND HEDONIC DATA IN GREEN TEA

YU PEIGEN
(B.Appl.Sc. (Hons.), National University of Singapore)

A THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

FOOD SCIENCE AND TECHNOLOGY PROGRAMME
DEPARTMENT OF CHEMISTRY
NATIONAL UNIVERSITY OF SINGAPORE

2016

CONFIDENTIALITY DISCLAIMER

This study is part of a collaborative research with Givaudan Singapore Pte Ltd, an industrial partner. Sections containing confidential information have been omitted from this version of the thesis. For more information regarding the omitted sections, the reader is referred to either the author or the Principal Investigators (Professor Zhou Weibiao and Dr Low Mei Yin).

DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety, under the supervision of Professor Zhou Weibiao (Food Science and Technology Programme, Department of Chemistry, National University of Singapore) and Dr Low Mei Yin (Asia-Pacific Innovation Centre, Givaudan Singapore Pte Ltd), between 9 January 2012 and 15 July 2016.

I have duly acknowledged all the sources of information which have been used in the thesis. This thesis has also not been submitted for any degree in any university previously.

Yu Peigen

15 July 2016

ACKNOWLEDGEMENTS

This PhD journey has truly been a life-changing experience for me, and I would not have been able to complete it without the support and encouragement from my mentors, colleagues, friends, and family.

First and foremost, I would like to express my appreciation and thanks to my doctoral supervisors Professor Zhou Weibiao and Dr Low Mei Yin for their continued guidance and support throughout this journey. Your expertise and understanding contributed immensely to this experience, and I have learnt so much from the both of you through these years on matters both relating and unrelated to this research.

This research would not have been possible without the funding from Givaudan Singapore Pte Ltd and Givaudan Flavors Corporation, as well as the NUS Industry Postgraduate Programme, and I would like to express my gratitude to the company and the university for the financial support provided.

I am very grateful to the staff members and interns at Givaudan Singapore Pte Ltd and Givaudan Flavours Corporation (Cincinnati). My gratitude goes to Ms Lim Xiao Hui and Ms Jenny Yong for their help with sensory profiling and sample production, and to Mr Rahul Siva, Dr Chun-Yen Cochrane, and Ms Mayette Salle for the very insightful discussions over the course of the entire project. Also, my appreciation goes to Ms Chong Ee Fah, Ms Lee Bee Keng, and Ms Lim Yoke Teng for their support in analytical work and logistics. Special thanks goes to Dr Gesa Haseleu for providing a much needed chemical standard and guidance, and Mr Ben Kranen and Mr Philip Chan for their expertise in flavour creation during the early stages of the project.

Many thanks to Ms Lee Chooi Lan, Ms Lew Huey Lee, Ms Jiang Xiaohui, Mr Rahaman, and Ms Lee Si Min for providing logistical support for my sensory evaluations at NUS FST. It would not have been such a smooth-sailing journey without your support.

I would like to especially thank the members of Prof Zhou's research group – Dr Huang Jen-Yi, Dr Sui Xiaonan, Dr Tammie Tham, Dr V. Kristina Ananingsih, Dr Turkay Kondakci, Ms Gao Jing, Ms Lin Jing, Ms Katja Krizman, Mr Yu Hang, Mr Craig D'Souza, and other grad students and interns. All of you have brought much joy and laughter into my time with the research group, and there has never been a dull moment in the lab and office with you guys around. Thank you all for the advice, friendship, and camaraderie all these years.

To my undergrad students Mr See Zhi Kai, Ms Lydia Sim, and Ms Angelin Yeo, thank you for your blood (figurative), sweat (literal), and tears (also figurative – I hope) while working on the final year projects. Your hard work is much appreciated, and without which, this project would not have been brought to completion.

Lastly, I would like to thank my family and friends for all their support and encouragement: my parents, who have always supported me unconditionally in all my pursuits; my sister, who has always lent a listening ear to my monologue on work and science; friends who have always been encouraging and reminding me that the light at the end of the tunnel is near; and most of all, my wife Amelia, whose love, encouragement, support, and understanding are what allowed me to undertake and complete one of the greatest journey in my life thus far.

Soli Deo gloria

TABLE OF CONTENTS

SUMMARY

Green tea (*Camellia sinensis*) is one of the world's most popular beverage, and its consumption is on the rise due to its associated health benefits. Beverage manufacturers have responded to this growing trend by increasing the availability of ready-to-drink (RTD) green tea products in the market. In order for manufacturers to keep up and stay relevant with consumer choices, products will have to be designed with consumer acceptability in mind. As such, the objectives of this research were to systematically develop a methodology for collecting, analysing, and utilising data through the use of the design of experiments (DOE) methodology and a suite of modelling techniques, in order to aid in green tea flavour creation. The study of chemical, sensory, and hedonic properties of RTD green tea is an information gathering process, and can be divided into three main stages: (i) the design of experiment; (ii) the collection of data through chemical analyses, sensory profiling, and consumer acceptance testing; and (iii) analysis and interpretation of data.

A study was first conducted to investigate the impact of volatile odours in RTD green tea beverages on consumer liking. Eight volatile flavour keys, each comprised of a mixture of volatile odourants found commonly in RTD green tea beverages, were combined at different levels based on an experimental design to obtain a series of green tea odours. The most well-liked sample was an odour match of a commercial sample (liking score of 6.65 ± 1.30), while the least-liked sample had a liking score of 3.65 ± 1.49. A linear stepwise regression

model was developed to objectively predict consumer liking using stepwise regression. Further analysis was conducted to identify flavour keys of lesser importance by performing stepwise regression on reduced experimental designs in order to reduce data dimensionality, such that odour keys with the greatest impact would be combined with taste keys in subsequent studies with limited sample sizes. Removal of the X_7 and X_8 flavour keys were found to the least impact on the resulting model structure, and were thus removed from subsequent studies.

In the second study, the key non-volatile compounds affecting the taste profile of RTD green tea were identified through a series of taste reconstruction and omission sensory experiments. Thirty-nine non-volatile compounds in seven ready-to-drink (RTD) green tea samples were analysed and quantified using liquid chromatography, of which, 13 compounds with dose-over-threshold (DOT) values greater than one were used to reconstruct the taste profile of commercial RTD green tea products with no significant differences. Subsequent omission experiments revealed that caffeine, epigallocatechin gallate (EGCG), and glutamic acid were the main tastants in RTD green tea.

Results obtained from the preliminary studies highlighted important aroma and taste keys with significant influences on the sensory profile and consumer acceptance of RTD green tea beverages, and were subsequently used in developing experimental designs and regression models for correlating chemical, sensory, and hedonic properties of RTD green tea. A linear partial least squares (PLS) regression model was developed to describe the effects of the eight flavour keys (six volatile keys, two non-volatile keys) on consumer

liking, with a coefficient of determination (R^2) of 0.709, and a root-mean-square error (RMSE) of 3.70%. The PLS model was further augmented with an artificial neural network (ANN) to establish a PLS-ANN hybrid model. The established hybrid model was found to give a better prediction of consumer liking scores, based on its R^2 (0.885) and RMSE (2.32%).

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

LIST OF PUBLICATIONS

Published full journal paper

Yu, P., Yeo, A. S. L., Low, M. Y., & Zhou, W. (2014). Identifying key non-volatile compounds in ready-to-drink green tea and their impact on taste profile. *Food Chemistry, 155*, 9-16.

Submitted journal paper

Yu, P., Low, M. Y., & Zhou, W. (2016). Development of a partial least squares-artificial neural network (PLS-ANN) hybrid model for the prediction of consumer liking scores of ready-to-drink green tea beverages.

Yu, P., Low, M. Y., & Zhou, W. (2017). Design of experiments and regression modelling in food flavour and sensory analysis: a review.

Published conference proceedings

Yu, P., See, Z., Low, M. Y., & Zhou, W. (2015). Impacts of storage on the chemical and sensory profiles of a heat treated green tea model system. In A. J. Taylor & D. S. Mottram (Eds.), *Flavour Science: Proceedings of the XIV Weurman Flavour Research Symposium* (pp. 425 – 430). Leicestershire, UK: Context Products Ltd.

Oral presentation

Yu, P., Yeo, A. S. L., Low, M. Y., & Zhou, W. (2013, December). Identifying key non-volatile compounds in ready-to-drink green tea and their impact on taste profile. *6th Joint Symposium on Food Science and Technology between National University of Singapore and Tokyo University of Marine Science and Technology*. Presented at National University of Singapore, Singapore.

Yu, P., See, Z., Low, M. Y., & Zhou, W. (2014, September). Impacts of storage on the chemical and sensory profiles of a heat treated green tea model system. *14th Weurman Flavour Research Symposium*. Presented at Queens College, University of Cambridge, *Cambridgeshire*, UK

Yu, P., Low, M. Y., & Zhou, W. (2016, February), Development of a partial least square-artificial neural network (PLS-ANN) hybrid model for the prediction of consumer liking scores of ready-to-drink green tea beverages. *Inaugural Joint Symposium on Food Science and Technology between National University of Singapore and Kasetsart University*. Presented at National University of Singapore, Singapore.

Poster presentation

Yu, P., Low, M. Y., & Zhou, W. (2014, August). Computer algorithmic approaches to food sensory analysis: a review. *17th World Congress of Food Science and Technology & Expo*. Presented at Montreal, Canada.

Yu, P., Low, M. Y., & Zhou, W. (2016, August). Development of a partial least squares-artificial neural network (PLS-ANN) hybrid model for the prediction of consumer liking scores of ready-to-drink green tea beverages. *18th World Congress of Food Science and Technology & Expo*. Presented at Dublin, Ireland.

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. Background

Tea, a beverage made by infusing the leaves of *Camellia sinensis* in hot water, is the second most popular drink in the world after water (Butt & Sultan, 2009). Tea can be classified into three major categories: unfermented green tea, partially fermented oolong tea, and fermented black tea. Tea fermentation is a consequence of the enzymatic action of polyphenol oxidase (PPO) found in tea, which catalyses the oxidation and condensation of polyphenols into complex quinones and tannins (Obanda, Owuor, & Mang'oka, 2001). Unfermented green tea undergoes little fermentation due to inactivation of PPO through drying and steaming. Partially fermented oolong tea undergoes a brief fermentation period by crushing leaves and releasing PPO, followed by a heating process to inactivate enzymes and dry the leaves. Black tea undergoes a full fermentation process, and majority of polyphenols present are condensed to form complex polyphenols, before inactivation of PPO is initiated through a "frying" (i.e. heating) process (Wang & Ruan, 2009).

Green tea is one of the most widely consumed beverages in East Asian countries, and is deeply ingrained in East Asian cultures, often with deep ties to the history of such countries and has been incorporated into many food products such as ice cream, baked goods, confectionary and as well as commercial and instant green tea beverages (Cabrera, Artacho, & Gimenez, 2006). There has been an

increasing worldwide popularity in consumption of green tea, due to its purported health benefits, and to a certain extent, medicinal benefits arising from its antioxidant (Cabrera et al., 2006) and free radical scavenging properties (Butt & Sultan, 2009). Studies have shown that tea consumption has a positive impact on the body in numerous ways, including lowered risks for hypertension and cardiovascular disease (Cabrera et al., 2006; Higdon & Frei, 2003), weight control (Cabrera et al., 2006; Wolfram, Wang, & Thielecke, 2006), and to a certain extent, cancer prevention (Cabrera et al., 2006; Higdon & Frei, 2003; Jankun, Selman, Swiercz, & Skrzypczak-Jankun, 1997). Such health benefits are largely attributed to the high polyphenol content present in tea (Higdon & Frei, 2003).

The growing trend of green tea consumption was reflected in the availability and sales of ready-to-drink (RTD) green tea products. Annual sales of RTD tea reached USD 6.7 billion in 2012 according to a market research report (Zegler, 2013), with the growth of consumption being projected to increase in the next five years. In order for manufacturers to keep up with consumer preferences and stay relevant in the industry, products will have to be designed with consumer acceptability and liking in mind. Tea quality is closely associated with the aroma, taste, appearance and other physical attributes of the product (Kengpol & Wangkananon, 2015). These sensory qualities of green tea products are typically determined by expert human panels, and as such, are affected by subjectivity and the physiological conditions of human panellists (Yu, Wang, Yao, Zhang, & Yu, 2008). With this in mind, several studies have attempted to investigate the relationship between volatile constituents which contribute to the aroma profile and non-volatile compounds which contribute to the sensory and

hedonic properties of green tea (Liang et al., 2008; Pongsuwan et al., 2008; Yu et al., 2008), allowing for objective predictions of green tea quality parameters.

Food sensory profiling and flavour analysis are key processes in product development and are essential in understanding consumers by helping to bridge the gap between product characteristics and consumer perception and acceptance. The study of the chemical, sensory, and hedonic properties of RTD green tea begins with an information gathering process, and can be divided into three main stages: (i) the design of experiments (DOE); (ii) the collection of data through chemical analyses, sensory profiling, and consumer acceptance testing; and (iii) modelling and interpretation of data. The design of an experiment is key to obtaining useful and representative data that can be used for subsequent analysis, which is especially so in regression model development. The use of the DOE approach employing classical techniques such as factorial designs and relatively newer methods such as optimal designs has been widely studied in various food products, but not in green tea related studies (Hewson, Hollowood, Chandra, & Hort, 2009; Knoop, Sala, Smit, & Stieger, 2013; Niimi, Overington, Silcock, Bremer, & Delahunty, 2016; Shiby, Radhakrishna, & Bawa, 2013). An adequate experimental design allows for proper estimation of the relationship between input factors and response variables. The use of classical methods may be limited in larger experiments due to a large number of experimental design points, which can be circumvented using computer-based methods such as optimal designs.

The collection of data through chemical and sensory analyses is another important challenge in new product development projects. Recent studies in

analytical methods are focused on development of rapid analytical methods allowing for high sample throughput (Ananingsih, Sharma, & Zhou, 2013; Jiang, Engelhardt, Thräne, Maiwald, & Stark, 2015). This extends to the use of sensory profiling methods, which is typically resource-intensive due to the amounts of time and money required in training an expert human panel (Yu et al., 2008). It would be an incentive for commercial companies to make use of quicker methods in analysing food samples, which would translate into lower costs and potentially greater profits.

Lastly, predictive modelling allows for an empirical understanding of food systems through regression models. While there have been numerous studies correlating chemical and sensory profiles to green tea quality (Jumtee, Komura, Bamba, & Fukusaki, 2011; Liang et al., 2008; Wang & Ruan, 2009), there have been limited studies to date correlating all three aspects of green tea simultaneously (Ikeda, Nagai, & Sagara, 2004). Furthermore, due to the nonlinear relationship between chemical and sensory profiles and hedonic properties, linear regression methods may only provide a limited insight to food systems (Krishnamurthy, Srivastava, Paton, Bell, & Levy, 2007). Nonlinear methods such as artificial neural networks and support vector machines have been used to develop nonlinear regression models to predict quality parameters in green tea (Kengpol & Wangkananon, 2015; Yu et al., 2008).

1.2. Research objectives

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

1.3. Overview of thesis structure

Chapter 1 describes the background and main objectives of the research, and provides a summary of the overall thesis structure.

Chapter 2 contains a review of current literature and research on the volatile and non-volatile compounds present in green tea, as well as design of experiments and predictive modelling methodologies used in flavour and sensory evaluation studies.

Chapter 3 presents results obtained in the preliminary study on volatile flavour keys used in developing RTD green tea beverages, and also identifying significant flavour keys contributing to an overall positive consumer liking.

Chapter 4 investigates the non-volatile chemical profile of seven commercially available RTD green tea beverages, and the key compounds responsible for the taste of these green tea products.

Chapter 5 covers the development of a hybrid regression model, which is comprised of a linear partial least square regression model augmented with a

nonlinear artificial neural network, and compares the model quality of the hybrid regression model with a linear PLS model and a nonlinear artificial neural network.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Chapter 8 examines the impact of storage on the chemical and sensory profile of a heat treated RTD green tea beverage model system.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

CHAPTER 2

LITERATURE REVIEW

2.1. Volatile compounds in green tea

Green tea quality can be determined by several factors, of which aroma is probably one of the most important. High quality green tea often contains a high concentration of volatiles, in particular terpenes and terpene alcohols (Kato & Shibamoto, 2001), and is characterised by characteristic "green" leafy and floral notes (Jumtee, Komura, Bamba, & Fukusaki, 2011). These compounds are found bound to sugar moieties during shoot formation, and are hydrolysed during the processing stage (Wang & Ruan, 2009). Due to the milder processing steps, the aroma of green tea is distinctively different from both oolong and black tea (Baptista, Tavares, & Carvalho, 1998) with fewer compounds contributing to a roasted odour. Instead, the aroma profile of green tea has been described to be a pleasant blend of sweet, floral notes and rancid odours (Baptista et al., 1998).

Volatile compounds present in green tea are generally the products of plant metabolism, although a few classes may be associated with high temperature processes during the drying stages. Aldehydes and alcohols represent some of the key odourants of green tea aroma. Short chain aliphatic aldehydes and alcohols are associated with a green odour, typical of grass and leaves (Baptista et al., 1998). Pyrazines and pyrroles may be found in higher concentrations in roasted green tea from Maillard reactions and Strecker degradations of amino

acids, and are responsible for a nutty, roasted aroma (Wang & Ruan, 2009). A summary of odour compounds commonly found in green tea is presented in Table 2.1.

The flavour of green tea is largely influenced by both volatiles and semi-volatiles present in the matrix and headspace (Baptista et al., 1998). The main mode of volatile analysis in green tea is gas chromatography coupled to a mass spectrometer (GC-MS), flame ionisation detector (GC-FID) or both (GC-FID-MS), as seen in many studies (Baptista et al., 1998; Jumtee et al., 2011; Kato & Shibamoto, 2001). While the analysis of green tea volatiles has mainly revolved around GC-MS or GC-olfactometry, the types of extraction methods employed may vary greatly, depending on the objectives and analytes of interest in the study. Solvent extraction and various distillation methods can be considered as some of the conventional methods employed in the analyses of flavour volatiles. With advances in technology and laboratory instrumentation, new methods such as solid phase extraction (SPE), solid phase microextraction (SPME), and solvent assisted flavour evaporation (SAFE) have gradually been adopted or replaced some of the conventional methods of sample preparation.

Table 2.1: Potent odour-active compounds extracted from three types of green tea. Adapted from Cheng, Huynh-Ba, Blank, & Robert, 2008; and Kumazawa & Masuda, (1999 and 2002).

Compound name	Odour quality	Tea type[†]				
		a	**b**	**c**	**d**	**e**
Alcohols, aldehydes and ketones						
(*Z*)-3-hexen-1-ol	woody, mushroom, green	●		●	●	●
geraniol	floral, woody	●	●	●	●	●
linalool	floral, green	●	●	●	●	●
p-cresol	phenolic			●	●	●
2-phenylethanol	floral, honey, sweet	●				
3-methylbutanal	stimulus			●	●	●
heptanal	grass, mushroom	●				
(*Z*)-4-heptenal	fatty, fish, hay-like	●	●	●	●	●
(*E*,*E*)-2,4-heptadienal	fatty		●	●	●	●
nonanal	floral, green, orange-like	●		●	●	●
(*E*)-2-nonenal	leather-like, green	●		●	●	●
(*E*,*E*)-2,4-nonadienal	fatty, green	●		●	●	●
(*E*,*Z*)-2,6-nonadienal	fatty, cucumber-like	●	●	●	●	●
(*Z*)-4-decenal	green		●			
(*E*,*E*)-2,4-decadienal	green, fatty	●	●	●	●	●
geranial	floral, leaves	●				
phenylacetaldehyde	honey-like	●	●	●	●	●
2,3-butanedione	buttery		●	●	●	●
2,3-pentanedione	buttery			●	●	●
1-hepten-3-one	grass, metallic	●				
1-octen-3-one	mushroom-like	●	●	●	●	●
(*Z*)-1,5-octadien-3-one	metallic	●	●	●	●	●
(*E*,*Z*)-3,5-octadien-2-one	green	●				
3-methylnonane-2,4-dione	green		●	●	●	●
β -damascone	honey-like		●			
β -damascenone	honey-like, floral	●	●	●	●	●
β -ionone	tea leaves, woody	●	●			
(*Z*)-jasmone	woody, floral	●	●	●	●	●
geranylacetone	tea leaves, floral	●	●	●	●	●
α-ionone	woody, floral	●				
β-ionone-5,6-epoxide	tea leaves, woody	●				
Acids, esters, and lactones						
acetic acid	acidic	●				
2-methylbutanoic acid	cheesy	●				
3-methylbutanoic acid	cheesy	●				
hexanoic acid	green, acid		●			
3-hexenoic acid	sour, cheesy	●				
ethyl 3-methylbutanoate	fruity	●				
(*Z*)-3-hexenyl (*Z*)-3-hexenoate	green		●			
methyl geranate	woody, leaf, floral	●				
(*E*)-methyl jasmonate	floral			●	●	●
4-nonanolide	sweet		●	●	●	●
jasmine lactone	sweet		●	●	●	●
coumarin	sweet, camphoraceous		●	●	●	●

(Cont.) Table 2.1

Compound name	Odour quality	Tea type[†, ‡]				
		a	**b**	**c**	**d**	**e**
N-containing compounds						
2-acetyl-1-pyrroline	roasty	●		●	●	●
2-acetylpyrazine	roasty, popcorn-like	●			●	●
2-ethylpyrazine	nutty		●			
2-isobutyl-3-methoxypyrazine	bell pepper, green, earth	●		●	●	●
2,3,5-trimethylpyrazine	nutty				●	●
2,5-dimethyl-3-ethylpyrazine	sweaty, roasty	●				
2-ethyl-3,5-dimethylpyrazine	roasty, nutty	●	●	●	●	●
2,3-diethyl-5-methylpyrazine	nutty			●	●	●
indole	animal-like		●	●	●	●
Other compounds						
3-hydroxy-4,5-dimethyl-2(5H)-furanone	caramel-like				●	●
methyl anthranilate	grape-like			●	●	●
vanillin	vanilla-like		●	●	●	●
eugenol	spicy		●	●	●	●
2-methoxy-4-vinylphenol	spicy		●	●	●	●
2-aminoacetophenone	grape-like		●	●	●	●
maltol	sweet		●			
4-hydroxy-2,5-dimethyl-3(2H)-furanone	caramel-like	●		●	●	●
guaiacol	spicy, smoky	●	●	●	●	●

[†] **a**, **c**: Sencha; **b**, **e**: Longjing tea; **d**: Kamairi-cha

[‡] **a**: Cheng et al., 2008; **b**: Kumazawa & Masuda, 1999; **c** – **e**: Kumazawa & Masuda, 2002

2.2. Non-volatile compounds in green tea

Unlike volatile compounds which determine the aroma profile of tea, the non-volatile components contribute to the taste profile of green tea. Analyses of the non-volatile components generally revolve around chromatographic methods and capillary electrophoresis, although in some studies other methods such as electronic tongue or near-infrared spectroscopy may be used as a form of non-destructive analysis.

2.2.1. Catechins

Catechins are the major polyphenols in green tea (12.7 to 54.9 mg/100 mL steeped green tea) (Higdon & Frei, 2003). The main catechins found are (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechingallate, (–)-epigallocatechingallate (EGCG) (Wang, Helliwell, & You, 2000; Zuo, Chen, & Deng, 2002). The structures of these compounds are illustrated in Figure 2.1. Recent studies have suggested that polyphenols, notably catechins, are responsible for a large range of health benefits in the human body (Higdon & Frei, 2003).

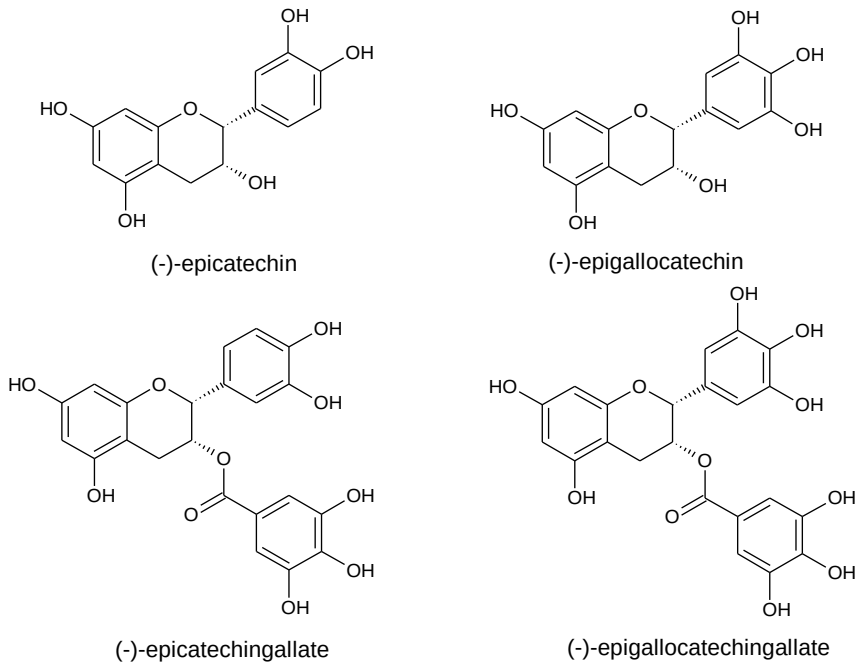

Figure 2.1: Structures of major catechins found in green tea.

Analyses of the non-volatile fraction of green tea are typically done using high performance liquid chromatography (HPLC) interfaced with ultraviolet absorption (El-Shahawi, Hamza, Bahaffi, Al-Sibaai, & Abduljabbar, 2012;

Friedman, Levin, Choi, Kozukue, & Kozukue, 2006; Wu, Xu, Heritier, & Andlauer, 2012), or a diode array detector (Wang & Helliwell, 2001; Wang et al., 2000; Zuo et al., 2002), although detectors such as mass spectrometer (Wu et al., 2012) and fluorescence detector may be used as well, depending on the sensitivity required. Other less common methods of analysis include capillary electrophoresis and its variants (Horie, Mukai, & Kohata, 1997; Hsiao, Chen, & Cheng, 2010; Peres, Tonin, Tavares, & Rodriguez-Amaya, 2011), Fourier transform near infrared spectroscopy (Chen, Zhao, Chaitep, & Guo, 2009) and electrode-based chemical sensors (Chen, Zhao, Guo, & Wang, 2010).

Wang et al. (2000) developed a protocol for the analysis of tea catechins (GC, EGC, C, EGCG, EC, GCG, ECG), caffeine and gallic acid using HPLC coupled to an UV detector. A methanol/water/orthophosphoric acid (20/79.9/0.1) isocratic solvent system was employed. All nine compounds were successfully separated under 53 min. A similar acetonitrile/water system was found to give a complete separation of EGC and C. Orthophosphoric acid was found to be essential in providing complete baseline separations of EGC/C and ECGC/EC. The authors also studied the effects of ethanol concentration used during sample preparation, and concluded that the concentration of ethanol should be kept below 15% to minimise interaction with the mobile phase in order to achieve good separation and quantitation.

Wu et al. (2012) utilised RP-HPLC-UV and RP-HPLC-MS to analyse and determine catechins and flavonol glycosides in Chinese teas. Separation was performed on a C_{18} column with a guard cartridge, using gradient elution with aqueous formic acid and acetonitrile as solvents. Electrospray ionisation (ESI)

was used as an ion source in the mass spectrometer. Constituents were identified using retention times and mass spectra, and quantified using RP-HPLC-UV.

2.2.2. Other phenolic compounds

Flavonols are another major class of phenolic compounds in green tea (7 to 9% of total flavonoid content) (Higdon and Frei, 2003), and are responsible for the yellow colour of green tea infusions. Studies have suggested that flavonols may have certain beneficial physiological effects in the human body, similar to that of catechins (Finger, Kuhr, & Engelhardt, 1992). Flavonols are often found bound to one or more carbohydrate moieties. Commonly found flavonol glycosides include quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside (Wu et al., 2012). Myricetin glycosides are present at lower amounts (Del Rio et al., 2004). It has been suggested that flavonol glycosides may impart a mouth-drying and mouth-coating sensation at very low concentrations (Scharbert, Holzmann, & Hofmann, 2004).

Theaflavins and thearubigins are condensation products of catechins, and are generally formed from the enzymatic fermentation of tea leaves through PPO-catalysed reactions. As a result, theaflavins and thearubigins are found in higher concentrations in oolong and black teas, and little to none in green tea.

Flavonol glycosides and gallic acid, like catechins, are usually analysed using HPLC coupled to a detector for separation and detection (Del Rio et al., 2004). UV, diode array and fluorescence detectors and mass spectrometers are some of

the more commonly used detectors. Although most recent studies focused on the profiling a single class of polyphenols, there is an increasing number of studies demonstrating simultaneous analysis of more than one class of tea components.

In a recent study, Peng, Song, Shi, Li, and Ye (2008) employed the use of HPLC coupled to a photodiode array detector in developing an improved method of analysing phenolic compounds, purine alkaloids and theanine simultaneously. A C_{16} column equipped with a guard column was used in place of a C_{18} column to separate tea infusions. Poor reproducibility was obtained using C_{18} columns due to the use of polar solvents required for analysing polar molecules (gallic acid and theanine). The optimum temperature for separation on the column was found to be 35 °C, following coelution of C and caffeine, and EGC and an unknown component, at 40 °C and 30 °C respectively. High linearity with correlation coefficient of greater than 0.999 for all components, and recovery (85.56 to 103.86%) for all components were obtained using this method.

Both Del Rio et al. (2004) and Wang, Lu, Miao, Xie, and Yang (2008) reported the use of HPLC-DAD-MS to separate polyphenols and purine alkaloids in tea. A two-step linear gradient elution programme was adopted by Del Rio et al. (2004), which according to the authors, allows for complete resolution of phenolic compounds with smaller peaks from larger peaks. On the other hand, Wang et al. (2008) made use of a single-step system, and their results indicated that there might be some coelution between GCG and an unknown compound, as well as a few other compounds.

2.2.3. Purine alkaloids

The distinct bitterness of tea is widely considered to be due to the presence of purine alkaloids (Figure 2.2) (Pongsuwan et al., 2008). Caffeine is present as the major alkaloid (Lin, Chen, & Harnly, 2008). Green tea contains normally 25 to 40 mg/g of caffeine, depending on geographical origin, and processing and steeping conditions (Cabrera, Gimenez, & Lopez, 2003). Theobromine and theophylline are present at concentrations about ten and hundred times lower, respectively (Finger et al., 1992). Besides instilling a bitter taste to tea, caffeine was also found to modify the mouthfeel of tea. A study conducted by Millin et al. (1969) found that caffeine decreased the astringency caused by polyphenolic compounds, and at the same time increased the mouthfeel of the caffeine/polyphenol mixture. At the same time, the bitterness of caffeine was somewhat negated by the presence of polyphenolic compounds, which suggested that caffeine may have some interactions with polyphenolic compounds.

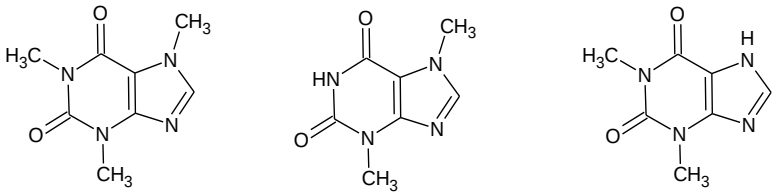

Figure 2.2: Structures of purine alkaloids found commonly in beverages.

A commonly used method of analysis of methylxanthines is reversed phase HPLC, using acetonitrile or methanol as a mobile phase, and coupled to a UV-vis spectrophotometer or a photodiode array detector for identification (Angelino & Gennaro, 2000; Horie & Kohata, 2000). Analysis of methylxanthines and other tea polyphenols can be done simultaneously, but such analyses may be very time consuming (Horie & Kohata, 2000). Polyphenolic compounds can be removed prior to methylxanthines analysis using polyvinylpolypyrroridone (PVPP) as an absorbent. Nakakuki, Horie, Yamauchi and Kohata (1999) developed a rapid method of removing polyphenols from tea using a column packed with PVPP connected upstream to the HPLC column.

Seeram et al. (2006) investigated catechin and caffeine content of green tea dietary supplements using a reverse-phase C_{18} column, coupled with a photodiode array detector. Aqueous and ethanolic-aqueous samples of green tea dietary products were not treated with PVPP, but instead catechins and caffeine were analysed concurrently and identified using known standards. Wang and Ruan (2009) determined concentration of caffeine in *Longjing* tea using a reverse-phase HPLC column. Detection of eluents was achieved using a UV-vis spectrophotometer at 280 nm. Caffeine content was determined simultaneously with tea catechins using internal standards.

2.2.4. Sugars

Tea polysaccharides are molecular complexes comprising of glycosidically linked monosaccharide residues with molecular weights of greater than 10 kDa.

Tea polysaccharides are mostly found covalently bound to polypeptides via O- or N- linkages (Nie & Xie, 2011). Recent studies have shown that tea polysaccharides may contribute towards several health benefits, including antihyperglycemic (Zhou et al., 2007), antimicrobial (Lee et al., 2006), and immuno-boosting effects (Monobe, Ema, Kato, & Maeda-Yamamoto, 2008). However, their exact physiological effect and composition and contribution towards taste in tea are still not well understood.

There have been limited studies on the determination of tea polysaccharides in tea, partly due to its complex structure. Wei et al. (2011) developed a method utilising resonance light scattering to provide quantitative determination of tea polysaccharides in the presence of a cetylpyridinium chloride-sodium hydroxide system, which was shown to have higher selectivity than traditional methods such as colourimetric methods (Wei, Xi, Wu, & Wang, 2011).

Monosaccharides are often found complexed to other components present in tea such as flavonol glycosides and terpenoid glycosides. There are limited studies on sugars in tea as well, possibly due to the fact that green tea, on its own, does not contain high amounts of simple sugars. Analysis of sugars is generally achieved through chromatographic means, although traditionally, it is done using colourimetric methods involving derivatisation with a chromophore. However, such methods usually involve the use of chemicals, some of which such as phenol may cause harm to human health. Other methods include techniques involving the use of enzymes and indicator compounds to determine sugar content colourimetrically, such as the glucose oxidase-peroxidase assay. The assay follows a two-step reaction as shown below:

$$\text{glucose} \xrightarrow{\text{glucose oxidase}} \text{glucono-}\delta\text{-lactone} + H_2O_2 \qquad \text{(Step 1)}$$

$$H_2O_2 + \text{indicator} \xrightarrow{\text{peroxidase}} \text{coloured compound} \qquad \text{(Step 2)}$$

Rovio, Yli-Kauhaluoma and Sirén (2007) developed a new capillary zone electrophoresis (CZE) for the separation determination of neutral carbohydrates in beverages. Separation was done using uncoated fused-silica capillaries, and detection was done using DAD set to measure in the wavelengths between 260 to 280 nm. Separation was achieved at pH 12.6, and a total of 12 carbohydrates were successfully resolved in under 2 min. Reproducibility was determined to be satisfactory, with correlation coefficients of greater than 0.94.

Ding, Yu, and Mou (2002) utilised a method involving anion-exchange chromatography to successfully separate and detect 22 amino acids and sugars, which included sucrose, fructose and glucose. Analyses were performed using an AminoPac PA10 analytical column on an HPLC system coupled with an ED50 electrochemical detector equipped with a thin-layer type amperometric cell. The method offered high sensitivities in the range of pmol for all analytes, and high reproducibility with correlation coefficients greater than 0.99.

2.2.5. Free amino acids

Theanine (5-*N*-ethyl-L-glutamine), a derivative of glutamic acid, is a non-proteinogenic amino acid found in tea that accounts for more than 50% of amino acids present (Wang et al., 2010). It is an important precursor in the synthesis of polyphenolic compounds in tea. Studies have shown that theanine is present

at a concentration between 4.9 to 10.9 mg/200 mL in green tea infusion, and up to 1 to 2% of the dry weight of green tea leaves (Wang et al., 2010). It has been suggested that theanine is responsible for the delicate, brothy, umami flavour of green tea, although other amino acids such as glutamic acid may contribute towards the umami taste as well (Pongsuwan et al., 2008). Strecker degradation of amino acids during processing or infusion may result in the formation of volatile aldehydes, which further contributes to the overall green tea flavour (Finger et al., 1992).

Analysis of free amino acids in tea can be achieved through chemical or chromatographic methods. Ninhydrin and 2,4-dinitrofluorobenzene are commonly used colourimetric methods. Chromatographic methods are usually slightly more complicated techniques, requiring some form of derivatisation due to the absence of a chromophore moiety on amino acids. Commonly used labelling reagents include dabsyl chloride, phenylisothiocyanate (PITC) and *o*-phthaldehyde (OPA). However, such reagents are not selective, and may react with other tea components such as catechins.

In the investigation of free amino acids in tea conducted by Wang et al. (2010), isolation of free amino acids was achieved using solid phase separation. The amino acid eluent was dried and concentrated before derivatisation with OPA. Free amino acids content was determined by HPLC-DAD using a reversed-phase column. Detector wavelength was set at 338 nm to detect derivatised amino acids. In another study conducted by Syu, Lin, Huang and Lin (2008) theanine and other amino acids were derivatised using dabsyl chloride. Analysis

was performed using a reversed-phase HPLC system, coupled to a UV-vis detector operating at 475 nm.

A rapid method of theanine analysis has been described in a study conducted by Ying, Ho, Chen, and Wang (2005), in which *o*-phthaldehyde was used as the derivatising agent. Similarly, a reversed-phase HPLC system was used to separate theanine from other tea components, and a fluorescence detector with excitation and emission wavelengths set at 330 and 418 nm respectively, was used. However, detection of other amino acids was not described in this study.

2.2.6. 5'-nucleotides

Umami is the fifth basic taste discovered after sweet, salty, sour and bitter, and is described as savouriness or broth-like. The amino acid glutamic acid was first isolated from seaweed as the source of an umami taste in soups. Following this discovery, the 5'-nucleotides guanosine monophosphate (GMP) and inosine monophosphate (IMP) were found to confer savouriness to foods as well. Disodium salts of GMP and IMP are currently used as flavour enhancer and potentiators (Ninomiya, 2002).

IMP and GMP are found in high amounts in seafood (especially shellfishes such as abalone and scallop) and meat products. Certain plants such as mushrooms and tomatoes are known to contain high amounts of taste enhancing 5'-nucleotides. The IMP and GMP profile in green tea has not been extensively studied, and there is little information in this regard. Existing literature utilised

HPLC as a method in analysing 5'-nucleotides in food samples (Ninomiya, 2002).

Koshiishi, Crozier, and, Ashihara (2001) investigated the nucleotide profile of fresh and manufactured tea leaves, and found that processed tea leaves had profiles significantly different from plants (including fresh tea leaves). Tea leaves were first treated using SPE and HPLC to obtain the 5'-nucleotides fraction, which was further analysed on an Asahipak GS 320-H HPLC column. Detection was achieved using a UV-vis detector measuring absorbance at 260 nm. Both GMP and IMP were separated and quantified using 10 mM NaH_2PO_4 (pH 4.6) as a mobile phase in isocratic mode.

2.3. Use of design of experiment methodologies

The design of experiments (DOE) methodology was first developed by Fisher in the 1920s, but it was not used until the last few decades when it was adapted for food sensory studies on a larger scale. A well-defined and well-structured experimental design allows for the study of input parameters (predictor variables) and the generated output (response variables), as well as various interactions that may exist between the input variables. In general, experimental designs are denoted by a matrix, with columns representing the independent variables associated with the study, and rows representing samples or experimental runs. Responses generated from an experimental design may be the sensory profile of a set of food products, or an optimisation process seeking to optimise a certain sensory and/or hedonic properties. The use of DOE in planning and conducting sensory studies allows the experimenter to obtain

useful information such as drivers of liking and dislike in a food product, which has important implications in consumer research.

Experimental designs range from the basic one-factor-at-a-time approach, to classical factorial designs, and more recently, computer-generated designs that were developed in the last three decades. The use of the DOE methodology has been reviewed in other fields such as analytical chemistry (Dejaegher & Vander Heyden, 2011; Hibbert, 2012; Candioti, De Zan, Cámara, & Goicoechea, 2014) and bioprocess control (Mandenius & Brundin, 2008), but not in sensory science. The following sections will provide an overview of use of classical and computer-generated experimental designs for use in food sensory studies. For a review of sensory evaluation methods, readers are referred to Murray, Delahunty, & Baxter (2001), and Valentin, Chollet, Lelievre, & Abdi (2012) for a comprehensive overview.

2.3.1. One-factor-at-a-time approaches

The one-factor-at-a-time (OFAT) approach is one of the oldest and simplest approaches when it comes to experimental design. In this approach, all variables are set at a constant level, and the effect of each variable is investigated by changing individual variables one at a time. While this may appear to be an ordered and sophisticated approach to evaluating sensory qualities of foods, it is in fact highly inefficient, and may produce inaccurate results since the effects of changing a single factor at a time may be very different from changing multiple effects together (Ellekjær, Ilseng, & Næs, 1996; Montgomery, 2008; Olsson, Gottfries, & Wold, 2004b).

Food systems, in most, if not all cases, are multivariate in nature, with individual sensory characteristics and liking being a combinatorial effect of multiple physical and chemical qualities. For example, in the hedonic evaluation of a citrus beverage product, consumer liking may be dependent on various taste (sweetness, sourness) and odour qualities (fruity, citrus), in addition to various other physical properties (presence of solid sediments, colour). As such, sensory analysis of a food system or product using the OFAT approach would thus require a large amount of resources in a multivariable food system to attain a comprehensive set of results. Furthermore, estimation of the effect of individual variables is based on the assumption that interactions between variables are not important or non-existent, which on the contrary, are extremely common in food systems, in relation to consumer perception of these food products.

2.3.2. Factorial designs approaches

Factorial designs are classical experimental designs that have been widely used in scientific experiments, based on a combination of factors, to investigate multiple factors and their interactions simultaneously while reducing the degree of biasness in experiments. The use of factorial designs and other variants is widespread in the field of chemometrics and analytical chemistry. Recent studies in sensory and flavour science utilising the factorial design approach have been summarised in Table 2.2.

Table 2.2: Applications of factorial designs in sensory and flavour studies.

Product/property	Analytical methodology	Experimental design	References
Umami taste	Quantitative descriptive analysis	Four-level full factorial design	Baryłko-Pikielna & Kostyra (2007)
Red wine	Flavour chemical analysis	Mixed-level factorial design	Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara (2009)
Polyphenol-rich beverages	Quantitative descriptive analysis and hedonic test	Mixed-level factorial design	Jaeger, Axten, Wohlers, & Sun-Waterhouse (2009)
Energy drink	Quantitative descriptive analysis	Three-level full factorial design	Tamamoto, Schmidt, & Lee (2010)
Extrusion product	Hedonic test	Mixture design	Lobato, Anibal, Lazaretti, & Grossmann (2011)
Odour-induced saltiness perception	Quantitative descriptive analysis	Mixed-level factorial design	Nasri, Beno, Septier, Salles, & Thomas-Danguin (2011)
Extrusion product	Quantitative descriptive analysis	Two-level full factorial design	Saeleaw, Dürrschmid, & Schleining (2012)
Extra virgin olive oil	Flavour chemical analysis	Two-level full factorial design and Box-Behnken design	Reboredo-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara (2012)
Yoghurt	Hedonic test	Mixed-level factorial design	Ramírez-Sucre & Vélez-Ruiz (2013)
Cross-modal taste interactions	Quantitative descriptive analysis	Two-level full factorial design	Knoop, Sala, Smit, & Stieger (2013)
Cheddar cheese taste	Quantitative descriptive analysis	Two-level fractional factorial design	Niimi et al. (2014)

Full factorial designs are orthogonal, balanced designs, allowing for the estimation of all main and interaction factors (the factors are said to be fully separated from each other), but would typically involve a large number of design points or samples, given by Equation 2.1:

$$n = m^k \qquad \text{(Equation 2.1)}$$

where n is the total number of samples, k is the number of factors, and m is the number of levels of each factor. Conventionally, most experiments utilising full factorial designs make use of two levels, along with a small number of factors, resulting in a 2^k design. The number of samples increases exponentially with the increase in number of factors, making it extremely costly to run such experiments, which is especially the case in sensory evaluations involving human panellists. Three-level factorial designs may serve better purposes in most cases, as they are able to generate second-order polynomial models for estimating curvature associated with the independent variables, but are more complicated than their two-level counterparts, and is especially so for a larger number of factors. When dealing with factors containing three or more levels, the number of samples required for complete estimation of main and interaction factors can reach a very high number, even when working with a small number of factors. For example, sensory profiling of a food product with four independent variables, each containing a low, mid, and high level, will require evaluation of 81 different samples. As such, two-level factorial designs are often used as screening designs to identify important factors, which are then studied in greater detail using experimental designs containing higher factor levels.

Fractional factorial designs, as the name suggests, are fractions of full factorial designs, and are used to predict main and interaction factors using a subset of the full design, by utilising a smaller number of samples. The number of

experimental points in an experiment containing two levels for all factors is given by Equation 2.2:

$$\boldsymbol{n} = 2^{\boldsymbol{k}-\boldsymbol{p}} \qquad \text{(Equation 2.2)}$$

where $\boldsymbol{p}$ represents the number of times the design is reduced by half, and the number of design generators used. Experimental designs for two-level fractional factorial designs are readily available in textbooks (Montgomery, 2008) and various online resources, and can be easily adapted for use by the experimenter. Chen, Sun, and Wu (1993) and Li, Zhang, and Zhang (2013) have published catalogues of three-level fractional factorial designs, categorised by the number of runs and factors. The Box-Behnken designs are examples of three-level designs used in the response surface methodology (Box & Behnken, 1960), with commonly-available resources for experimenters to use. The centre point in Box-Behnken designs are often replicated in order to approximate the experimental error.

Fractional factorial designs may not be able to provide clear estimations of main and interaction factors as some of them may be confounded or aliased, depending on the degree of resolution of the experimental design. This is true when the number of factors involved in the experiment becomes large, which is typical in food-related sensory studies. The experimenter would then have to make a choice between utilising a larger amount of resources in obtaining in-

depth knowledge of the system, and sacrificing information collected in exchange for resources spent.

2.3.3. Computer algorithmic approaches to design of experiments

Computer algorithmic designs are computer generated, non-standard experimental designs which are commonly used in place of classical designs in the following situations (Montgomery, 2008): (i) the experimental design space is constrained or irregular; (ii) a non-standard model is required; or (iii) there is a limitation on the number of experimental runs or sample size, which is a common phenomenon where the number of model variables (***k***) is greater than the number of observations (***n***).

These limitations are encountered in the food sensory studies and experiments, and especially so for the third scenario. In the sensory evaluation of food products, the large number of independent variables may result in a very large factorial experimental design, and assessment of all experimental runs would likely result in sensory fatigue among panel members, and would require a large amount of resources in terms of time and money. Furthermore, symmetrical designs such as factorial designs or response surface methodologies may not necessarily produce the most ideal experimental design, as the relationship between physiochemical attributes in foods and sensory qualities may not be linear in nature. In sensory experiments, the levels of independent variables included in an experiment may be different due to constraints related to the nature of the experiment. Although mixed levels factorial designs may be developed to suit this purpose, it may result in unbalanced and non-orthogonal

designs. The use of a computer algorithm allows for greater flexibility in the construction of customised experimental designs, according to the experimental parameters (Périnel & Pagès, 2004).

A set of design points is chosen from a candidate set, which may correspond to a classical design such as a full factorial design, or may be a specific experimental design customised by the experimenter, due to limitations or constraints in the experiment (Nguyen & Miller, 1992; Olsson et al., 2004b). As the name suggests, these methods employ the use of an algorithm in the selection of the ideal set of experimental design points.

The candidate matrix refers to the matrix containing a set of experimental observations for the algorithm to select from. In classical designs, which may serve as the candidate design, there are $\boldsymbol{m^k}$ experimental points spanning over the entire design matrix, where $\boldsymbol{m}$ represents the number of factor levels, and $\boldsymbol{k}$ representing the number of variables in the mathematical model forming the basis of the design (Olsson et al., 2004b). Given that the optimal experimental design of choice should contain much fewer numbers of experimental points than the candidate design, this leads to an arbitrarily large number of possible combinations when $\boldsymbol{k}$ becomes large. It should be noted that the number of design points chosen is dependent on the model to which data obtained will be fitted.

A model containing $\boldsymbol{k}$ variables, excluding the constant term, should be estimated from an experimental design containing at least $\boldsymbol{k}$ + 1 design points for adequate degrees of freedom. For example, in a sensory evaluation of a beverage product containing five variables at three levels each, a full factorial

design contains 243 design points, which translates to 243 different products to be evaluated. The experimenter may choose to reduce this to a reasonable experimental design with 21 runs to prevent panel sensory fatigue, and to reduce the potential strain on resources spent on conducting the sensory evaluation. However, this results in $\binom{243}{21} \approx 10^{30}$ possible combinations of experimental runs, of which exists a number of optimum subsets that ideally, would be used for experimentation.

A brute force method of analysing all possible combinations of design points may not be possible even with a computer, and even so, is not recommended due to limitations in terms of time and cost (Nguyen & Miller, 1992; Veira, Sanchez, Kienitz, & Belderrain, 2011). As such, the best subset of design points should be selected based on a set of criterion based on a computer algorithm. Amongst the various criteria, optimal designs are perhaps the most commonly used in food sensory evaluation studies. Other less frequently employed computer algorithmic approaches used in experimental designs are space filling designs and Latin hypercube designs, which also rely on a computer algorithm for generating the experimental design.

2.3.3.1. Optimal designs

In optimal designs, there exist several criteria on which the optimality is based on, and of which, the D-optimality criterion is the commonly employed in food sensory studies. Other criteria include A-optimality and G-optimality (de Aguiar, Bourguignon, Khots, Massart, & Phan-Than-Luu, 1995; Montgomery,

2008). However, as these criteria are less often used due to greater computational requirements (Carlsson & Martinsson, 2003), this section will instead focus on D-optimal designs.

The D-optimal design is a computer generated design that seeks to select a subset of design points, **X**, from a matrix of candidate points, **M**, such that the determinant of the Fisher information matrix, given by $|\mathbf{X}'\mathbf{X}|$ ($\mathbf{X}'$ refers to the transpose of **X**), is maximised (Mentré, Mallet, & Baccar, 1997). Both the candidate and optimal sets of design points are defined by matrices containing $\boldsymbol{N} \times \boldsymbol{k}$, and $\boldsymbol{n} \times \boldsymbol{k}$ design points respectively, where $\boldsymbol{N} > \boldsymbol{n}$. Maximising the determinant is equivalent to minimising the determinant of the inverse of the information matrix, such that:

$$|\,(\boldsymbol{X_0}'\,\boldsymbol{X_0})^{-1}| = \underset{X|X \ni M}{min}\ (|\,(\boldsymbol{X}'\,\boldsymbol{X})^{-1}|) \qquad \text{(Equation 2.3)}$$

The D-optimal algorithm chooses an ideal subset from all possible combinations, and in doing so significantly reduces the number of required experiments, compared to standard design types, while maximising the prediction accuracy and minimising aliasing of regression model coefficients (Mitchell, 1974). A regression model correlating predictors to dependent variables would be of the general form:

$$f(\mathbf{X}) = \boldsymbol{\beta}\mathbf{X}' + \mathbf{e} \qquad \text{(Equation 2.4)}$$

where f(**X**) is an $\boldsymbol{n} \times 1$ matrix containing $\boldsymbol{n}$ observations, $\boldsymbol{\beta}$ is a $\boldsymbol{k} \times 1$ matrix containing unknown model coefficients (which is dependent on the model on which the experimental design is based upon), and $\boldsymbol{e}$ is an $\boldsymbol{n} \times 1$ matrix containing prediction errors of observations. The type of regression model is dependent on the purpose of the analysis. Often, it is a second-order polynomial equation which allows for estimation of main effects, potential two-factor interaction effect between the independent variables, and quadratic effects of the independent variables, which in turn allows for estimation and identification of maximums or optimal points. Higher order interaction effects may be disregarded as they often contribute insignificantly to the regression model.

2.3.3.2. D-optimal design selection algorithm

The selection of a D-optimal subset is based on a computer algorithm. Direct comparison of all possible subsets of points from a candidate set requires a significant amount of computing resources when taking into consideration all possible permutations, as discussed above. The use of an algorithm significantly reduces the load on computational power.

There are several algorithms present in the literature for selecting a D-optimal subset of experimental design points from a candidate set, such as: (i) Fedorov's exchange algorithm (Fedorov, 1972); (ii) modified Fedorov's exchange algorithm (Ogungbenro, Graham, Gueorguieva, & Aarons, 2005); (iii) DETMAX algorithm (Mitchell, 1974); and (iv) sequential designs (Dror & Steinberg, 2008), to name a few.

Fedorov's exchange algorithm is one of the most utilised exchange algorithms in optimal design theory, and has been implemented in several statistical and mathematical software packages. In brief, the algorithm is as follows:

1. An $\boldsymbol{n} \times \boldsymbol{k}$ subset, containing $\boldsymbol{n}$ design points such that $\boldsymbol{n} > \boldsymbol{k}$, is chosen from a candidate set, and the determinant of the information matrix for this subset is calculated.
2. The variance function of each design point in the selected subset and candidate set is calculated, and differences between all possible pairs between the subset ($\mathbf{x_i}$) and candidate set ($\mathbf{x_j}$) are determined.
3. The pair of design points that has the largest positive difference is identified and exchanged, which leads to a larger determinant of the new information matrix.
4. Steps 2 and 3 are repeated until the difference in variance functions between the subset and candidate set are the same, and there is no further change in the determinant of the information matrix.

2.3.3.3. Quality indicators of experimental designs

Several parameters can be used for the estimation of experimental design quality. The D-efficiency of an experimental design, which is a measurement of D-optimality, is given by Equation 2.5:

$$\text{D-efficiency} = \frac{|(\mathbf{X}'\,\mathbf{X})|^{\frac{1}{\mathbf{k}}}}{\mathbf{n}} \qquad \text{(Equation 2.5)}$$

where **X** is the matrix of design points scaled to the range of -1 to 1, ***k*** is the number of factors, and ***n*** is the number of design points. The value of D-efficiency ranges from 0 (not D-optimal) to 1 (fully D-optimal). Classical symmetrical designs such as full factorials, fractional factorials, and response surface methodology are fully D-optimal (de Aguiar et al., 1995; Olsson et al., 2004b), and therefore have D-efficiencies of one. Comparison of D-efficiencies between different experimental designs allows the experimenter to select a design for having greater confidence in estimating regression coefficients. However, it should be noted that the calculation of D-efficiency using Equation 2.5 is only applicable to two-level designs. For designs with more than two levels per variable, a scaling factor should be applied to the calculated D-efficiency in order to convert the D-efficiency to a range of zero to one, such that comparisons to two-level designs can be made.

Although fractional factorial designs may provide an experimental design with D-efficiency equals to one, and are readily available, they suffer the drawback of being unable to fully resolve confounded variables, especially for designs with lower resolutions. Such aliasing can be represented in a correlation table which compares the degree of correlation between a pair of model variable, as shown in Figure 2.3.

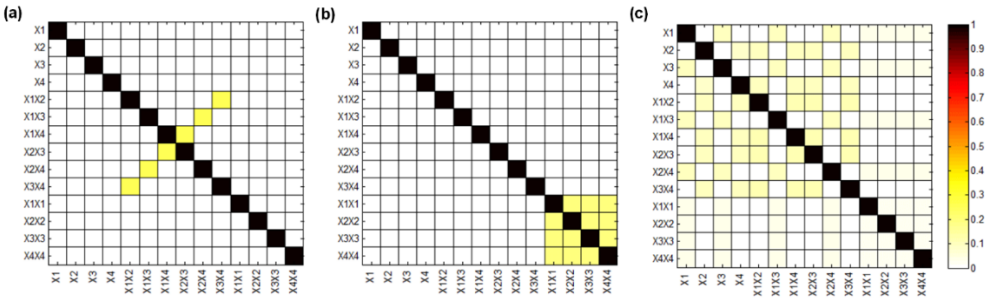

Figure 2.3: Correlation plots for (a) fractional factorial design; (b) Box-Behnken design; (c) D-optimal design for four factors and three levels. Correlation between model variables (linear, interaction, and quadratic effects) up to the second degree is indicated using a colour scale.

This can be reduced through utilisation of Box-Behnken or optimal designs. However, Box-Behnken designs for a large number of design variables may result in a large number of samples, which falls into the problem of large number of experimental runs. D-optimal designs are able to reduce the degree of severe correlations between model variables, but are often not balanced, and their generation is dependent on a computer algorithm for the reasons stated above. As such, the experimenter will have to decide between the convenience of standard, classical designs such as fractional factorial or Box-Behnken designs, and the greater computing complications of the more versatile optimal designs.

2.3.3.4. Applications of D-optimal experimental designs

There has been some recent applications of D-optimal designs in sensory, flavour, and shelf life-related studies, as summarised in Table 2.3.

Table 2.3: Applications of D-optimal designs in sensory and flavour studies.

Product/property	Purpose for using D-optimal designs	Experimental design	References
Taste interactions in citrus beverages	Sample size reduction	Three-factor mixed-level, 16 runs	Hewson, Hollowood, Chandra, & Hort (2008)
Multimodal taste interactions in carbonated beverages	Sample size reduction	Three-factor mixed-level, 18 runs	Hewson, Hollowood, Chandra, & Hort (2009)
Salad dressing	Sensory analysis and shelf life study	Three-factor mixed-level, 14 runs	Jang, Park, & Park (2011)
Red wine ageing process	Sample size reduction and use of a nonstandard model	Two-factor mixed-level, 20 runs	Puškaš & Miljić (2012)
Energy drink	Sensory analysis and shelf life study	Two-factor mixed-level, 13 runs	Shiby, Radhakrishna, & Bawa (2013)

Hewson and co-workers (2008 and 2009) made use of D-optimal designs in reducing the number of beverage samples for sensory evaluation in the investigation of taste-aroma-trigeminal interactions. In the first study, odour-taste interactions were investigated using a combination of lactic or citric acids, glucose or fructose, and two levels of citrus flavour, which resulted in a three-factor, mixed-level full factorial design. A D-optimal subset of the candidate design was identified to reduce the total number of samples that were evaluated from 24 (four levels for glucose, three levels for lactic acid, and two levels for flavour) to 16. Four predictive models, each for a combination of a sugar and an acid, were developed to predict flavour intensity, sweetness, and sourness. In another similar study involving the use of a D-optimal subset for the reduction in total number of samples, a mixed-level design containing 18 runs was used.

In another study conducted by Puškaš and Miljić (2012) on the quality of aged red wine, a D-optimal subset was used to reduce the number of samples

investigated from 42 to 20. There were a total of six levels investigated for seed content added to the samples, and seven levels corresponding to the total ageing time. Results obtained were fitted into third-order polynomial regression models to predict total phenols, anthocyanin content, flavan-3-ol content, colour, and hue of the aged red wine samples, as a function of seed content and ageing time.

Shiby, Radhakrishna, and Bawa (2013) made use of D-optimal designs in the development of whey-fruit juice energy drink mixes to reduce the number of samples from a two-factor, mixed-level mixture design with a design constraint. Whey was mixed with either grape or pomegranate juice with each component at a minimum of 20% and a maximum of 80%, and freeze dried to produce an energy drink dry mix, which was then stored for shelf life and sensory evaluations.

In another study conducted by Olsson, Gottfries, and Wold (2004a), D-optimal designs were utilised in a so-called D-optimal onion design to select a subset of compounds from a candidate set of synthesised compounds with peroxisome proliferator-activated receptor (PPAR) α and γ activity, in order to investigate structural-efficacy relationships. D-optimal onion design was named as such due to the division of the candidate set into multiple layers based on the Euclidean distance of a design point from the centre point. A D-optimal selection was made for individual layers, which were combined together to form an experimental design, resembling the multi-layered nature of onions. The onion design was shown to perform better than standard D-optimal designs, based on the root-mean-squared errors of prediction. It provided greater control of the inner design space, compared to standard D-optimal designs which tend

to select extreme points in order to maximise the determinant. Although this study fell beyond the scope of flavour and sensory science, it is worth mentioning as the same principles can be adapted for use in designing experiments or sensory trials.

2.4. Use of multivariate analysis techniques

Raw datasets obtained from experiments often present little significance until useful information and relationships between factors can be extracted. The use of one-dimensional statistical methods such as analysis of variance (ANOVA) and correlation tests may provide experimenters with trends and patterns within a set of data, but limited information relating multiple datasets, such as between that of a set of sensory attributes and the consumer liking of a product, can be inferred from such methods. To put it simply, the experimenter is only able to obtain information on differences between products or linear trends between different product attributes, as suggested by Zielinski et al. (2014). Mathematical and computer methods have been used in signal calibration in chemometrics, and tasks involving pattern recognition, classification, and regression, and have found applications in many fields including medicine, engineering, and image processing, to name a few.

Univariate regression methods such as one-factor analysis of variance (ANOVA) are often insufficient in finding patterns between food physicochemical attributes, sensory profiles, and hedonic properties, due to a multitude of compounds and physical attributes that are present in food products (Zielinski et al., 2014). Furthermore, the presence of masking and synergistic effects,

especially between taste and odour properties of foods creates a nonlinear relationship between these factors (Noble & Ebeler, 2002). Often, a single product property such as texture or flavour may be related to several sensory attributes, as perceived by the human brain (Perrot et al., 2006). All these combine to give a highly complex relationship, which cannot be easily analysed using univariate methods. Multivariate statistical methods become essential for such analyses, and can be loosely categorised into linear and nonlinear methods, which will be briefly discussed in the next two sections.

2.4.1. Linear regression

Linear regression is used to study the linear relationship between a group of independent variables (predictor variables), and a set of dependent variable(s). In general, linear model is of the form (Equation 2.6):

$$\mathbf{Y} = \beta_0 + \beta_1\mathbf{x}_1 + \beta_2\mathbf{x}_2 + ... + \beta_n\mathbf{x}_n + \mathbf{E} \qquad \text{(Equation 2.6)}$$

where **Y** is a vector of dependent variables, **β** is a vector of regression coefficients, **x** is a matrix for independent variables, and **E** is a vector associated with errors of the estimation. For example, the intensity of citrus attribute in an orange flavoured beverage may be a function of the concentrations of sucrose, ascorbic acid, and total amounts of terpenes present in the product. Likewise, this can be extended to prediction of consumer acceptance, where the liking of

a green tea drink may be positively correlated to the floral and green sensory attributes, and negatively correlated to bitterness.

There are a number of statistical methods for multiple linear regression, of which the most commonly encountered techniques in the fields of chemometrics and sensory science are multiple linear regression, partial least squares regression (PLSR), and internal and external preference mapping, both of which are variants of principal component analysis. Examples of recent studies utilising these methods have been summarised in Table 2.4.

Table 2.4: Application of linear regression methods in flavour and sensory studies.

Product	Purpose	Analytical methodology	References
Strawberries	Correlate consumer acceptability scores with sensory attribute intensities	Multiple linear regression using principal components	Ares, Barrios, Lareo, & Lema (2009)
Bread	Relate sensory characteristics to volatile composition, and correlate consumer perception to chemical composition	Partial least squares regression	Heenan, Dufour, Hamid, Harvey, & Delahunty (2009)
Dry-cured ham	Identify sensory characteristics driving consumer acceptability	External preference mapping using principal components	Resano, Sanjuán, Cilla, Roncalés, & Albisu (2010)
Ice cream	Correlate descriptive attributes and hedonic judgements, and investigate effects of formula composition on sensory profile	Partial least squares regression	Soukoulis, Lyroni, & Tzia (2010)
Oolong tea	Investigate relationship between chemical composition and sensory profile	Multiple linear regression using principal components	Wang et al. (2010)

Product	Purpose	Analytical methodology	References
White wine	Correlate volatile and sensory data sets	Partial least squares regression	González Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara (2011)
Pomelo juice	Correlate instrumental data and sensory profile	Partial least squares regression	Cheong, Liu, Zhou, Curran, & Yu (2012)
Black tea	Correlate chemical and aroma profile, and to develop measurement models for tea aroma quality	Stepwise multiple linear regression	Pang et al. (2012)
Fruit smoothies	Investigate effects of chemical and physical attributes on overall sensory properties	Partial least squares regression	Keenan, Brunton, Mitchell, Gormley, & Butler (2012)
Sweet potato	Correlate sensory properties to carotenoid and dry matter contents	Stepwise multiple linear regression	Tomlins, Owori, Bechoff, Menya, & Westby (2012)
Blanched apple slices	Correlate texture attributes to rheological properties	Partial least squares regression	Loredo, Guerrero, & Alzamora (2014)
Lingonberry	Correlate sensory characteristics with chemical composition	Partial least squares regression	Viljanen, Heiniö, Juvonen, Kössö, & Puupponen-Pimiä (2014)
Beer	Correlate sensory profile and higher alcohol and ester production during fermentation	Partial least squares regression, artificial neural network, support vector machine	Dong et al. (2014)
Oranges, pineapples, and grapes	Correlate consumer liking and sensory attributes to physical and physicochemical properties	Univariate regression and multiple linear regression	Corrêa et al. (2014)
White wine	Correlate sensory properties to chemical components	Partial least squares regression	Liu et al. (2015)

2.4.1.1. Multiple linear regression

Multiple linear regression refers to the prediction of a single dependent variable from multiple independent variables, and can be considered as the most basic form of linear regression. Linear regression using a single independent variable is often insufficient, and is unlikely to yield a satisfactory explanation of the relationship between the independent and dependent variables, given that the number of predictor variables encountered in sensory evaluation of food can be numerous.

The most often used approach to multiple linear regression is the least squares approach, which is to fit a line through a plot of independent versus dependent variables in a multidimensional hyperspace, minimising the errors of the sum of squares of deviation. Computation of the regression coefficients is a degree of freedom issue, that is to say, the number of observations or samples has to be greater than the number of independent variables ($\boldsymbol{n} > \boldsymbol{k}$), without which, the $\boldsymbol{k} \times \boldsymbol{k}$ covariance matrix of $\mathbf{X}'\mathbf{X}$ is singular, and there is rank deficiency and insufficient information for estimation of model coefficients.

Stepwise multiple linear regression is a linear regression technique that uses feature selection in building a regression model. In a nutshell, the regression procedure is divided into multiple steps, during which a model term is either added or removed from the regression model. The F-statistic is used to compare the new regression model against the model at the start of each step. Model terms with the most significant change in the F-statistic will be added or removed, such that only the most relevant features will be used in model building. Stepwise regression with only added model terms is known as forward

stepwise regression; stepwise regression that only allows for removal of model terms, commonly from an initial full model, is known as backward regression. A combination of both types of feature selection is the most commonly type of stepwise regression.

Stepwise multiple linear regression may be used as an alternative to full multiple linear regression due to its feature selection property, allowing model selection to stop when the addition or deletion of model terms does not improve the quality of the resulting model. This property of stepwise multiple linear regression is independent of the number of model terms or observations, thus allowing $\boldsymbol{k} \leq \boldsymbol{n}$ criterion in regression problems to be satisfied or ignored.

A general algorithm for forward-backward stepwise multiple linear regression is as follows:

1. Specify type of regression model for fitting, as well as model terms that should be included at the initial step.
2. At each step, the F statistic of each model term, when included into or removed from the model, is determined.
3. The model term that results in the most significant F statistic will be included or excluded from the model.
4. Steps two and three are repeated until there is no significant change in the F statistic.

2.4.1.2. Partial least squares regression

Partial least squares regression (PLSR), or sometimes referred to as "projection onto latent structures", was developed in the 1970s as a tool in economics, but has since been adopted in a number of fields, notably chemometrics, as well as the social sciences, as a statistical method. In this section, we will briefly discuss the basic concepts of PLSR as a form of regression tool in sensory science. Reviews discussing the technical aspects of PLSR in greater details can be found elsewhere (Abdi, 2003; Geladi, 1988; Höskuldsson, 1988; Noble & Ebeler, 2002; Rosipal & Krämer, 2006; Wold, Sjöström, & Eriksson, 2001).

PLSR is a linear regression method that can be thought of as a combination between multiple linear regression and principal component analysis. Instead of using independent variables for regression, PLSR constructs new latent variables known as principal components, which are linear combinations of the original variables, and are not directly observed or measured (Rosipal & Krämer, 2006).

One of the most important features of PLSR in sensory science and chemometrics is its ability to deal with big data. As mentioned previously in Section 2.4.1.1, multiple linear regression is not feasible if the number of variables is greater than the number of observations or samples. In such cases, there may be a few latent variables that are sufficient in explaining most of the variation in the dependent variables. PLSR uses an extracted set of latent variables from the original independent variables to predict latent variables extracted from the original dependent variables, thereby indirectly predicting response variables using the predictors. Like multiple linear regression, PLSR

seeks to fit the model given in Equation 2.6, where **Y** is a matrix of $\boldsymbol{n}$ observations by $\boldsymbol{q}$ dependent variables, **X** is a $\boldsymbol{n}$ by $\boldsymbol{p}$ matrix, **β** is a $\boldsymbol{p}$ by $\boldsymbol{q}$ matrix containing the PLSR regression coefficients, and **E** is noise or residuals associated with both independent and dependent variables.

The nonlinear iterative partial least squares (NIPALS) and statistically inspired modification of PLS (SIMPLS) algorithms are two of the most commonly used algorithms for PLSR. For a more technical discussion on the algorithms, we refer readers to the articles by Geladi and Kowalski (1986) and de Jong (1993).

The number of components chosen to compute PLSR is an important factor in PLSR. While it is possible to use all components in constructing the PLSR model, this is not often done as components of higher degrees often explain a lower amount of variances, and may contain experimental noise, which is screened out in the earlier components. However, data containing a higher degree of nonlinearity may require a larger number of components to explain the nonlinearities associated with the data. Data transformation methods may be applied prior to PLSR to remove the nonlinearities, which may decrease the number of components required to construct PLSR models (Geladi & Kowalski, 1986).

PLSR has been used in a wide range of studies in analysis of flavours and sensory properties in food products. In most cases, PLSR was used to establish correlations between chemical components including taste and odour active compounds and sensory perceptions, sensory attributes and consumer preferences or acceptance, as well as between chemical components and hedonic properties. Gao et al. (2015) developed a PLSR model to correlate

concentrations of phenolic compounds in red wines with sensory attributes, which included appearance, fragrance, mouthfeel, and overall impressions, as determined by a trained sensory panel. The first two principal components were found to explain up to 62% of variance in appearance, and 40.1% of variance in mouthfeel perceptions. In another study related to wines, Liu et al. (2015) investigated the relationship between tastants and odourants, and sensory attributes of Slovak white wines using PLSR. The first two components were found to explain 44% of variance in *X* (chemical components) and 78% of variance in *Y* (sensory attributes). Rather than using selected odourants based on the odour activity values (OAV), the chemical data were used entirely to allow for consideration of potential interactions between compounds with OAV of less than one.

Bindon et al. (2014) investigated the relationship between sensory properties and consumer preference in Cabernet Sauvignon wine. Chemical, sensory, and consumer liking data were obtained through chemical analyses, sensory profiling by a trained sensory panel, and consumer testing. PLSR models were developed to correlate chemical data to sensory data, as well as to associate sensory attributes and consumer liking to consumer demographics. The number of principal components selected for model development was based on the residual variance explained by each component. Validation of the PLSR model was done using the leave-one-out cross validation.

Preference mapping is an extension of principal component analysis and other related methods (principal component regression etc.) that is used in producing a visual representation of sensory and consumer data from which significant

trends and observations such as consumer segmentation and drivers of liking can be easily deduced from. Both internal and external preference mapping has been widely used in sensory studies, and an extensive discussion of preference mapping can be referred to Greenhoff and MacFie (1994).

2.4.2. Nonlinear regression models

Linear regression models are often used in correlating different data sets within food systems. However, the information present in food systems may be inherently nonlinear in nature, resulting in the need for data transformation or other techniques in order to transform a nonlinear relationship into one that can be modelled by linear techniques. One of the most commonly used transformation method is the Box-Cox transformation, as well as the other types of power transformation. However, the transformed variables or model coefficients may be difficult to interpret. The use of nonlinear regression methods allows users to correlate different data sets with nonlinear relationships without the need to perform data transformation. While linear regression, and in some cases of nonlinear regression such as modelling the growth rate of microorganisms, often translates to determination of model coefficients based on a known model structure (e.g. Equation 2.6), there may not be a predetermined nonlinear model in most cases, such as in the relationship between chemical constituents and sensory characteristics of a food product. Machine learning and artificial intelligence methods such as fuzzy logic and artificial neural network may be used in such examples, and will be briefly discussed in the following sections.

2.4.2.1. Fuzzy logic

Fuzzy logic is a decision making and classification tool that is modelled after the human thought process by generating complex decisions based on imprecise information. It has been used in sensory analysis to derive conclusions regarding consumer acceptance, ranking of food products, as well as identifying important factors for discrimination. Results obtained from sensory evaluation is dependent on the accuracy of human panels. As sensory results are often highly variable, subsequent analysis using statistical methods may produce results with low accuracy, precision, as well as repeatability (Mukhopadhyay et al., 2013). The uncertainty associated with such sensory results can be treated with computation methods involving the use of fuzzy logic.

Briefly, fuzzy logic refers to probability-based logic where there is a lack of definite or absolute values in the data, as opposed to classical logic, where a value is either false (denoted by 0) or true (denoted by 1). Considering this, probability-based fuzzy logic allows for values in between 0 and 1, allowing for data to be 'partially true or false'. The lack of an absolute value is an important aspect in sensory evaluation. For example, a fruit juice may not necessarily be absolutely sweet or sour, but may instead be partially sweet with a tinge of sourness. Furthermore, the nonlinear relationship between food constituents and sensory perception requires statistical analysis using nonlinear methods. Fuzzy logic is an important tool for analysing vague and fuzzy data that are frequently encountered in sensory data, and can be used to derive conclusions from sensory and hedonic properties of foods (Sinija & Mishra, 2011).

Fuzzy logic has been largely used for classification and ranking purposes in the food sensory domain. Sinija and Mishra (2011) made use of a fuzzy logic classifier on sensory data to rank the quality of instant green tea powder and granules, as well as to identify key product attributes affecting consumer perception of the green tea samples. Triplet scores for each green tea sample were calculated using the triangular membership function, based on a six-point acceptance scale for colour, flavour, taste, and strength. Flavour, taste, and colour were determined to be the most important product attributes affecting product quality, according to similarity values calculated from the membership functions of these attributes. Likewise, the similarity values of the tea samples were used to determine the ranking of green tea samples evaluated by the sensory panel.

Liu, Dong, Wang, Yin, and Li (2012) developed a fuzzy system to reduce the subjectivity of human sensory evaluation on Chinese beers, and to transform complex sensory data into a common index for comparison and ranking. Similarly, membership functions for beer attributes were determined and converted into fuzzy weight vectors, which were then used in fuzzy comprehension evaluation to determine the quality of beer samples, based on sensory input from a consumer panel consisting of 30 male and 30 female consumers.

Fuzzy logic has also been used in the analysis of sensory data in other food products and applications, such as consumer acceptability of Indian yoghurt (Routray & Mishra, 2012), sensory authentication of extra virgin olive oil

(Aparicio, Calvente, & Morales, 1996), and discrimination of red wines using adaptive fuzzy partition, which is derived from fuzzy logic (Piclin et al., 2008).

2.4.2.2. Artificial neural network

Artificial neural networks (ANNs) are a type of nonlinear method which were designed to mimic pattern recognition and information storage processes performed by the brain and biological nervous system, with individual nodes modelled after biological neurons, and weighted connections mimicking axons and synapses. This can be contrasted with fuzzy logic, which also attempts to simulate complex human thought processes. An ANN consists of a network of connected neural units, also known as nodes, which are typically divided into the input, output, and hidden layers.

The input layer often corresponds to the independent variables, and its size is determined by the dimensionality of the input data set. The output layer generates the output of the ANN, which in most cases, is the predicted value of a dependent variable in a regression, or classification groups in classification problems. The hidden layer is a layer of nodes in between the input and output layers, and has no direct interaction with input and output data. This layer is the main 'workhorse' of the neural network, and is the main driving force behind ANN's ability to solve complex nonlinear problems.

Input to individual neurons is determined by the sum of outputs from neurons in the preceding layer, which in turn is affected by weights of individual neural connections and biases of preceding neurons. Weights and biases of an ANN

represent how information is processed by the neural network. The net input is then processed by the neuron transfer function, which is the same for neurons within the same layer, and information, or signal, is passed on to neurons in the subsequent layer. There are a number of transfer functions used in ANN, with the linear, and sigmoidal functions being the more commonly used transfer functions. The general workings of a single neural unit can be summarised by Equation 2.7, and shown in Figure 2.4:

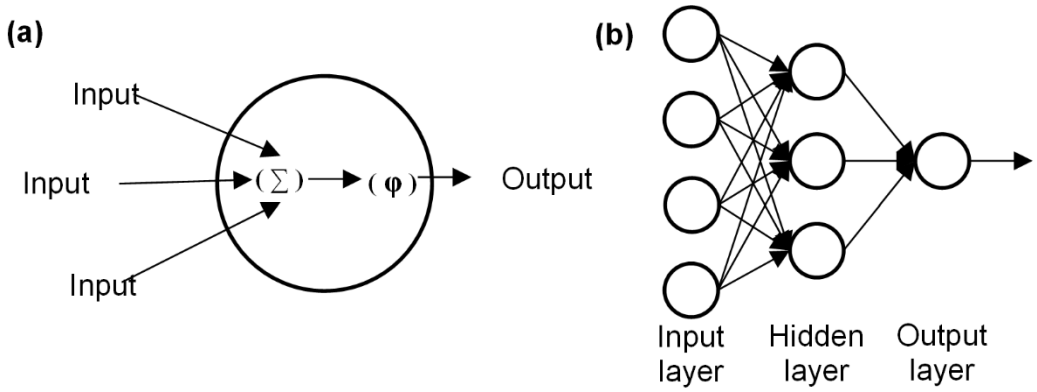

Figure 2.4: Illustration of (a) a single artificial neural unit, with the main processes of summation and transformation denoted by ∑ and **φ**, respectively; and (b) a three-layer artificial neural network with a 4-3-1 architecture, with each layer consisting of artificial neural units.

$$\mathbf{Y} = \boldsymbol{f}\left(\sum \mathbf{w}\,\mathbf{X} + \mathbf{b} \right) + e \qquad \text{(Equation 2.7)}$$

where **Y** is the output of the neural unit, ***f*** refers to the transfer function, **w** is the connection weight of the input signal, **X** is the input signal, **b** is the bias associated with the neural unit, and e is the noise of the input. Given that a typical ANN consists of a number of such neural units, the entire neural network

is able to model highly nonlinear data and theoretically approximate any function, linear or nonlinear in nature.

2.4.2.2.1. ANN training algorithms

The back propagation (BP) algorithm is one of the most commonly used algorithm for training neural networks. Neural networks were considered to be a dying trend in the 1960s and 70s due to hype and limitations in the early conceptions of the neural network and perceptron. The initial perceptron algorithm was limited in solving nonlinear classification problems (Wythoff, 1993). However, the development of the BP algorithm and incorporation of an additional hidden layer overcame this limitation, and led to a growth in the use of ANN as niche functions for classification and function approximation.

Using the BP algorithm, ANNs are able to learn by processing inputs and comparing against desired outputs. Input data is initially fed forward through the input layer, hidden layer, and finally the output layer, with data transformation taking place in the hidden and output layers. The resulting output (predicted values or classification results) is compared against the observed or experimental output. The calculated errors between the predicted and observed output are propagated back throughout the network, and weights of connections between nodes and biases associated with nodes in the hidden and output layers are adjusted accordingly to lower the error function. Each cycle of processing and propagation is known as an epoch, and it is not uncommon for ANNs to have thousands of epochs.

A potential drawback of the BP algorithm is the tendency for the algorithm to be stuck in a local minimum while searching for a global minimum for the error function (Huang, Kangas, & Rasco, 2007). The error of the network is decreased with each training epoch, as connection weights and biases are adjusted to achieve the global minimum of the error function. As such, users would be required to perform several rounds of network training and validation to ensure that training of the ANN has not resulted in local minima. Despite of this drawback, ANNs holds great potential in computing due to their ability to perform tasks beyond simple pattern recognition.

In training an ANN, the number of samples required should theoretically be at least equal to the number of variables present in the problem. Due to the presence of connection weights and biases, the number of observations required is very much higher in order to obtain a unique solution, compared to the regression or classification problem. However, this is unlikely to achieve in real life due to the difficulty in evaluating a large number of food products in a sensory evaluation, leading to multiple solutions when the network is trained repeatedly, as well as poor prediction capabilities using new, unseen data, and a lack of robustness (Huang et al., 2007). Another key factor affecting the robustness of the ANN is the size of the hidden layer. Although several rules for determining the number of nodes present are available in the literature, none of it have been fully established to be a good rule of thumb when training an ANN. One method which can be considered is to repeatedly train a network to search for the size of the hidden layer with the lowest error (mean square error, root mean squared error, and so on). The use of a hidden layer with a small number of nodes may be unable to fully model complex relationships in datasets,

while including too many nodes in the hidden layer may lead to overfitting and poor generalisation of the overall relationship between independent and dependent variables.

2.4.2.2.2. Applications of ANN

ANNs have been utilised for prediction, function approximation and generation of patterns unachievable by conventional statistical or modelling methods (Cimpoiu et al., 2011; Khanchi et al., 2007; Yu & Wang, 2007). Recent applications of ANN in flavour-related studies have been summarised in Table 2.5.

Krishnamurthy, Srivastava, Paton, Bell, and Levy (2007) investigated the use of artificial neural networks in predicting consumer liking of ten commercial beef bouillons from sensory profiles obtained from trained sensory panels. ANN was compared with linear regression methods including stepwise multiple linear regression, principal component regression, and partial least squares regression for predicting consumer liking scores. Two data sets were used for training two ANNs, the first consisted of raw data, while the other consisted of data with a transformation function applied to it. The ANN trained using the transformed data was found to be the best in predicting consumer liking, along with the best tolerance to variations in trained panel scores.

Bahramparvar, Salehi, and Razavi (2014) evaluated the use of an ANN in predicting consumer acceptance of ice cream made from three different stabilisers. Consumer acceptance was predicted based on the magnitudes of six

sensory attributes determined by a trained panel. The size of the hidden layer was established as 10 nodes in a single layer by determining the mean absolute error, mean square error, and normalised mean square error of networks containing a different number of nodes in the hidden layer.

ANN is often used alongside electronic noses and tongues in the analyses of odour and taste active compounds in food products as a tool for correlating the nonlinear digital signals to sensory characteristics or hedonic properties (Cevoli et al., 2011). The electronic nose is an array of metal oxide gas sensors, capable of detecting mixtures of volatile compounds and representing the differing levels of volatiles as a multivariate output signal. In recent years, ANN has been used in the interpretation of electronic nose and gas chromatography data in the study of volatile compounds in cheese (Cevoli et al., 2011), wines (Kruzlicova et al., 2009; Lozano et al., 2008), coffee (Michishita et al., 2010), green tea (Yu et al., 2008), and pear (Zhang et al., 2008).

Table 2.5: Applications of ANN for regression and pattern recognition in flavour and sensory studies.

Product	Purpose	Analytical methodology	References
Green tea	Rapid determination of quality grade	Cluster analysis and artificial neural network for interpreting electronic nose data	Yu, Wang, Yao, Zhang, & Yu (2008)
Red and white wines	Wine discrimination	Probabilistic neural network for classification purposes using electronic nose data	Lozano, Santos, & Carmen Horrillo (2008)
White wine	Discrimination of white wine varieties	Classification of GCMS data using back propagation ANN	Kruzlicova et al. (2009)
Coffee	Evaluation and development of ready-to-drink espresso aroma	Regression of sensory data from GC-O and E-nose data using ANN	Michishita et al. (2010)
Pear	Development of fruit quality indices	ANN for prediction using E-nose data	Zhang, Wang, & Ye (2008)
Cheese	Classification of cheeses based on ripening times	Classification of GCMS data using back propagation ANN	Cevoli et al., (2011)
Beef bouillon	Prediction of consumer liking	ANN for prediction using trained sensory panel data	Krishnamurthy, Srivastava, Paton, Bell, & Levy (2007)
UHT milk	Prediction of changes in sensory profiles during storage	Back propagation ANN for prediction using concentrations of various chemical indicators	Singh, Ruhil, Jain, Patel, & Patil (2009)
Yoghurt	Prediction of consumer acceptance and purchase intent	ANN for prediction using several hedonic and sensory quality indicators	Cruz et al. (2011)
Ice cream	Prediction of consumer acceptance	ANN for prediction using sensory attributes as input data	Bahramparvar, Salehi, & Razavi (2014)
Soy sauce	Classification according to fermentation and geographical region	Back propagation ANN for classification using key variables of GCMS data	Xu et al. (2014)

2.5. Conclusions

In this chapter, several popular experimental design and regression methodologies have been presented and discussed, along with newer but less commonly applied computer algorithm-related techniques. While classical techniques such as fractional factorial designs and partial least squares regression have been widely used in food flavour and sensory related studies, these techniques may be inadequate in fully describing a complex and potentially nonlinear system found in food products. Computer-aided experimental designs such as optimal designs are gradually seeing greater use in food sensory evaluations. The selection of an experimental procedure in sensory and flavour science is critical to obtaining an accurate set of data for interpretation through regression models or other methods. Such observations are important in understanding the relationships between food products and consumers, which in turn are key in determining the direction of growth and research for businesses. The computer algorithm-related techniques discussed in this review may be able to provide experimenters with a greater depth of information over existing techniques. However, this requires an initial investment in the computational power and software licensing costs, which may be significant at the beginning. Furthermore, newer computer-based methods are still very much in development, and it is crucial that these methods should be furthered explored in order to be readily established and accepted in food flavour and sensory related studies.

CHAPTER 3

IDENTIFICATION OF KEY VOLATILE FLAVOUR KEYS IN THE DEVELOPMENT OF A READY-TO-DRINK GREEN TEA BEVERAGE

Abstract

Eight volatile flavour keys, each representing an aroma dimension of green tea and comprised of a mixture of volatile odourants found commonly in ready-to-drink green tea beverages, were mixed together using an olfactometer which allowed for controlled blending of mixtures of flavours into a single aroma profile, based on a 50-point D-optimal design to obtain a series of green tea odours. A consumer acceptance test was conducted to obtain liking information on these green tea odours. The most well-liked sample was an odour match of a commercial sample (liking score of 6.65 ± 1.30), while the least-liked sample had a liking score of 3.65 ± 1.49. A linear regression model was developed to objectively predict consumer liking based on the chemical formulation using stepwise regression, with a resulting coefficient of determination (R^2) of 0.890, and training and validation root-mean-squared errors (RMSE) of 0.175 and 0.629, respectively. Further analysis was conducted to identify flavour keys of lesser importance by performing stepwise regression on reduced experimental designs. Removal of the X_7 and X_8 flavour keys were found to have the least impact on the resulting model structure, and were thus fixed at the mid-level for subsequent studies.

Keywords: green tea; consumer liking; stepwise regression; olfactometer; experimental design

3.1. Introduction

Tea, a beverage made by infusing the leaves of *Camellia sinensis* in hot water (Higdon & Frei, 2003), is the most popular drink in the world after water with an annual production of 3.95 million tonnes in 2007 (Butt & Sultan, 2009; Higdon & Frei, 2003). Ready-to-drink (RTD) green tea beverages are becoming increasingly popular, due to the health benefits associated with the consumption of green tea, such as reduction in risks of diabetes and cardiovascular diseases, as well as its anti-carcinogenic and anti-obesity properties (Butt & Sultan, 2009). In light of this, beverage manufacturers have responded to this growing trend by increasing the production and availability of RTD green tea beverages (Zegler, 2013).

In order to increase sales of food and beverage products, the extrinsic (brand, nutritional labelling, advertising and marketing) and intrinsic (flavour and texture profiles) properties of food products will have to be taken into consideration (Fernqvist & Ekelund, 2014). Food aroma properties have been widely accepted to play a critical role in consumer liking and acceptability, and is one of the key considerations in the development of new food and beverage products (Leclercq & Blancher, 2012). Volatile odourants are perceived by humans in the olfactory epithelium orthonasally (through the nose) or retronasally (from the mouth via the pharynx), giving rise to a perception of flavours from the external environment or from the mouth respectively (Small,

Gerber, Mak, & Hummel, 2005). Due to the differences in mechanisms in which odourant molecules reach receptors in the olfactory mucosa, the efficiency in which an odour is detected by orthonasal and retronasal stimulus is different. A previous study by Pierce and Halpern (1996) suggested that odour detection via the orthonasal route led to a higher degree of accuracy in the detection and identification of volatile compounds. The Virtual Aroma Synthesiser (VAS) is an olfactometer developed by Givaudan to deliver controlled amounts of food odourants in a gas phase through a nose piece which will be detected by sniffing through the smelling port. This allows for a rapid evaluation of samples, which when contrasted to traditional sensory evaluation of food products, requires less time and resources in the collection of sensory data (Leclercq & Blancher, 2012).

In recent years, there has been an increase in the number of studies regarding the use of electronic nose (e-nose) and tongue (e-tongue) in the analysis of chemical constituents in green tea (Chen, Zhao, Chen, Lin, & Zhao, 2011; Huo et al., 2014). However, it is limited in its use in discriminating green tea quality, due to fundamental differences between detection of chemical compounds by an instrument and perception of an odour by biological organisms. Although the relationship between volatile odourants in green tea brews and leaves, and consumer acceptability has been studied extensively (Kumazawa & Masuda, 2002; Lin, Dai, Guo, Xu, & Wang, 2012; Wang & Ruan, 2009), there are limited studies focusing on RTD green tea beverages. Kim et al. (2007) studied the effects of heating on the chemical constituents and colour changes of RTD green tea beverages, without considering effects on consumer acceptability and liking. It would be of relevance and interest to manufacturers of RTD green tea beverage to identify key chemical compounds affecting consumer liking.

As such, the main objectives of this study were to identify the key volatile aroma keys affecting hedonic properties of RTD green tea, and to develop a regression model for predicting consumer liking. This information would subsequently be used for dimension reduction in future studies involving non-volatile tastants and their effect on the flavour profile and consumer acceptance of RTD green tea.

3.2. Materials and Methods

3.2.1. Materials

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

3.2.2. Sample preparation

Eight green tea flavour keys comprising of mixtures of different volatile flavour chemicals were developed for the MiniVAS, which is a portable version of the VAS. These flavour keys were designed by in-house flavourists to be able to reproduce the aroma profile of seven commercial RTD green tea products obtained from supermarkets in the People's Republic of China. The MiniVAS is a proprietary tool developed by Givaudan allowing for rapid and automated

mixing of several flavour blocks, reducing the amount of time required for evaluating a large number of samples. The eight flavour keys represent aromas associated with the commercial RTD green tea samples in general, and are summarised in Table 3.1.

Flavour keys were dosed into polyethylene tubes containing polystyrene foam particles according to the required amount. The polyethylene tubes were then mixed on an orbital shaker for 30 min to allow for adequate distribution of the flavour keys, followed by storage at 4 °C until subsequent use on the MiniVAS.

Table 3.1: In-house formulated flavour keys used in the MiniVAS.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

3.2.3. Sensory analysis

A consumer acceptance study was conducted using a D-optimal experimental design consisting of 58 design points. Forty-two points were generated from the OPTEX procedure in the SAS package (SAS Institute Inc., North Carolina, USA) to form a D-optimal design that allowed for estimation of all main effects and two-factor interactions at two factor levels. Eight variables were included, corresponding to the eight green tea flavour keys that were developed. In

additionally, eight design points were added to allow for a quadratic fit to the eight flavours keys. The resulting three-level experimental design is summarised in Table 3.2. The aromas of three commercial RTD green tea samples were mimicked using the eight flavour keys (F52 to F54), and together with another design point (F51) consisting of mid-levels were included in the design as part of the validation set, which was evaluated in triplicate (once per session). Lastly, two dummy points were also included for calibration purposes.

A total of 126 untrained panellists from the National University of Singapore were recruited through online (emails, online surveys) and offline means (approached directly). Prior to joining the sensory panel, participants were required to complete a pre-sensory survey to provide information on frequency of consumption of green tea, and other demographics. Participants that disliked or did not regularly consume green tea were excluded from the consumer preference study. Only participants who were frequent consumers of RTD green tea beverages (at least once per week) were recruited to participate in the sensory evaluation of the green tea model systems. Panellists were each assigned a random three-digit judge code.

Samples were evaluated in duplicate over two sessions, on a liking scale of one to nine, with one denoting an extreme dislike for the odour, and nine denoting an extreme liking for the odour. Presentation order of the samples was based on a 54 × 54 Latin square. Panellists were required to place their nose at the smelling port of the MiniVAS, following which an odour would be emitted from the instrument. Facilitators were present to input scores for the panellists while they were evaluating green tea odour samples. Panellists were instructed to

breathe into a polyethylene cup filled with wet paper towels (source of humidified air) in order to remove carry-over odours in between samples.

3.2.4. Data and statistical analyses

Consumer liking scores were presented as mean ± standard deviation. Analysis of variance (ANOVA) was performed using MATLAB version 7.12 (The MathWorks, Inc., USA), with Tukey's Honestly Significant Difference as a post-hoc test. Differences were considered to be statistically significant at $p < 0.05$. The correlation between chemical formulation and consumer liking was investigated using regression analysis. All variables were normalised to a range of 0.1 to 0.9 based on the minimum and maximum values of each variable to ensure numerical consistency.

Forward stepwise multiple linear regression was performed using MATLAB version 7.12. The coefficient of determination of the regression line (R^2) and root-mean-squared errors of predicted values (RMSE) were used to describe reliability of the predictive power of regression models.

Table 3.2: Fifty-point D-optimal design for eight factors and three levels.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

3.3. Results and discussion

3.3.1. Consumer preference study

A consumer acceptance study was conducted to obtain liking scores for aromas associated with RTD green tea beverages using a 50-point D-optimal design. The most well liked sample was sample F54 (odour match of RTD green tea sample no. 3; 6.65 ± 1.30), while the least liked was sample F17 (3.65 ± 1.49). The resulting distribution of scores is shown in the form of a box-and-whiskers plot in Figure 3.1.

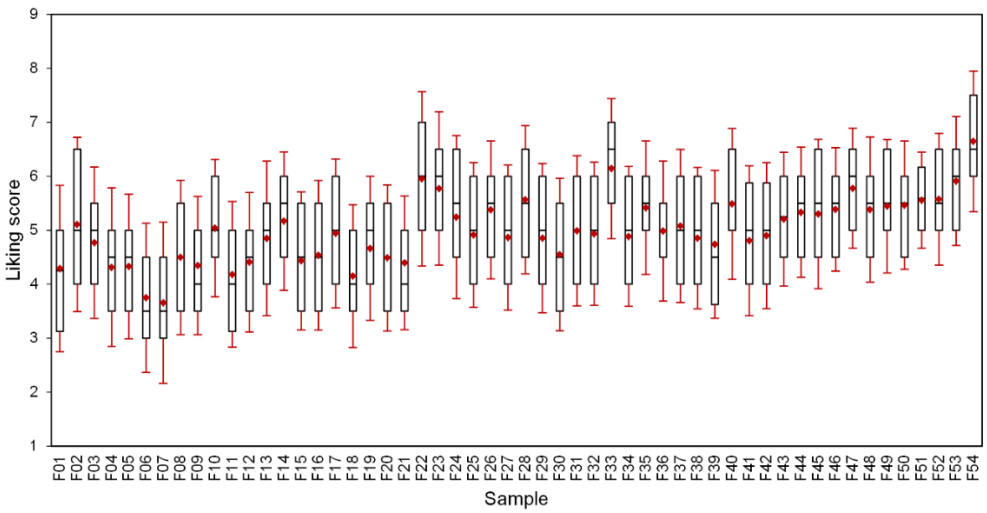

Figure 3.1: Box-whisker plot of average consumer liking scores. First quartile, median and third quartile scores are denoted by the box; mean scores for samples are represented by diamond within box; whiskers show one standard deviation above and below mean of data. n = 126.

One-way ANOVA results indicated that the observed consumer liking scores for 54 green tea odours differed significantly ($p < 0.05$), despite of the relatively narrow range of liking scores. Post-hoc test was conducted using Tukey's Honestly Significant Difference, and results obtained were summarised in Table

3.3. A total of 24 groups were obtained, and significant differences were detected at a significant difference of 0.69 between the most well-liked odour sample (F54) and the sample in the subsequent group (F22), and a significant difference of 0.67 in the least-liked samples (F07 and F05) in the groups with the lowest consumer liking score, according to ANOVA.

Table 3.3: ANOVA post-hoc test results obtained using Tukey's Honestly Significant difference.

Sample	Liking score																								
F07	3.65 ± 1.49																								x
F06	3.75 ± 1.38																							w	x
F18	4.15 ± 1.32																						v	w	x
F11	4.18 ± 1.35																					u	v	w	x
F01	4.29 ± 1.54																				t	u	v	w	x
F04	4.31 ± 1.47																			s	t	u	v	w	x
F05	4.33 ± 1.34																		r	s	t	u	v	w	
F09	4.35 ± 1.28																		r	s	t	u	v	w	
F21	4.40 ± 1.24																	q	r	s	t	u	v	w	
F12	4.41 ± 1.29																p	q	r	s	t	u	v	w	
F15	4.43 ± 1.28																p	q	r	s	t	u	v		
F20	4.49 ± 1.36															o	p	q	r	s	t	u	v		
F08	4.50 ± 1.43															o	p	q	r	s	t	u	v		
F16	4.54 ± 1.39														m	o	p	q	r	s	t	u	v		
F30	4.55 ± 1.41														m	o	p	q	r	s	t	u	v		
F19	4.66 ± 1.33													l	m	o	p	q	r	s	t	u	v		
F39	4.74 ± 1.37												k	l	m	o	p	q	r	s	t	u	v		
F03	4.77 ± 1.40											j	k	l	m	o	p	q	r	s	t	u	v		
F41	4.81 ± 1.39										i	j	k	l	m	o	p	q	r	s	t	u	v		
F13	4.85 ± 1.31									h	i	j	k	l	m	o	p	q	r	s	t	u			
F29	4.85 ± 1.38									h	i	j	k	l	m	o	p	q	r	s	t	u			
F38	4.85 ± 1.43									h	i	j	k	l	m	o	p	q	r	s	t	u			
F27	4.87 ± 1.35									h	i	j	k	l	m	o	p	q	r	s	t				
F34	4.88 ± 1.29								g	h	i	j	k	l	m	o	p	q	r	s	t				
F42	4.90 ± 1.35							f	g	h	i	j	k	l	m	o	p	q	r	s	t				
F25	4.91 ± 1.34							f	g	h	i	j	k	l	m	o	p	q	r	s	t				
F32	4.93 ± 1.33							f	g	h	i	j	k	l	m	o	p	q	r	s	t				
F17	4.94 ± 1.38							f	g	h	i	j	k	l	m	o	p	q	r	s	t				
F36	4.98 ± 1.30							f	g	h	i	j	k	l	m	o	p	q	r	s					
F31	4.99 ± 1.39							f	g	h	i	j	k	l	m	o	p	q	r						
F10	5.04 ± 1.27							f	g	h	i	j	k	l	m	o	p	q							
F37	5.08 ± 1.42							f	g	h	i	j	k	l	m	o	p								
F02	5.11 ± 1.61						e	f	g	h	i	j	k	l	m	o									
F14	5.17 ± 1.28						e	f	g	h	i	j	k	l	m										
F43	5.21 ± 1.24						e	f	g	h	i	j	k	l	m										
F24	5.24 ± 1.51					d	e	f	g	h	i	j	k	l											
F45	5.30 ± 1.38				c	d	e	f	g	h	i	j	k	l											
F44	5.33 ± 1.20				c	d	e	f	g	h	i	j	k	l											
F26	5.38 ± 1.14				c	d	e	f	g	h	i	j	k												
F48	5.38 ± 1.28				c	d	e	f	g	h	i	j	k												
F46	5.38 ± 1.34				c	d	e	f	g	h	i	j	k												
F35	5.42 ± 1.24				c	d	e	f	g	h	i	j													
F49	5.44 ± 1.24				c	d	e	f	g	h	i														
F50	5.46 ± 1.19		b	c	d	e	f	g	h	i															
F40	5.49 ± 1.40		b	c	d	e	f	g	h																
F51	5.56 ± 0.89		b	c	d	e	f	g																	
F28	5.56 ± 1.37		b	c	d	e	f																		
F52	5.57 ± 1.22		b	c	d	e	f																		
F23	5.77 ± 1.42		b	c	d	e																			
F47	5.78 ± 1.11		b	c	d	e																			
F53	5.91 ± 1.19		b	c	d																				
F22	5.95 ± 1.62		b	c																					
F33	6.14 ± 1.30	a	b																						
F54	6.65 ± 1.30	a																							

[†] Products with same lower-case letters are not significantly different.

3.3.2. Modelling of analytical and hedonic datasets

Multiple linear regression model using forward stepwise regression was used to identify the main drivers of liking. All main effect, interaction and quadratic terms were excluded from the initial model, and at each step, variables were added or deleted one at a time until an acceptable regression model was obtained. Addition or deletion of variables was dependent on the F-test, which compares quality of the model at any given step against the previous step. Entrance and exit tolerances were set at $p = 0.05$. In this study, main effects were prioritised over interaction and quadratic terms, i.e. as long as the p of a main effect was less than 0.05, the variable would be included in the model, even though there were other interactions or quadratic terms with smaller p. Although pairwise interactions of model variables were important, model factors consisting of a single variable would be able to provide a parsimonious interpretation of the relationship between volatile flavour keys and consumer liking of the overall aroma profile.

The eight flavour keys were used as predictor variables while the mean consumer liking scores between the two sessions were used as response variables. Fifty samples comprising of the experimental design and eight quadratic points were used for model training, and the remaining four points consisting of three matches of commercial samples and the average of mid-level sample were used for model validation. The dummy points were used as a calibration for consumers at the start of each evaluation session, and were not used in model development. A regression model containing only the statistically significant terms was developed (Equation 3.1). The derived model is as such:

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Predictor inputs and predicted liking scores were normalised to a range of 0.1 to 0.9. The coefficient of determination and root mean square error of the model were calculated as 0.890 and 0.175 respectively. Cross-validation of the model using the validation set resulted in a RMSE of 0.629. Residuals analysis revealed a fairly random distribution between bands, indicating that forward stepwise regression provided a good fit to the data. X_1 and X_3 flavour blocks were determined to be the main positive drivers of liking, suggesting that samples with stronger fruity and floral notes were positively correlated to liking within the panel of consumers. X_5 and X_6 flavour blocks were negative drivers of liking. A quadratic term for the X_3 flavour block was determined to be significant, indicating that there was a maximum dosage of the X_3 flavour block which produced an optimum liking score.

3.3.3. Dimensional reduction in experimental design

The experimental design for the preliminary study investigated the effects of eight flavour keys, for which there would be a need for reduction in the number of flavour keys for an increase in the degrees-of-freedom to account for non-

volatile tastants in the next stage of the study. Increasing the number of predictor variables is not an option, due to the increase in size of experimental design to cater for additional variables, which would result in an increase in the amount of resources required for conducting both trained and untrained panel sensory sessions.

Reduction in dimensionality of the analytical data comes in the form of removing insignificant flavour keys from the experimental design by fixing these factors at the mid-level such that they were no longer variables, but were still present as they still contribute to the overall aroma profile of RTD green tea beverages. The approach adopted in this study was to compare model quality for different sets of predictor variables, each with one or more variables removed. Stepwise regression was used to construct the regression models, in a manner similar to that mentioned previously. The R^2 and RMSE of the resulting regression models with one or more removed variables are shown in Figure 3.2.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 3.2: (a) Training set R^2; (b) training set RMSE; and (c) validation set RMSE of original model and regression models developed using experimental designs with one or more removed flavour keys.

From Figure 3.2, it can be seen that up to two flavour keys in combinations of X_2, X_7, and X_8, could be removed without significantly affecting model quality. Removal of the X_1, X_3, X_5, and X_6 flavour keys individually resulted in decreases in model R^2 and an increase in RMSE of both training and validation sets, highlighting the importance of these flavour keys relative to other keys or combination of keys. Removal of the X_4 flavour key seemingly resulted in a model of comparable quality to the original stepwise regression model, similar to that of removing the X_2, X_7, and X_8 flavour keys. However, this was probably due to an inadequate range of concentration used in this study. In-house evaluation of commercial RTD green tea beverage samples by flavourists, from whom the eight flavour keys were designed by, suggested that the X_4 flavour key was an important flavour aspect of the commercial samples. As such, it was recommended that the flavour key should be included in subsequent studies. Combinations of X_2, X_7 and X_8 flavour keys were selected to be removed. Figure 3.3 shows a comparison of the significant terms in stepwise regression models between the different sets of flavour keys removed.

Stepwise regression models developed from the removal of X_2 and X_8 flavour keys, and X_7 and X_8 flavour keys were the most similar to the original regression model (Equation 3.1), among the four data sets evaluated. The main effect terms were unchanged, with only slight differences in magnitudes. Removal of X_2 and X_7 flavour keys, and all three flavour keys resulted in a regression model containing very different significant terms, indicating a large change in terms of model stability. As such, the X_7 and X_8 flavour keys were chosen to be removed ultimately as the regression model quality was better than that of the data set with X_2 and X_8 flavour keys removed.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 3.3: Coefficients of significant regression model terms derived from stepwise regression based on experimental designs with (a) all variables intact; (b) X_2 and X_7 removed; (c) X_2 and X_8 removed; (d) X_7 and X_8 removed; and (e) X_2, X_7, and X_8 removed.

3.4. Conclusions

In conclusion, a linear regression model was developed to objectively predict consumer liking of green tea odour from mixtures of chemical flavour keys using stepwise regression. The X_1 and X_3 keys were found to be key drivers of liking, suggesting that fruity, floral smelling green teas were well-received compared to less fruity and floral smelling samples. Among the eight flavour keys that were used in this study, the X_7 and X_8 flavour keys were found to be of the least importance, and were removed from the experimental design in subsequent studies and replaced with two other non-volatile flavour keys.

CHAPTER 4

IDENTIFYING KEY NON-VOLATILE COMPOUNDS IN READY-TO-DRINK GREEN TEA AND THEIR IMPACT ON TASTE PROFILE

Abstract

Thirty-nine non-volatile compounds in seven ready-to-drink (RTD) green tea samples were analysed and quantified using liquid chromatography. Taste reconstruction experiments using thirteen selected compounds were conducted to identify the key non-volatile tastants. Taste profiles of the reconstructed samples did not differ significantly from the RTD tea samples. To investigate the taste contribution and significance of individual compounds, omission experiments were carried out by removing individual or a group of compounds. Sensory evaluation revealed that the astringent- and bitter-tasting (–)-epigallocatechin gallate, bitter-tasting caffeine, and the umami-tasting L-glutamic were the main contributors to the taste of RTD green tea. Subsequently, the taste profile of the reduced recombinant, comprising of a combination of these three compounds and L-theanine, was found to not differ significantly from the sample recombinant and RTD tea sample. Lastly, regression models were developed to objectively predict and assess the intensities of bitterness and astringency in RTD green teas.

Keywords: green tea; taste reconstruction; taste omission; half tongue test; sensory evaluation; mathematical modelling

4.1. Introduction

Tea quality is usually evaluated by professional tea tasters based on the appearance, aroma and taste of the tea brew, as well as appearance of the dry and infused leaves (Liang et al., 2008). Due to the subjectivity and inconsistency of this evaluation method, several studies have attempted to correlate the chemical constituents and sensory characteristics to the perceived quality index evaluated by professional tea tasters (Liang et al., 2008; Pongsuwan et al., 2008). Volatile compounds contribute to the aroma profile, while non-volatile components contribute to the taste profile of green tea, which includes the characteristic bitterness and astringency. It has been generally accepted in the literature that astringency is a tactile sensation felt on the tongue caused by the interaction between tea polyphenols and salivary proteins (Brossaud, Cheynier, & Noble, 2001). However, Rossetti, Bongaerts, Wantling, Stokes, and Williamson (2009) found that astringency is not entirely a tactile perception caused by the loss of lubrication in the oral cavity, but may instead involve other mechanisms, such as the inhibition of sodium ion channels on epithelial cells, as suggested by Simon, Hall, and Schiffman (1992).

Several groups of non-volatile compounds have been found to have potential activity on the taste profile of tea, including phenolic compounds, purine alkaloids, amino acids, nucleotides, carbohydrates, organic acids, ions and others (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006; Liang et al., 2008; Scharbert & Hofmann, 2005; Wang & Ruan, 2009). Tea polyphenols, particularly tea catechins, have been extensively researched, and were found to have an effect on the bitterness and astringency of tea (Narukawa, Kimata, Noga,

& Watanabe, 2010). Purine alkaloids, in particular caffeine, are the other major contributors to bitterness in tea. Although the bitter taste in tea may be attributed to a range of varying non-volatile compounds, the bitter taste transduction pathway varies between different tastants. Green tea catechins are known to activate the human bitter taste receptors hTAS2R14 and TAS2R39 (Roland et al., 2013; Yamazaki, Narukawa, Mochizuki, Misaka, & Watanabe, 2013) in a dose dependent manner, with nonlinear responses at low and high concentrations. On the other hand, caffeine and other methylxanthines have been suggested to induce a bitter taste without activating bitter taste receptors (Rosenzweig, Yan, Dasso, & Spielman, 1999).

On top of the commonly associated bitterness and astringency, green tea is often associated with having a unique umami taste quality, which might largely be contributed by L-glutamic acid and L-aspartic acid. L-theanine (5-*N*-ethyl-L-glutamine), a non-proteinogenic amino acid that makes up more than 50% of the free amino acids content in green tea leaves, has been reported to have sweet, brothy and umami characteristics, and has also been described by many studies to be taste-active in green tea (Ekborg-Ott, Taylor, & Armstrong, 1997; Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999).

While many of the past studies have focused on black tea, a limited number of studies were performed on green tea with an emphasis on its taste profile (Chaturvedula & Prakash, 2011). In light of the increasing demand for ready-to-drink (RTD) green tea in East Asian countries, companies may choose to focus their attention on the younger consumers who are frequently on-the-go and may therefore prefer products offering a greater degree of convenience

(Chen, Zhu, Tsang, & Huang, 2001). Hence, RTD green tea was chosen as a model beverage system in the present study to explore the relationship between non-volatile components and sensory perception.

As such, the main objective of this study was to identify the main non-volatile compounds affecting the taste profile of RTD green tea, and to develop regression models for the objective prediction of various taste attributes of RTD green tea. This was achieved through the following: (i) quantifying several groups of non-volatile compounds in commercially available RTD green tea samples; (ii) studying the taste activity of each compound; (iii) validating the taste contribution of the key compounds via sensory assessments consisting of taste reconstruction and omission experiments.

4.2. Materials and Methods

4.2.1. Samples, reagents, and standards

Seven types of ready-to-drink (RTD) bottled, unsweetened green tea samples, with no other added flavours unrelated to green tea (e.g. lemon, honey), were obtained from supermarkets in China and Japan. Unopened green tea samples were stored at 25 °C away from direct sunlight in their original packaging. Samples were opened prior to analyses, and were not reused. Green tea samples were filtered through a 0.45 μm membrane prior to chromatographic separation, and used directly for sensory analyses.

The following reagents and standards were obtained commercially: *o*-phosphoric acid 85%, HPLC-grade acetonitrile (ACN) and HPLC-grade

methanol (MeOH) from Merck KGaA (Darmstadt, Germany); caffeine, gallic acid, (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)-epigallocatechin (EGC), (−)-gallocatechin (GC), (−)-epicatechin (EC), (+)-catechin (C), L-aspartic acid (Asp), L-glutamic acid (Glu), amino acids standard, guanosine-5′-monophosphate disodium salt hydrate (GMP), inosine-5′-monophosphate disodium salt octahydrate (IMP) and sodium hydroxide solution 50% from Sigma-Aldrich Chemical Co. (Missouri, USA); L-theanine (Thea) from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan); ascorbic acid from DSM Nutritional Products Ltd. (Basel, Switzerland); AccQ-Fluor Reagent Kit and AccQ-Tag Eluent A Concentrate from Waters Corporation (Massachusetts, USA); skimmed milk from CP-Meiji Co. Ltd. (Bangkok, Thailand).

4.2.2. Quantitative analyses

4.2.2.1. High performance liquid chromatography (HPLC) system

All HPLC analyses were performed on an Agilent 1100 Series HPLC system (Santa Clara, USA), which consisted of a micro vacuum degasser, a quaternary pump, an autosampler, a thermostatted column component, a diode array detector, a variable wavelength detector, and a refractive index detector.

4.2.2.2. Analyses of catechins, gallic acid and caffeine

Standard solutions of catechins, gallic acid and caffeine were prepared by dissolving the required weight of each compound in deionised water. Ascorbic acid was added at a concentration of 5 mg/10 mL sample. All samples were filtered through a 0.45 μm membrane prior to HPLC analyses. Analysis was performed on the HPLC system equipped with an Agilent ZORBAX Eclipse XDB-C18 HPLC column (250 mm × 4.6 mm, 5 μm), according to Wang and Zhou (2004) with slight modifications described below. Mobile phase A consisted of 0.01% phosphoric acid in water, while mobile phase B was 100% methanol. Column temperature was set at 25 °C. Catechins, gallic acid and caffeine were detected at 230 nm and identified by comparison of retention times and spectrum of standard solutions. Analytes were quantified by external calibration standards. Performance of the HPLC method was validated through accuracy and precision tests.

4.2.2.3. Analyses of free amino acids

Precolumn derivatisation of free amino acids was performed using the AccQ-Fluor Reagent Kit according to the manufacturer's specifications. Separation was performed on the HPLC system equipped with a Waters AccQ Tag reversed-phase HPLC column (150 mm × 3.9 mm, 4 μm), according to the manufacturer's specifications with slight modifications. Briefly, mobile phase A consisted of AccQ Tag Eluent A Concentrate in deionised water (1:10 v/v), while mobile phase B consisted of 60% ACN in deionised water. A gradient programme was used for the separation of amino acids: 0–0.5 min, linear

gradient from 0 to 2% B; 0.5–15 min, linear gradient from 2 to 7% B; 15–19 min, linear gradient from 7 to 10% B; 19–32 min, linear gradient from 10 to 33% B; 32–33 min, 33% B; 33–34 min, linear gradient from 33 to 100% B; 34–40 min, 100% B; 40–42 min, linear gradient from 100 to 0% B. Postrun time was 2 min. Sample injection volume was 10 μL. Flow rate was 1.0 mL/min. Column temperature was set at 37 °C. Amino acids were detected at 248 nm, and identified by comparison of retention times and spectrum of standard solutions of amino acids kit and L-theanine. Quantification was done via external calibration curves. Performance of the HPLC method was validated through accuracy and precision tests.

4.2.2.4. Analysis of 5'-nucleotides

GMP and IMP were analysed on the HPLC system equipped with an Agilent ZORBAX SB-AQ HPLC column (250 mm × 4.6 mm, 5 μm), using an isocratic elution system. Mobile phase A consisted of 20 mmol potassium dihydrogen phosphate in deionised water, adjusted to pH 7 using sodium hydroxide. Total run time was 30 min. Postrun time was 2 min. Sample injection volume was 10 μL. Flow rate was 0.5 mL/min. Column temperature was set at 30 °C. GMP and IMP were detected at 254 nm, and identified by comparison of retention times and spectrum of standard solutions. Analytes were quantified through the use of external calibration curves. Performance of the HPLC method was validated through accuracy and precision tests.

4.2.3. Sensory analyses

4.2.3.1. Sensory design

A sensory panel constituting of eight experienced panellists was trained to recognise and quantify the intensity of bitterness, umami taste and astringency using the following compounds dissolved in deionised water: caffeine (48.5 mg/100 mL) for bitterness, L-glutamic acid (132.3 mg/100 mL) for umami, and EGCG (32.6 mg/ 100 mL) for astringency. The panellists were familiar with sensory experiments, and had prior experiences in sensory evaluations. A five-point scale ranging from 0 (not detected) to 4 (very intense) was used in the sensory analyses. Deionised water was used as an anchor point for point 0, while the standard solutions used for training were used as anchor points for point 4 on their respective scale. A modified version of the half tongue test (Scharbert & Hofmann, 2005) was employed as a means for sensory testing. Two samples (each containing 1 mL of solution) were delivered onto the left and right sides of the tongue simultaneously while keeping the head tilted back, using disposable pipettes. Panellists were then asked to rate the taste intensities of one of the solutions, relative to the other solution which was used as a reference. Nose clips were used to prevent any potential taste-odour interactions. The reference solutions used were deionised water, RTD green tea samples, and sample recombinant, depending on the objective of evaluation.

To prevent excessive sensory fatigue and the carry-over effect of astringency, panellists were required to cleanse their palates with skimmed milk, followed by deionised water, before tasting the subsequent sample. In addition, samples were presented in a sequence of increasing concentration of catechins to reduce

any possible carry-over effect. Panellists were also required to take a 30 min break after every three samples to prevent sensory fatigue and adaptation.

4.2.3.2. Taste reconstruction experiment

Three RTD green tea samples (samples nos. 1, 2 and 7) were selected, based on analytical results, to represent the extremes and average of the green tea samples, respectively, and hence were used for sensory analyses. Thirteen taste compounds (caffeine, gallic acid, EGCG, GCG, ECG, CG, EGC, GC, EC, C, Asp, Glu and Thea) were selected based on their taste activity in the seven RTD green tea samples (Table 4.1; refer to Section 4.2) and dissolved individually in deionised water to obtain stock solutions. The stock solutions were then added together to obtain sample recombinants matching the chemical profiles of tea samples nos. 1, 2 and 7. The panellists were then asked to rate the taste intensities of the sample recombinants with reference to the respective green tea samples using the half tongue test. Samples were evaluated within 30 min of sample preparation, and were expectorated after tasting.

4.2.3.3. Taste omission experiment

To investigate the taste contribution and significance of the individual taste compounds, seven partial taste recombinants were prepared by omitting a single taste compound or a group of taste compounds from the complete taste recombinant of tea sample no. 2. Seven omission samples were prepared by removing: (a) all catechins; (b) gallic acid and all catechins except EGCG; (c)

caffeine; (d) Thea; (e) Glu and Asp; (f) Glu; and (g) Asp, from the complete recombinant. Panellists were required to rate the taste intensities of the partial sample recombinants with reference to the complete recombinant using the half tongue test, and samples were expectorated after tasting.

4.2.3.4. Reduced recombinant testing

Stock solutions of four taste compounds (caffeine, EGCG, Thea, Glu) were mixed together, according to the results obtained in Section 4.3, to obtain a reduced recombinant matching the chemical profile of tea sample no. 2. Similarly, panellists were asked to rate the taste intensities of the reduced sample recombinant with reference to the recombinant of green tea sample no. 2 using the half tongue test. Samples were expectorated after tasting.

4.2.4. Data and statistical analyses

All HPLC analyses were performed in triplicates. Sensory evaluations comprised of a panel consisting of eight experienced panellists. Results were presented as mean ± standard deviation. Analysis of variance (ANOVA) was performed using Excel 2010. Differences were considered to be statistically significant at $p < 0.05$, unless otherwise specified. Forward stepwise multiple linear regression was performed using MATLAB version 7.12 (R2011a, The MathWorks, Inc.).

4.3. Results and discussion

4.3.1. Non-volatiles profile of RTD green tea and dose-over-threshold (DOT) values

In order to study the relationship between non-volatile compounds and the taste profile of RTD green tea, the concentrations of eight catechins, gallic acid, caffeine, 18 amino acids and two nucleotides were quantitatively determined. Dose-over-threshold (DOT) values for each compound were determined as the ratio of the concentration of compound to the taste threshold determined by previous studies (Table 4.1). Compounds in RTD green tea samples with DOT greater than one are highlighted in bold.

Preliminary sensory evaluations of the seven green tea samples showed that sweetness, saltiness and sourness were not detected in them. As such, non-volatile tastants responsible for these tastes were not chemically quantified and were excluded from subsequent sensory analyses.

Table 4.1: Experimental results obtained from HPLC analyses of seven RTD green tea samples. Compounds in samples with DOT greater than one are highlighted in bold.

Compound	Threshold ($\mu g\ g^{-1}$ (ppm))[†]	Concentration in RTD green tea sample ($\mu g\ g^{-1}$ (ppm))						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Gallic acid	34[a]	4.49 ± 0.11	16.0 ± 0.2	5.73 ± 0.05	2.53 ± 0.03	13.7 ± 0.1	6.42 ± 0.02	7.48 ± 0.20
EGCG	87[b]	16.7 ± 1.7	85.4 ± 10.5	23.4 ± 0.6	26.4 ± 2.5	29.2 ± 0.7	22.0 ± 1.7	27.9 ± 2.2
GCG	179[b]	11.5 ± 0.5	68.7 ± 1.8	21.1 ± 0.8	12.2 ± 0.7	21.6 ± 0.6	21.8 ± 0.2	20.6 ± 1.6
EGC	159[b]	9.96 ± 1.62	34.6 ± 4.7	18.5 ± 1.8	21.5 ± 0.7	21.3 ± 3.7	25.2 ± 3.1	30.9 ± 2.3
GC	165[b]	19.1 ± 0.8	55.1 ± 1.3	28.6 ± 1.1	22.9 ± 1.2	44.4 ± 1.4	51.4 ± 2.7	56.2 ± 1.4
ECG	115[b]	4.77 ± 0.15	27.3 ± 2.1	7.52 ± 0.47	6.60 ± 0.57	8.36 ± 0.54	6.22 ± 0.57	5.50 ± 0.45
CG	111[b]	5.99 ± 0.38	31.8 ± 1.9	11.8 ± 0.9	4.75 ± 0.10	10.2 ± 1.0	14.7 ± 1.1	11.6 ± 0.3
EC	270[b]	7.55 ± 0.38	28.2 ± 1.3	12.6 ± 0.8	13.6 ± 0.8	15.4 ± 0.8	15.3 ± 0.6	18.3 ± 0.2
C	119[b]	8.51 ± 0.30	24.3 ± 1.1	10.3 ± 0.2	6.77 ± 0.13	16.0 ± 0.7	21.8 ± 1.9	16.2 ± 0.5
Total catechins	-	84.1 ± 3.7	355 ± 8	134 ± 3	115 ± 2	166 ± 2	178 ± 3	167 ± 3
Caffeine	97[c]	**112 ± 5**	**243 ± 8**	**120 ± 9**	**97.1 ± 6.0**	**146 ± 10**	**128 ± 6**	**125 ± 2**
Asp	24[d]	14.5 ± 1.6	12.7 ± 1.2	5.82 ± 0.71	13.3 ± 2.2	17.0 ± 2.0	9.03 ± 2.33	9.48 ± 1.03
Ser	1500[e]	7.39 ± 0.64	3.59 ± 0.64	2.26 ± 0.14	6.35 ± 0.98	8.35 ± 1.58	3.54 ± 0.98	4.05 ± 0.71
Glu	9[d]	**19.0 ± 2.3**	**17.1 ± 1.1**	8.65 ± 0.97	**14.0 ± 3.1**	**22.3 ± 4.2**	**11.3 ± 2.6**	**12.2 ± 1.6**
Gly	1300[e]	n.d.	0.405 ± 0.046	n.d.	n.d.	1.18 ± 0.14	0.406 ± 0.018	0.406 ± 0.032
His	191[d]	3.20 ± 0.25	1.65 ± 0.28	0.590 ± 0.034	3.57 ± 0.19	3.68 ± 0.39	0.824 ± 0.253	1.70 ± 0.42
Arg	209[d]	6.09 ± 0.64	6.27 ± 0.45	3.36 ± 0.42	5.04 ± 0.34	11.3 ± 0.7	3.88 ± 0.56	5.64 ± 0.46
Thr	2600[e]	2.19 ± 0.88	2.44 ± 0.52	1.23 ± 0.42	2.04 ± 0.27	4.01 ± 1.34	1.49 ± 0.50	1.82 ± 0.97
Ala	600[e]	1.96 ± 0.21	1.86 ± 0.16	0.946 ± 0.232	1.83 ± 0.15	4.58 ± 0.68	1.83 ± 0.41	1.72 ± 0.11
Pro	1738[d]	0.712 ± 0.138	0.490 ± 0.081	n.d.	0.709 ± 0.076	1.63 ± 0.36	0.409 ± 0.037	0.409 ± 0.016
Thea	1045[c]	36.0 ± 4.2	42.1 ± 3.5	19.6 ± 2.2	39.4 ± 1.5	40.5 ± 2.6	19.7 ± 1.7	28.3 ± 1.8
Cys	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tyr	906[c]	1.31 ± 0.07	2.20 ± 0.56	1.07 ± 0.31	1.49 ± 0.15	3.47 ± 0.48	1.73 ± 0.32	1.73 ± 0.36
Val	400[e]	1.64 ± 0.21	1.85 ± 0.15	1.27 ± 0.84	1.62 ± 0.19	3.41 ± 0.28	1.46 ± 0.44	1.69 ± 0.27
Met	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lys	104[d]	1.43 ± 0.19	0.921 ± 0.089	0.420 ± 0.056	1.49 ± 0.52	5.35 ± 1.42	0.638 ± 0.130	1.11 ± 0.12
Ile	900[e]	1.60 ± 0.58	0.868 ± 0.402	0.600 ± 0.116	1.11 ± 0.35	2.09 ± 0.39	0.843 ± 0.184	0.802 ± 0.273
Leu	846[d]	1.01 ± 0.25	0.424 ± 0.070	n.d.	0.814 ± 0.130	3.97 ± 0.83	0.427 ± 0.097	0.717 ± 0.142
Phe	900[e]	1.34 ± 0.12	0.899 ± 0.133	n.d.	1.52 ± 0.09	2.70 ± 0.19	0.504 ± 0.069	0.726 ± 0.052
Total amino acids	-	99.4 ± 8.0	95.7 ± 7.5	45.6 ± 3.6	94.2 ± 8.3	136 ± 15	57.6 ± 9.2	72.4 ± 5.7
GMP	109[a]	n.d.	0.808 ± 0.011	0.767 ± 0.005	n.d.	0.632 ± 0.012	0.647 ± 0.038	1.10 ± 0.02
IMP	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[†] Threshold values were obtained from the following sources: [a] Kaneko et al. (2006); [b] Scharbert, Holzmann and Hofmann (2004); [c] Scharbert and Hofmann (2005); [d] Schiffman, Sennewald and Gagnon (1981); [e] Kim and Lee (2003) (S.-H. Kim & Lee, 2003; Schiffman, Sennewald, & Gagnon, 1981)
n.d. = not detected

The total catechin content in all seven RTD green tea samples ranged from 84.10 to 355.39 ppm, with sample no. 1 containing the lowest amount of catechins, and sample no. 2 containing the highest. Catechins can be broadly categorised into two groups: EGCG, GCG, EGC and GC in the first group, which were present in higher concentrations in the samples; and ECG, CG, EC and C, which were present in lower amounts. Caffeine and gallic acid contents ranged from 97.14 to 355.39 and 4.49 to 16.00 ppm, respectively. Tea sample no. 2 contained the highest amount of caffeine, while tea sample no. 4 contained the least. The DOT values of all catechin species and gallic acid were less than 1.0, with the exception of EGCG in tea sample no. 2 (approximately 1.0), indicating that the concentrations of catechin species were below taste threshold values. The DOT values of caffeine in all the samples were equal to or greater than 1.0, with values ranging from 1.0 to 2.5.

Regarding free amino acids, tea sample no. 3 had the lowest total free amino acid content with 45.64 ppm, while tea sample no. 5 had the highest with 135.51 ppm. L-theanine was found to be the most abundant free amino acid in all the samples, with its concentrations ranging from 19.64 to 42.11 ppm. L-glutamic acid and L-aspartic acid were the next most abundant free amino acids present in the sample. The DOT values of L-glutamic acid were greater than one in all the samples except for sample no. 3, indicating a possible contribution towards an umami taste by L-glutamic acid. The DOT values of L-theanine and L-aspartic acid were less than one in all the samples, suggesting a lack of taste activity by both compounds in these samples.

GMP was found to be absent or present at very low concentrations in the seven green tea samples, while IMP was not present at all. This suggested that the 5′-nucleotides did not contribute towards the taste profile of the samples.

Although the DOT values of catechins were less than one in the RTD samples, with the exception of EGCG in tea sample no. 2, astringency in tea samples no. 1 and no. 7 was still detected by the panel. This could have indicated the presence of an unknown compound contributing towards tea astringency, or an additive effect by the presence of catechins. This was further investigated by conducting taste reconstruction and omission experiments, which are discussed below.

4.3.2. Sensory evaluation of RTD tea samples and taste reconstruction experiments

Sensory evaluation of the RTD tea samples was conducted to obtain the taste profile of individual samples. Panellists were required to evaluate the RTD tea samples based on the bitterness, umami taste and astringency of the samples, using a five-point scale ranging from 0 (not detectable) to 4 (very intense). Anchors used in panel training were below the maximum taste intensity in order to prevent excessive sensory fatigue, especially in the case of the astringent-causing tastants, due to carry over effects. Results obtained are summarised in Table 4.2. Generally, bitterness and astringency were perceived to have a higher intensity, as compared to umami taste. Tea sample no. 2 scored the highest for all the three taste attributes, and was perceived to have similar astringency as

sample no. 6. Tea sample no. 1 was the least bitter and astringent, while tea sample no. 4 was the least umami.

Taste reconstruction experiments were performed to identify the key non-volatile compounds contributing significantly to the individual taste attributes, using tea samples nos. 1, 2 and 7, which were chosen to represent the bottom, top and middle levels of taste intensities. Taste recombinants of these three samples were prepared using 13 non-volatile tastants selected based on their DOT values in the RTD green tea samples: gallic acid, eight species of catechins, caffeine, L-aspartic acid, L-glutamic acid and L-theanine. Although the DOT values of L-theanine were less than 0.1 in all the tea samples, it was included in the sensory analyses to further investigate its contribution towards the various taste attributes.

Sample recombinants were compared to their respective original RTD green tea samples, and results obtained are summarised in Table 4.2. The intensities of taste attributes of the sample recombinants were found to be similar to those of the original samples, with the exception of astringency in recombinant tea no. 1. A one-way ANOVA was used to test for differences among the tea samples and their reconstructed samples. A significant difference ($F = 7.23$, $p < 0.05$) was detected in the astringency of tea sample no. 1 and its reconstructed sample. This was attributed to the low astringency associated with tea sample no. 1, which resulted in decreased sensitivity towards the mouth puckering effect, and thus was likely to be below the threshold of accurate determination by the panel. Other than astringency in tea sample no. 1, other taste attributes were not found to be statistically different between the RTD samples and their corresponding

reconstructed samples. As such, the 13 non-volatile tastants were concluded to be sufficient in reproducing the taste profile of RTD green tea.

Table 4.2: Taste profiles obtained from sensory evaluation of seven RTD green tea samples and the reconstructed samples of tea nos. 1, 2 and 7.

Sample type		Intensity of taste[†]		
		Bitter	**Umami**	**Astringent**
RTD green tea	Sample 1	1.13 ± 0.83^{a}	1.00 ± 0.76^{ab}	1.38 ± 0.92^{a}
	Sample 2	2.56 ± 1.02^{b}	2.13 ± 0.64^{b}	2.81 ± 0.84^{c}
	Sample 3	2.00 ± 0.93^{ab}	1.50 ± 0.76^{ab}	2.75 ± 0.89^{bc}
	Sample 4	1.88 ± 0.64^{ab}	0.875 ± 0.641^{a}	1.63 ± 0.52^{ab}
	Sample 5	1.88 ± 0.83^{ab}	1.63 ± 0.52^{ab}	1.88 ± 0.64^{abc}
	Sample 6	2.31 ± 0.46^{ab}	1.88 ± 0.99^{ab}	2.81 ± 0.92^{c}
	Sample 7	1.50 ± 0.53^{ab}	1.50 ± 0.76^{ab}	1.75 ± 0.46^{abc}
Sample recombinant	Sample 1	1.06 ± 0.18	0.750 ± 0.707	0.438 ± 0.623
	Sample 2	2.94 ± 1.02	2.38 ± 0.88	3.06 ± 0.62
	Sample 7	1.88 ± 0.64	1.50 ± 0.53	2.06 ± 0.86

[†] Values are expressed in terms of mean ± standard deviation. n = 8 panellists.
[a, b, c] One-way ANOVA conducted at $\alpha = 0.05$. Post-hoc test conducted using Tukey's honestly significant difference test.

4.3.3 Sensory evaluation of sample recombinant and taste omission experiments

After determining the key non-volatile tastants responsible for the taste profile of the RTD tea samples, omission experiments were conducted to further narrow down the list of compounds that contribute significantly to taste. Tea sample no. 2 recombinant was used as the basis for comparison as it scored high in all the three taste attributes, and was thus easier for the panellists to identify differences that might be present between the omission samples and the recombinant sample. The omission samples were prepared by removing an individual or a group of compounds from the sample no. 2 recombinant. The panellists were required to evaluate and assess the taste intensities of the omission samples, with reference to the sample no. 2 recombinant, using the

half-tongue test. Seven omission samples were prepared by removing the following compound(s): (a) all catechins; (b) gallic acid and all catechins except EGCG; (c) caffeine; (d) L-theanine; (e) L-glutamic acid and L-aspartic acid; (f) L-glutamic acid; and (g) L-aspartic acid. Intensity scores of the three taste attributes were compared to that of the sample no. 2 recombinant (Figure 4.1).

The omission of all eight catechin species from the sample recombinant resulted in a reduction in all three taste attributes (Figure 4.1a). A significant reduction in the astringency (-1.63) of the sample ($F = 19.9$, $p < 0.001$) was detected. The bitterness and umami tastes of the omission sample were found to be lower than those of the original recombinant (by -0.75 for both) as well, but at an insignificant level. The sensory results in this research were consistent with results from previous studies, that catechins, as a group, elicited an astringent sensation, and to a certain extent, bitterness in green tea (Peleg, Gacon, Schlich, & Noble, 1999; Scharbert & Hofmann, 2005). Although gallic acid was included in this omission sample, the intensity of astringency was still significantly lower than that of the sample recombinant, suggesting a lack of contribution towards astringency. While the reduction in intensities of bitterness and astringency were expected, a reduction in umami taste was not expected, as a diminished bitterness and astringency would allow for a greater ease of perception of the umami taste. Furthermore, catechins have been suggested to be astringent- and bitter-causing, and are not commonly associated with an umami taste. However, in a study conducted by Narukawa et al. (2010), a few panellists associated EC with umami and salty tastes. As such, there is no definite conclusion that catechins do not impart an umami taste. Nevertheless, results obtained from this set of omission experiments showed that catechins

were the main group of compounds contributing to astringency in the RTD green tea samples.

As EGCG was the only catechin species with a DOT value approximately equal to one, its contribution to the taste profile of RTD green tea was investigated by removing gallic acid and the other seven catechins in the second set of the omission experiments (Figure 4.1b). All the three taste attributes of the omission sample were less intense than those of the sample recombinant, but did not differ significantly ($p > 0.05$) from the recombinant sample, which suggested EGCG being the main contributor to astringency in the RTD tea samples. EGCG has a lower astringency threshold than the other species of catechins, according to the literature (Scharbert et al., 2004), which further supports the importance of EGCG in contributing to astringency in relation to the excluded compounds.

Significant reductions in the intensities of bitterness (-1.44) and umami taste (-1.25) were observed with the exclusion of caffeine from the sample recombinant (Figure 4.1c; $F = 5.87$, $p < 0.05$ for bitterness; $F = 8.54$, $p < 0.05$ for umami taste). Caffeine is the primary bitter-causing compound in most beverages, including green tea. Therefore, its omission resulted in the decrease in the intensity of bitterness. However, a similar reduction in the intensity of umami taste was not expected, as caffeine has not been shown to confer an umami taste. Furthermore, a reduction in bitterness would generally unmask umami taste, allowing it to be perceived better. As such, there is a possibility of bitter and umami taste-taste interaction within a mixture, or interactions between caffeine and umami-causing amino acids that has yet to be studied.

L-theanine has been suggested to have a complex taste profile, influencing the basic tastes (sweet, bitter, umami) and physical sensations (astringency) within the oral cavity (Ekborg-Ott et al., 1997; Juneja et al., 1999). As shown in Figure 4.1d, the difference in umami intensities between its omission sample and the recombinant sample was large, but insignificant (difference of -1.0, $F = 4.07$, $p = 0.063$). Bitterness and astringency were similar in both samples (difference of -0.38 for bitterness and -0.5 for astringency). Contrary to previous studies, L-theanine was not shown to be a major contributor to the taste profile of RTD green tea, which may be due to the low DOT values of L-theanine in the samples.

Removal of aspartic acid and L-glutamic acid, both individually and as a group, resulted in a decrease in umami intensity. This was in agreement with previous studies reporting the umami-causing qualities of both amino acids (Kaneko et al., 2006; Yamaguchi & Ninomiya, 2000). However, removal of L-glutamic acid resulted in a greater reduction in the taste intensity of umami taste (by -1.06), and it was therefore concluded that L-glutamic acid played a greater role than aspartic acid in contributing to the umami taste of RTD green tea (Figures 4.1f, 4.1g). The differences in the intensities of bitterness and astringency between the sample recombinant and omission samples were found to be insignificant.

Umami-causing tastants have been shown to exhibit remarkably high synergistic effects, for example in the case of L-glutamic acid and 5′ nucleotides (Yamaguchi, 1998), such that the total effect is greater than the sum of the effects caused by the individual compounds alone.

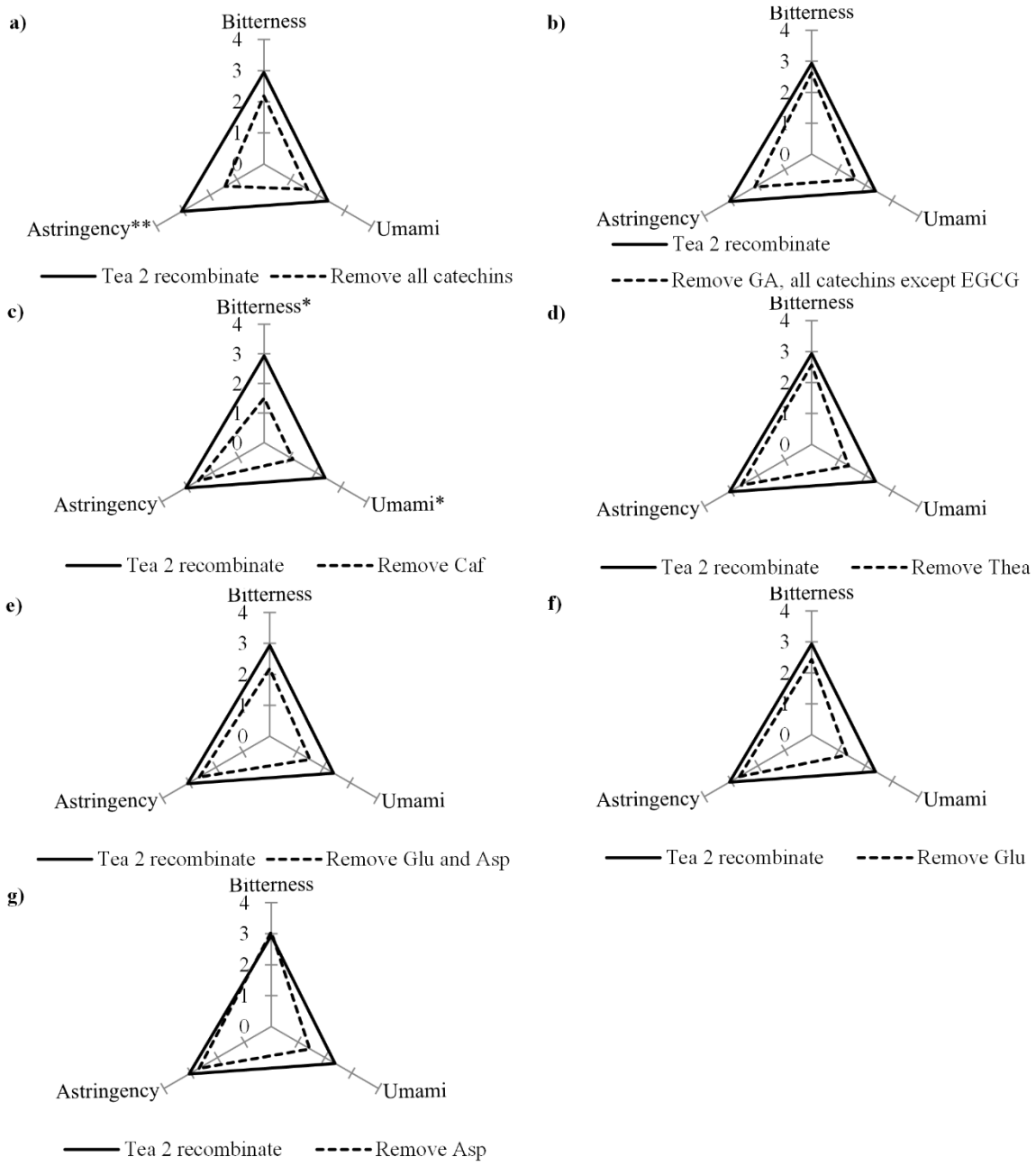

Figure 4.1: Taste profiles of tea 2 recombinant and omission experiments: (a) removing all catechins; (b) removing gallic acid and all catechins except EGCG; (c) removing caffeine; (d) removing L-theanine; (e) removing L-glutamic acid and aspartic acid; (f) removing L-glutamic acid; and (f) removing aspartic acid. Values are represented by mean scores of the sensory evaluation. n = 8 panellists.
* Significant difference detected using one-way ANOVA ($p < 0.05$).
** Significant difference detected using one-way ANOVA ($p < 0.01$).

Simultaneous omission of both aspartic acid and L-glutamic acid was done to investigate the potential synergistic effect between the two amino acids (Figure

4.1e). However, results obtained suggested no taste synergism between the two compounds, as the reduction in the intensity of umami taste in the omission sample (e) was not greater than that of either samples (f) or (g). Interestingly, removal of both L-glutamic acid and aspartic acid resulted in a greater reduction in bitterness intensity, suggesting the possibility of the presence of a synergistic effect in affecting bitterness. The difference in bitter intensity in the omission sample (e) was found to be approximately twice that of sample (f), although it was still statistically insignificant.

As such, EGCG was concluded to be the main contributor to astringency in RTD green tea, caffeine as the main contributor to bitterness, and L-glutamic acid to be the main contributor to umami taste.

4.3.4. Sensory evaluation of reduced sample recombinant

A reduced recombinant consisting of just EGCG, caffeine, L-glutamic acid and L-theanine was prepared to validate the significance of the key non-volatile tastants identified from the omission experiments. L-theanine was included in the reduced recombinant due to its complex taste profile in order to obtain a "rounded" profile, despite having a DOT value of less than one. The four compounds were added based on their concentrations in tea sample no. 2. The panellists were required to evaluate the reduced recombinant using the half tongue test.

The taste attributes of the reduced sample recombinant did not differ significantly from the sample recombinant and the RTD tea sample (Figure 4.2;

bitterness: F = 0.4422, p = 0.649; umami: F = 1.26, p = 0.306; astringency: F = 1.26, p = 0.306), suggesting that the four compounds used to construct the reduced sample recombinant were able to create a mixture to sufficiently replicate the taste profile of the RTD green tea sample.

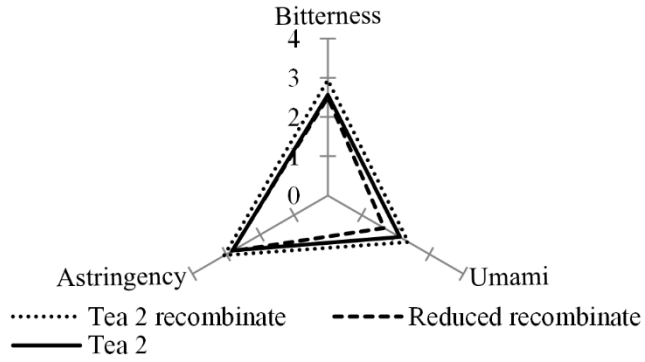

Figure 4.2: Taste profiles of RTD tea sample no. 2, sample no. 2 recombinant and reduced sample no. 2 recombinant. n = 8 panellists.

4.3.5. Regression analysis of chemical and sensory profiles

The taste reconstruction and omission experiments were conducted to identify the key non-volatile compounds that contributed to the taste profile of RTD green tea. Forward stepwise multiple linear regression (MLR) was employed to supplement these findings by exploring and describing the relationship between the chemical composition and the perceived sensorial characteristics, and regression models were established to provide an objective prediction of the taste attributes in RTD green teas. There is no literature on the use of mathematical modelling to predict the intensities of taste attributes in RTD green tea products.

Regression was performed after normalising the chemical and sensory data to a range of 0.1 and 0.9. The following two models relating bitterness and astringency to the concentrations of chemical components were obtained from forward stepwise MLR using observations from the original RTD tea samples and omission experiments as a training set:

$$\text{Bitterness} = 1.37 + 0.00421 \times \text{caffeine} + 0.000587 \times \text{glutamic acid} - 0.00335 \times \text{aspartic acid} + 0.000859 \times \text{EGCG} - 0.00127 \times \text{aspartic acid*glutamic acid} + 0.000308 \times \text{glutamic acid*EGCG} \quad \text{(Equation 4.1)}$$

$$\text{Astringency} = 1.38 + 0.00775 \times \text{EGCG} + 0.161 \times \text{CG} - 0.00902 \times \text{GC} - 0.00084 \times \text{EGCG*GC} \quad \text{(Equation 4.2)}$$

A satisfactory regression model for umami taste was not obtained from forward stepwise MLR, possibly due to the complexity of the taste attribute, as well as the relatively weak umami taste that could have been masked by the stronger bitter taste and astringency in the samples. This was further supported by the lack of significant differences in the omission experiments involving the umami-tasting non-volatile compounds.

The regression models obtained (Equations 4.1 and 4.2) explained the dependency of the intensities of bitterness and astringency on the different chemical constituents present. Regression Equation 4.1 indicated that caffeine

was the main driver for bitterness, followed by EGCG, and L-glutamic acid, while aspartic acid was negatively proportional to bitterness. The magnitude of coefficient of L-glutamic acid was an order of magnitude lower than caffeine, suggesting a lesser contribution to bitterness by L-glutamic acid. On its own, L-glutamic acid seemed to contribute very slightly to bitterness, but the interaction between aspartic acid and glutamic acid was found to negatively affect the intensity of bitterness. Variance explained by Equation 4.1 was 78.9%, with a root-mean-squared-error (RMSE) of 0.232. The bitterness model was validated with a subset of the data consisting of the sample recombinants and reduced recombinant, with a RMSE of 0.370. Caffeine and EGCG have been reported to confer bitterness, and caffeine is the main bitter-causing compound in beverages. Both umami tastants were not expected to contribute significantly to bitterness. Previous studies suggested the possibility of binary taste interactions and interactions between glutamic acid and bitter-tasting compounds, although results were not conclusive (Warmke & Belitz, 1993).

EGCG, CG and GC were found to significantly affect the intensity of astringency, with EGCG and CG positively driving astringency. The EGCG*CG interaction term was found to drive astringency negatively, along with CG. The coefficient of determination obtained from this regression showed that 84.1% of variance was explained by the regression model, with a training RMSE of 0.209. Validation of the regression model using the recombinant samples resulted in a RMSE of 0.934, which was almost three times higher than that of the bitterness regression model. A large part of the error was attributed to the tea sample no. 1 recombinant, which was perceived to be significantly less astringent than the original tea sample by the panellists. The low intensity

of astringency could be a factor that led to the decreased sensitivity of the panellists. Removal of this anomaly decreased the RMSE of the astringency validation data to 0.562.

One-factor-at-a-time robustness measurements were performed to assess the robustness of the regression models. The model coefficients of input parameters were adjusted by a factor of 10% and the corresponding model outputs were compared to those of the unadjusted model outputs (Figure 4.3). Adjustments to parameter coefficients in the bitterness regression model resulted in less than 5% change in the model output for all coefficients, while the astringency model had a change of up to 9.41%, indicating greater robustness of the bitterness regression model.

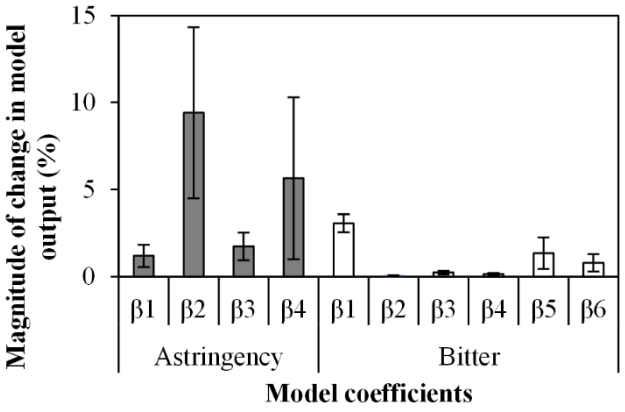

Figure 4.3: Magnitudes of change in model output corresponding to an adjustment of 10% in model parameter coefficients.

Parameter sensitivity analysis was also conducted by determining the sensitivity index (SI) of individual input parameters in both regression models, using the following equation (Hamby, 1994):

$$SI = \frac{D_{max} - D_{min}}{D_{max}} \qquad \text{Equation (3)}$$

Where D_{min} is the model output when all inputs are at minimum values, and D_{max} is the model output when a single input is at maximum value (Table 4.3). Sensitive input parameters are denoted by their high SI, and negative SI denotes a negative correlation with the model output. While important inputs have high sensitivities, sensitive inputs may not be always important (Hamby, 1994). For the bitterness regression model, caffeine was determined to have the highest SI compared to the other three inputs, which was in agreement with the omission experiments. The SI of glutamic acid was almost two orders of magnitude smaller than that of caffeine, indicating that the bitterness model was not sensitive to the concentration of glutamic acid. For the astringency model, SI of all three input parameters were relatively high, indicating high sensitivity. However, results from the omission experiments indicated that CG and GC were shown to only have slight contributions to the intensity of astringency. As such, it was concluded that although the model output was highly sensitive to the concentrations of GC and CG, these two catechins were still less important than EGCG in affecting intensity of astringency.

Table 4.3: Sensitivity indices of model parameters for bother bitterness and astringency regression models.

Regression model	Model parameter	Sensitivity index
Bitterness	Caffeine	0.427
	Glutamic acid	0.00943
	Aspartic acid	-0.0459
	EGCG	0.0507
Astringency	EGCG	0.324
	CG	0.787
	GC	-0.580

4.4. Conclusion

In conclusion, a series of quantitative studies, taste reconstruction and omission experiments revealed that the bitter-tasting and astringent-causing EGCG, bitter-tasting caffeine, umami-tasting glutamic acid were the key non-volatile compounds that contributed to the taste profile of RTD green tea. Subsequent multiple linear regression using forward stepwise regression identified caffeine as the main contributor to bitterness, and EGCG as the major contributor to astringency. Two regression models correlating the concentrations of the key compounds to the intensities of bitterness and astringency were established, and can be used to objectively predict sensory scores for bitterness and astringency in other RTD green tea samples.

CHAPTER 5

DEVELOPMENT OF A PARTIAL LEAST SQUARES-ARTIFICIAL NEURAL NETWORK (PLS-ANN) HYBRID MODEL FOR THE PREDICTION OF CONSUMER LIKING SCORES OF READY-TO-DRINK GREEN TEA BEVERAGES

Abstract

In order to develop products that would be preferred by consumers, the effects of the chemical compositions of ready-to-drink green tea beverages on consumer liking were studied through regression analyses. Green tea model systems were prepared by dosing solutions of 0.1% green tea extract with differing concentrations of eight flavour keys deemed to be important for green tea aroma and taste, based on a D-optimal experimental design, before undergoing commercial sterilisation by heating at 138.0 ± 1.5 °C for 15 s. Sensory evaluation of the green tea model system was carried out using an untrained consumer panel to obtain hedonic liking scores of the samples. Regression models were subsequently trained to objectively predict the consumer liking scores of the green tea model systems. A linear partial least squares (PLS) regression model was developed to describe the effects of the eight flavour keys on consumer liking, with a coefficient of determination (R^2) of 0.709, and a root-mean-square error (RMSE) of 3.69%. The PLS model was further augmented with an artificial neural network (ANN) to establish a PLS-ANN hybrid model. The established hybrid model was found to give a better

prediction of consumer liking scores, based on its R^2 (0.816) and RMSE (2.32%).

Key words: Green tea; consumer liking; regression; partial least squares; artificial neural network; optimisation

5.1. Introduction

Green tea is a beverage prepared by steeping the leaves of *Camellia sinensis* in hot water, and is one of the most popular beverages, especially in East Asian countries, due to the health benefits associated with consumption of green tea (Butt & Sultan, 2009). In order to support the modern, convenience-seeking lifestyles of students and working adults, food manufacturers have responded to the growing demand and popularity by increasing the availability and variety of ready-to-drink (RTD) and ready-to-eat food products (Euromonitor International, 2015). Annual sales of RTD tea products have reached staggering sales of USD 6.7 billion in 2012 according to a market research report (Zegler, 2013), and in order for food and beverage manufacturers to stay relevant in this growing industry, products will have to be designed with consumer acceptability and likability in mind.

The consumer liking and acceptance of RTD green tea beverages and other food products are largely dependent on several extrinsic and intrinsic factors. Extrinsic factors, which may include variables such as price of the product, nutritional information, consumer demographics, and brand or company reputability and credence, are largely unaffected by sensory properties of food

products (Bae, Lee, & Kim, 2015; Fernqvist & Ekelund, 2014). Intrinsic factors are largely driven by the sensory and chemical properties of the product, such as the sensory profile and presence of off-flavours (Shimoda, Shigematsu, Shiratsuchi, & Osajima, 1995), profiles of volatile odourants and non-volatile tastants (Chapter 4), as well as physical appearance of the product (Hurling & Shepherd, 2003). As the odour and taste properties of a product are affected by its chemical constituents, there have been numerous studies focusing on correlating the sensory profile of green tea beverages to its chemical profile, as well as the effects of the chemical profile on the overall acceptability of the product (Ikeda et al., 2004; Wang et al., 2010; Wang & Ruan, 2009).

Predictive modelling allows for an empirical understanding of food systems through regression models correlating the chemical, sensory, and hedonic properties of green tea and other food products. Linear multiple regression techniques such as multiple linear regression and partial least squares (PLS) regression have been used for the prediction of hedonic properties of green tea beverages from both the chemical and sensory profiles (Jumtee et al., 2011; Lin et al., 2012; Wang & Ruan, 2009). However, as the relationship between chemical constituents and sensory and hedonic properties may not necessarily be linear, the linear regression methods may fail to take into account the nonlinear relationships between human perception and food properties (Krishnamurthy et al., 2007). Nonlinear methods such as artificial neural networks (ANN) have been used in recent years in exploring the nonlinear relationship between sensory properties and consumer perception in green tea beverages (Ikeda et al., 2004). However, one of the primary drawbacks of ANN as a regression tool is its ‘black box’ nature, from which it may be difficult to

derive useful relational information from ANNs. Hybrid models comprised of a linear portion and a nonlinear part have been suggested in modelling food properties (Therdthai & Zhou, 2002), in order to derive useful information such as drivers of liking and dislike from the linear model, while ensuring that the nonlinear relationships are well-modelled by the nonlinear ANN.

As such, the main aims of this study were (i) to develop linear and nonlinear regression models for an objective prediction of consumer liking scores for RTD green tea beverages from the chemical formulation; (ii) to develop a hybrid model by augmenting the linear model with a nonlinear model; (iii) to assess and compare model qualities; and (iv) to optimise the RTD green tea formulation using the developed models as objective functions.

5.2. Materials and Methods

5.2.1. Materials

The following materials were used in the preparation of the RTD green tea beverage model systems: ascorbic acid and epigallocatechin gallate (EGCG)-enriched green tea extract (94% EGCG content) was obtained from DSM Nutritional Product Ltd (Basel, Switzerland), sodium bicarbonate was obtained from Merck KGaA (Darmstadt, Germany), caffeine was obtained from Givaudan International AG (Dübendorf, Switzerland), green tea extract (GTE) was obtained from Damin Foodstuff (Zhangzhou) Co. (Fujian, China), and other volatile flavouring ingredients were obtained from Givaudan Singapore Pte Ltd (Singapore, Singapore). For the sensory evaluation of RTD green tea

samples, crackers from Carr's Table Water, United Biscuits (UK) Ltd. (UK) and skimmed milk from CP Meiji Ltd. (Bangkok, Thailand) were used.

5.2.2. Sample preparation

The RTD green tea beverage model systems were formulated based on a 22-run Bayesian D-optimal experimental design (H01 to H22) for eight factors at three levels (low, mid, high), based on a linear second-degree polynomial model to allow for estimation of linear, two-factor interaction, and quadratic effects, using JMP (SAS Institute Inc., Cary, NC, USA). An additional five samples corresponding to a mid-level sample (H23), a calibration sample without any flavour keys added, and three matches of commercial RTD green tea samples (H25 to H27) were included in the experimental design, giving a total of 26 samples. The eight factors were based on flavour keys which significantly contributed to the overall flavour profile of RTD green teas, according to results obtained in Chapters 3 and 4, and comprised of six volatile odour keys (each consisting of a group of volatile compounds based on an in-house formulation) and two non-volatile tastant keys, summarised in Table 5.1. X_1 represented a fruity, berry-like odour; X_2 represented an ocean-like odour; X_3 represented a floral, jasmine-like odour; X_4 represented a nutty, roasted odour, X_5 represented a pea-like, green vegetal odour, X_6 represented a dry, sulphury odour, X_7 represented a bitter taste; while X_8 represented an astringent, mouth-drying sensation. The eight flavour keys were mixed with green tea extract, ascorbic acid, sodium bicarbonate according to the experimental design and dissolved in deionised water. The mixture was sterilised by ultra-high temperature (UHT)

processing using a UHT/HTST system (Armfield Limited, Ringwood, UK) at 138.0 ± 1.5 °C for a holding time of 15 s, before being rapidly cooled to a temperature of 15.0 ± 2.0 °C, and then subsequently filled into sterile polyethylene terephthalate (PET) bottles (330 mL) in a laminar flow aseptic filling cabinet. Bottled green tea samples were immediately stored at 4 °C until subsequent use within two weeks.

Table 5.1: In-house formulated flavour keys used in preparation of RTD green tea beverage model systems.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

5.2.3. Sensory evaluation

5.2.3.1. Panel recruitment and selection

Consumer acceptance test was performed using 146 untrained panellists (aged 18 to 35, Chinese ethnicity), recruited from the student population of the National University of Singapore and staff members of Givaudan Singapore Pte Ltd. Panellists were required to complete a questionnaire to obtain demographical information and data pertaining to consumption of RTD beverages. Only participants of the questionnaire who were frequent consumers of RTD green tea beverages (at least once per week) were recruited to

participate in the sensory evaluation of the green tea model systems. Each panellist was assigned a random three-digit panellist code.

5.2.3.2. Sensory evaluation procedure

The green tea samples were evaluated by each panellist over two sessions to minimise the effects of sensory fatigue, with 14 or 15 samples being evaluated during each session. A random three-digit code was assigned to each of the 27 green tea samples. Approximately 40 mL of each sample was poured into polypropylene cups (2 fl. oz.; SKP Pte Ltd., Singapore), and samples were evaluated by panellists in individual evaluation booths at an ambient temperature of 23.0 ± 1.0 °C. The presentation order of the samples was based on a 26 × 26 Latin square. After tasting five samples, panellists were given a 5 min break before evaluating the next set of five samples. Panellists were provided with drinking water (23.0 ± 1.0 °C), skimmed milk (23.0 ± 1.0 °C) and crackers, and were instructed to consume small quantities of crackers and water after every sample, and small quantities of skimmed milk, cracker, and water during each break to reduce the carry-over effect of astringency.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 5.1: Structure of a typical evaluation session.

The format of a typical evaluation session is summarised in Figure 5.1. Scoring of the green tea samples was done using FIZZ (Biosystèmes, Couternon, France), based on a 9-point scale with 1 indicating 'like extremely', and 9 indicating 'dislike extremely'.

5.2.4. Statistical analysis and mathematical modelling

Consumer liking scores were presented as mean ± standard deviation. Analysis of variance (ANOVA) was performed using MATLAB version 7.12 (The MathWorks, Inc., USA), with Tukey's Honestly Significant Difference as a post-hoc test. Differences were considered to be statistically significant at $p < 0.05$. The correlation between chemical formulation and consumer liking was investigated using regression analysis. All variables were normalised to a range of zero to one based on the minimum and maximum values of each variable to ensure numerical consistency. PLS regression models, ANNs, and PLS-ANN hybrid models were constructed and trained using the Neural Network Toolbox in MATLAB, and model optimisation was performed using a genetic algorithm found in the Global Optimisation Toolbox in MATLAB version 7.12.

5.3. Results and discussion

5.3.1. Consumer liking scores

The observed liking scores of the RTD green tea samples were summarised in Figure 5.2. In general, the liking scores of the samples ranged from 4.79 (sample

H05) to 5.99 (sample H14). One-way ANOVA results indicated that the observed liking scores differed significantly among the samples ($p < 0.05$), which suggested that while the range of consumer liking scores was relatively narrow, products were significantly different. Post-hoc test was conducted using Tukey's Honestly Significant Difference, and results obtained are summarised in Table 5, which showed that significant differences in consumer liking scores were present at differences in absolute scores of 0.80.

Table 5.2: ANOVA post-hoc test results obtained using Tukey's Honestly Significant difference.

Sample	Consumer liking score	ANOVA post-hoc test results[†]				
H14	5.99					e
H21	5.94				d	e
H26	5.92				d	e
H27	5.77			c	d	e
H12	5.62		b	c	d	e
H15	5.53	a	b	c	d	e
H02	5.51	a	b	c	d	e
H13	5.51	a	b	c	d	e
H16	5.49	a	b	c	d	e
H08	5.47	a	b	c	d	e
H07	5.44	a	b	c	d	e
H23	5.41	a	b	c	d	e
H19	5.36	a	b	c	d	e
H10	5.29	a	b	c	d	e
H04	5.27	a	b	c	d	e
H09	5.21	a	b	c	d	e
H01	5.18	a	b	c	d	
H18	5.04	a	b	c	d	
H20	5.00	a	b	c		
H03	4.99	a	b	c		
H22	4.94	a	b			
H06	4.86	a	b			
H25	4.83	a	b			
H11	4.82	a	b			
H05	4.79	a				
H17	4.79	a				

[†] Products with same lower-case letters are not significantly different.

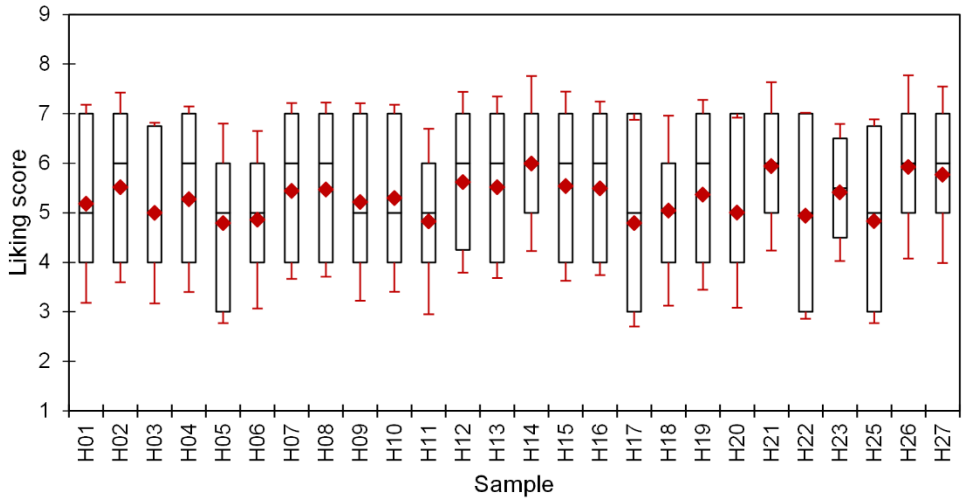

Figure 5.2: Box and whiskers plot of observed consumer liking scores for RTD green tea samples. Bottom and top of box indicate first and third quartiles, while band within the box indicates the median score. Means of liking score are indicated by ♦, and standard deviations are shown as whiskers. n = 146.

5.3.2. Partial least squares regression analysis of flavour keys and consumer liking

PLS regression is a linear regression method that can thought of as a cross between multiple linear regression and principal component analysis, and was carried out to relate the observed consumer liking scores to the concentrations of the flavour keys as given by the Bayesian D-optimal experimental design, based on a linear second order polynomial model, with eight independent variables corresponding to the eight flavour keys. The RTD green tea samples were divided into a model training set comprising of the first 22 samples (H01 to H22) that was used to train the regression model, and a validation set comprising of four samples (H23, H25 to H27) that was used as an unseen set to validate the trained model. Leave-one-out cross validation was used to

develop a PLS model for the training data. The quality of the resulting PLS regression model is shown in Figure 5.3.

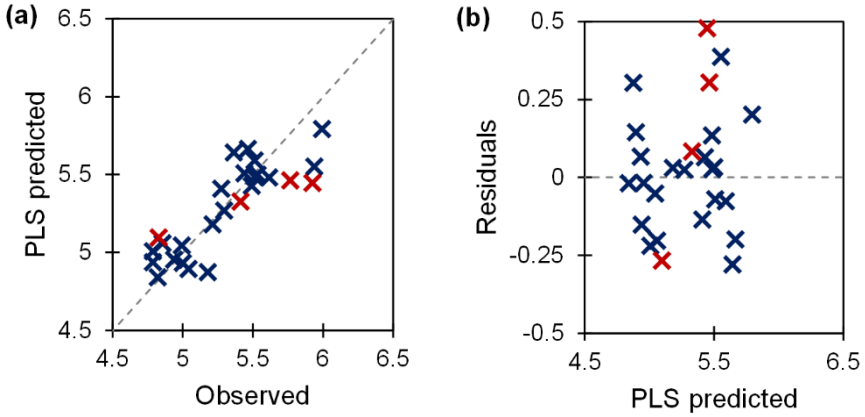

Figure 5.3: (a) Plot of predicted liking scores against observed liking scores, based on a two-component PLS regression model; (b) residuals plot of two-component PLS regression model. Training samples are indicated as × while model validation samples are indicated as ×.

The first two components of the PLS regression model explained approximately 77% of the total variance in the training set, 44% in the validation, and had an overall R^2 of 0.709. The RMSE of the training and validation sets were 3.11% and 5.93% respectively, with an overall error of 3.70%. Although the training data was well-modelled using a two-component PLS model, the low R^2 and high RMSE associated with the unseen validation set suggested that the predictive capability of the regression model did not extend well into unseen data, as seen in the skewed distribution of the validation sample about the line of equality (i.e. y = x line). This could suggest either an overfitting of the PLS model, in which the model had been fitted to include noise or error that was inherently present in the data, or there was a degree of nonlinearity that was present within the

relationship between chemical constituents and consumer liking, that was not well-modelled by a linear model.

Residuals analysis was carried out on the prediction errors of the PLS model to determine the prediction quality of the two-component PLS model, using Levene's Test for homoscedasticity and the Wald-Wolfowitz runs test for randomness. Although results obtained suggested that the models residuals were randomly distributed ($p > 0.10$), variances for the model residuals were heteroscedastic ($p < 0.25$), as seen from the unequal variances in Figure 5.3b. The heteroscedasticity associated with the model residuals indicated that the regression model gave poorer predictions of consumer liking scores at higher scores, compared to lower liking scores, which together with the poor R^2 and RMSE of the validation set, suggested that the two-component PLS regression model did not provide an adequate prediction of consumer liking of the RTD green tea samples.

Nonetheless, additional information concerning the main drivers of liking and dislike of RTD green tea can be estimated from the regression model, based on parameter uncertainties. The confidence intervals of the PLS model coefficients were estimated using a modified jack-knife method, according to Martens and Martens (2000), as the uncertainty of model variables was not readily available due to the nature of PLS regression. The coefficients of the model parameters, along with the associated uncertainty are shown in Figure 5.4. Model parameters were considered to be significant if the uncertainty did not include zero.

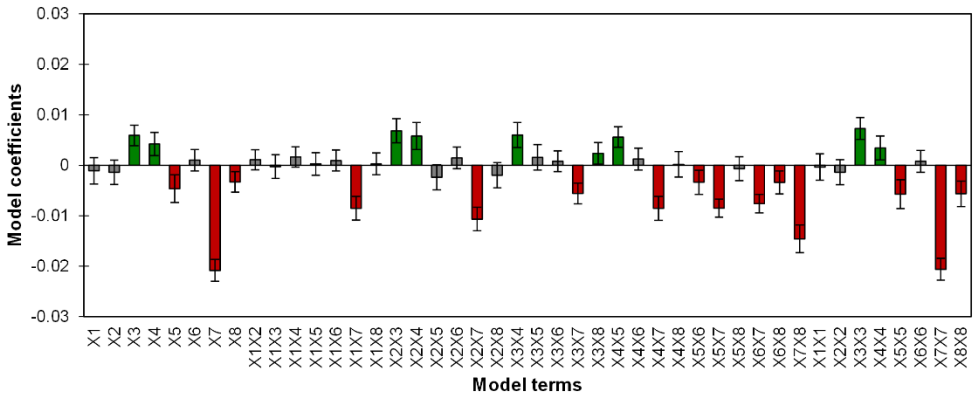

Figure 5.4: Coefficients of two-component PLS regression model terms. Significant drivers of liking are represented in green, and significant drivers of dislike are given in red.

The PLS regression model obtained explained the dependency of consumer liking on the concentrations of flavour keys used in formulating the RTD green tea samples. From Figure 5.4, it can be seen that the main linear drivers of dislike for RTD green tea were associated with the bitter and astringent flavour keys (X_7 and X_8). The model coefficient of the first-order term of X_7 was generally larger in magnitude as compared to the other independent variables, suggesting a particular dislike for the bitter-associated flavour key by the untrained consumer panel. First order interaction terms containing the X_7 flavour key were found to negatively drive liking, suggesting the negative impact of this flavour key on the overall consumer liking of the RTD green tea model system. On the other hand, variables X_3 and X_4 were the main linear drivers of liking of RTD green tea for the consumer panel based on the significance of the first-order model terms for these two variables. These variables were observed to have significant interactions with other flavour keys which mostly positively affected consumer liking, such as X_2X_3, X_2X_4, and X_3X_4. Results obtained from regression analysis using a linear PLS model thus

suggested that the consumer panel preferred RTD green tea beverages that were formulated with ingredients associated with floral and roasted, nutty attributes, while ingredients associated with bitterness and astringency should be kept to a minimum.

5.3.3. Structure of the PLS-ANN hybrid model

In order to improve the predictive ability of the regression model, the linear two-component PLS model was augmented with an ANN to yield a parallel PLS-ANN hybrid model, comprising of both linear and nonlinear moieties. In theory, the hybrid model should have the advantages of both its constituents, while reducing the drawbacks associated with linear models and ANNs. The relationship between the flavour keys and consumer liking modelled using PLS can be described as:

$$\mathbf{Y} = \mathbf{f(X)} + \mathbf{e}' = \hat{\mathbf{Y}}' + \mathbf{e}' \qquad \text{(Equation 5.1)}$$

where **Y** is the observed liking scores, **X** is the concentration of flavour keys as given in the experimental design, $\hat{\mathbf{Y}}'$ is the modelled liking scores expressed as a linear function of **X**, and $\mathbf{e}'$ is the prediction error of the linear PLS model. The linear prediction error, $\mathbf{e}'$, was used as the output for training the ANN model, where:

$$\mathbf{e'} = \mathbf{g(X)} + \mathbf{e}$$

$$= \mathbf{\Delta\hat{Y}} + \mathbf{e} \qquad \text{(Equation 5.2)}$$

where $\mathbf{\Delta\hat{Y}}$ is the prediction error modelled by the augmented ANN model, expressed as a function of **X**, and **e** is overall prediction error. By combining Equations 5.1 and 5.2, the overall function describing the PLS-ANN hybrid model was obtained:

$$\mathbf{Y} = \mathbf{f(X)} + \mathbf{g(X)} + \mathbf{e}$$

$$= \mathbf{\hat{Y}'} + \mathbf{\Delta\hat{Y}} + \mathbf{e}$$

$$= \mathbf{\hat{Y}} + \mathbf{e} \qquad \text{(Equation 5.3)}$$

where $\mathbf{\hat{Y}}$ is the modelled liking score based on the PLS-ANN hybrid parallel model. The structure of the hybrid parallel model is shown in Figure 5.5.

In brief, the linear PLS model provided an estimation of consumer liking as well as insights based on the drivers of liking and dislike based on the flavour keys, while the ANN provided a means to model the nonlinearity that exists between odourants and tastants and the human perception of these chemical constituents, which may not be well-captured by the linear PLS model.

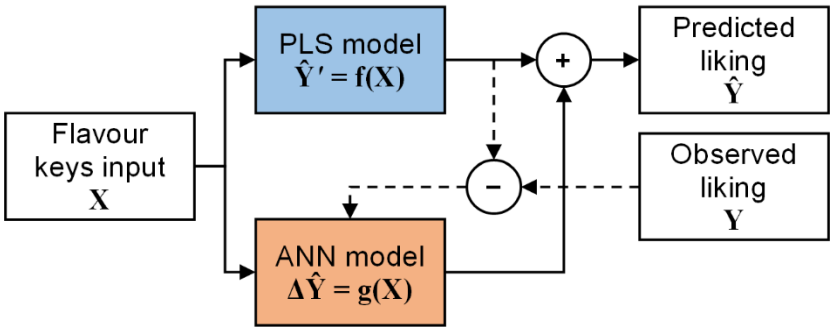

Figure 5.5: Structure of a PLS-ANN hybrid model.

5.3.4. Determination of ANN hidden layer

The linear PLS model provided an estimation of consumer liking as well as insights based on the drivers of liking and dislike based on the flavour keys. This can be augmented with an ANN to provide a means to model the nonlinearity that exists between odourants and tastants and the human perception of these chemical constituents, which may not be well-captured by the linear PLS regression model.

Prior to building and training an ANN for use in regression, the number of neurons in the hidden layer (i.e. size of the hidden layer) was first optimised. Using a hidden layer containing too few neurons may result in underfitting, in which nonlinearity in the data may not be adequately modelled, while using too many neurons may result in overfitting of the data. The size of the hidden layer will therefore have to be carefully chosen to avoid both scenarios. This is typically achieved through a trial-and-error process by systematically changing the number of neurons and training duration (Bernados & Vosniakos, 2007), commonly known as the grid search method, and is simple to implement and reliable in low dimensional spaces (Bergstra & Bengio, 2012). In addition to

grid searching, other methods such as random search and evolutionary methods can be used in tuning the architecture and learning parameters of ANNs when the number of dimensions corresponding to network parameters involved is high (Bergstra & Bengio, 2012; Leung, Lam, Ling, & Tam, 2003).

The size of hidden layer of the hidden layer together with the network training parameters (training duration, learning momentum and other associated parameters) were determined through a random search in the five-dimensional space. The following parameters were considered: two to ten neurons in the hidden layer, a training duration of five to 50 epochs, learning momentum (μ) of one to 30, μ increase of one to 10, and μ decrease of 0.01 to one. These learning parameters are associated with the Levenberg-Marquadt algorithm for ANN training. A multilayer feedforward, backpropagation ANN containing the tangent-sigmoid transfer function in the hidden layer and the linear transfer function in the output layer was trained according to the combinations of parameters derived from the random search. The ANNs were trained using leave-one-out cross validation with the training data set. Results obtained are presented in Figure 5.6.

In general, the learning parameters μ, μ increase, and μ decrease did not exhibit any observable trends, according to sub-figures (i), (ii), and (iii) of Figures 5.6a and 5.6b. This indicates that while these learning parameters might have an effect on the overall predictive quality of the ANN regression model, it may be dependent on other network parameters such as the architecture and the training duration. For the PLS-ANN hybrid model (Figure 5.6a), RMSE for networks decreased with an increase in the number of neurons in the hidden, before

increasing, with a minimum observed at around five to seven neurons in the hidden layer. This could suggest that networks containing fewer than five neurons in the hidden layers were unable to adequately recognise and model the nonlinearity that was present in the data. The increase in the RMSE as the hidden layer size increased beyond seven neurons may suggest a degree of overfitting, based on leave-one-out cross validation. The trend for training duration was less clear, but it appeared that a minimum in the training error was observed at the range between 10 to 15 epochs.

For the ANN model, the learning parameters μ, μ increase, and μ decrease did not have any clear effect on the training error of the regression model. Similarly, this could be due to the learning parameters being dependent on the other network parameters. The optimum size of the hidden layer was found to be between five to seven neurons, while training duration did not have an effect on the overall training error.

Based on results obtained through leave-one-out cross validation on the training set, the lowest RMSE corresponded to an optimum hidden layer size of six neurons, with a training duration of six epochs for the pure ANN model; and an optimum hidden layer size of five, with a training duration of seven epochs for the hybrid model. As such, an ANN with a network architecture of 8-5-1 was then used as an augmentation to the PLS model developed in the previous section, while an 8-6-1 neural network was used for the ANN model.

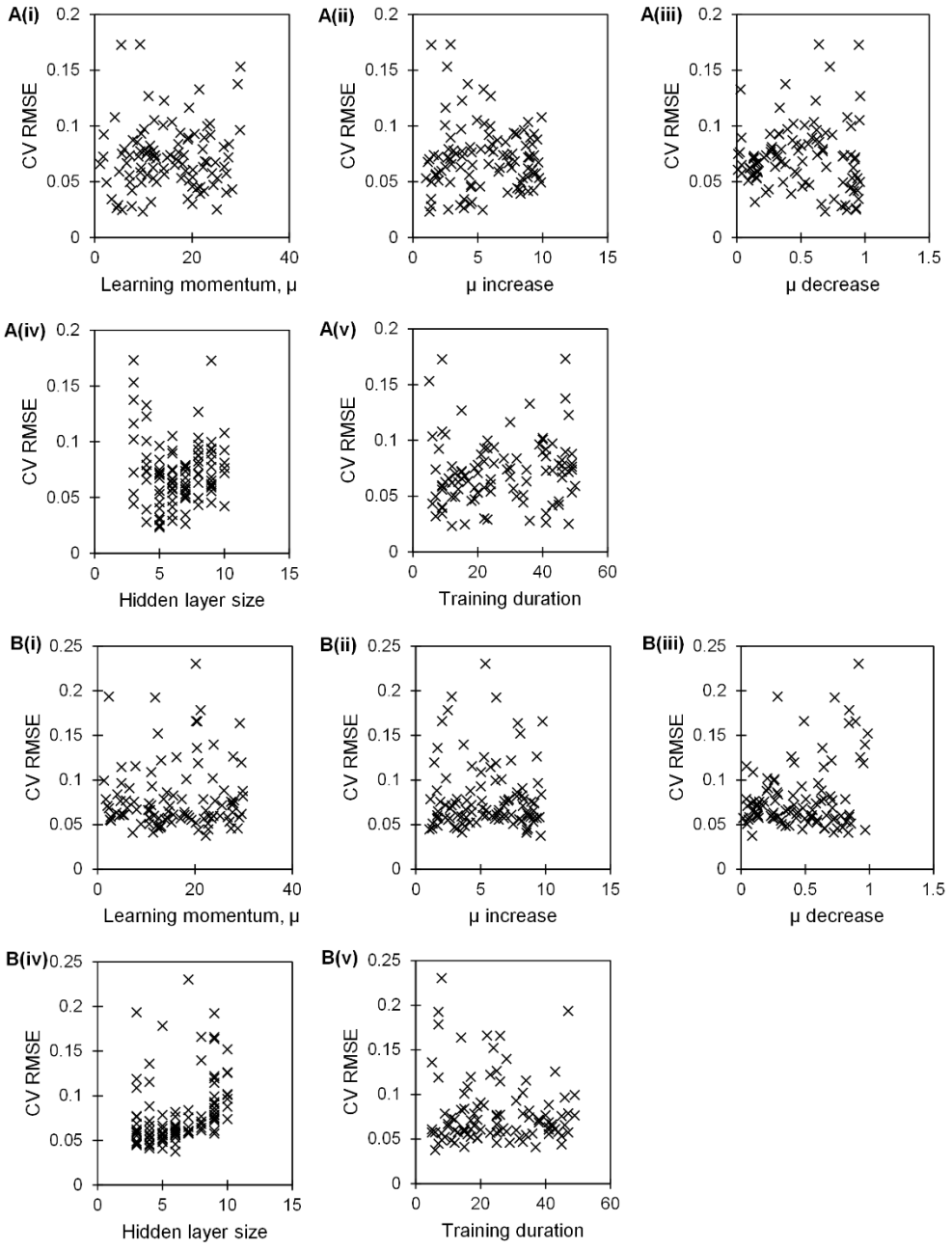

Figure 5.6: Training RMSE of the (A) PLS-ANN hybrid model and (B) ANN model based on (i) learning momentum; (ii) increase in learning momentum; (iii) decrease in learning momentum; (iv) size of the hidden layer; and (v) training duration during optimisation of neural network training parameters.

5.3.5. Model qualities of the ANN and PLS-ANN hybrid models

The RTD green tea samples were divided into training and validation sets in the same manner described in Section 5.3.2. Regression models were trained using the training set, and validated using the validation set. Results obtained are shown in Figure 5.7. The ANN model (Figure 5.7a) was found to have better prediction quality compared to the PLS model, as indicated by a total explained variance of approximately 89.9%, and an overall prediction error of 2.18%. The training R^2 and RMSE were 0.908 and 1.96%, while the validation R^2 and RMSE were 0.843 and 1.67%. This suggested that there might be a degree of nonlinearity which was not captured by the linear PLS model.

For the PLS-ANN hybrid regression model (Figure 5.7c), the resulting R^2 of the training and validation data set were 0.845 and 0.792 respectively, with an overall R^2 of 0.840. Compared to the standalone two-component PLS model, the addition of an ANN portion as an augmentation improved the overall variance explained by approximately 20%. Similarly, the overall RMSE of the PLS-ANN hybrid model was 2.73%, which was lower than that of the PLS model. The RMSE of the training set (2.53%) and validation set (3.50%) were lower compared to the two-component PLS model.

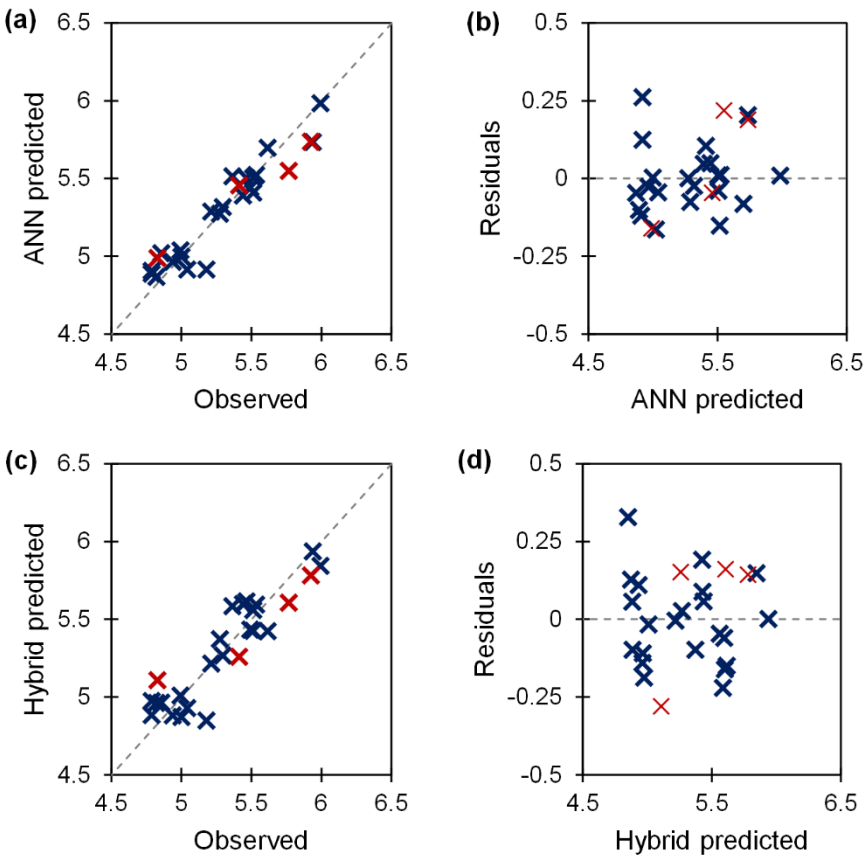

Figure 5.7: (a) Plot of predicted liking scores against observed liking scores, based on an 8-6-1 ANN; (b) residuals plot of ANN model; (c) plot of predicted liking scores against observed liking scores, based on a PLS-ANN hybrid model; and (d) residuals plot of PLS-ANN hybrid model. Training samples are indicated as × while model validation samples are indicated as ×.

Regarding residuals analysis of the ANN model and PLS-ANN hybrid model (Figures 5.7b and 5.7d), results obtained indicated that residuals for both models were randomly distributed. However, residual variances for both models were heteroscedastic, indicating that similar to the PLS regression model, poorer predictions were expected for products with lower consumer liking scores. The test for residuals normality using the Lilliefors test (Lilliefors, 1967) indicated that residuals for the ANN model were normally distributed, while normality was not detected in the residuals of the PLS model and PLS-ANN hybrid model.

Between the PLS model and PLS-ANN hybrid model, the PLS-ANN hybrid model produced the best performance based on model quality. The PLS model had lower R^2 and higher RMSE values, indicating poor predictive ability, relative to the other two ANN-based models, and demonstrating the ability of ANN to model nonlinearity, which was not well-modelled in linear models. Although the model qualities of the ANN and PLS-ANN hybrid model were very similar, the ANN model had a slightly higher overall R^2 and lower overall RMSE. However, the PLS-ANN hybrid model has an advantage of being a 'grey-box' model, as opposed to a 'black-box', in the case of the pure ANN model. This allowed for a better understanding of the relationship between chemical ingredients and consumer liking of the RTD green tea beverages.

5.3.6. Model optimisation

Model optimisation was performed to determine the optimal chemical formulation for the RTD green beverages. This was achieved using a genetic algorithm with the PLS and PLS-ANN hybrid models as objective functions. Genetic algorithms were chosen as the optimisation method of choice due to the nonlinear nature of ANNs, as well as the highly complex model structure associated with neural networks. In brief, genetic algorithms are a set of computer algorithms that are designed to solve complex problems by mimicking natural evolutionary processes, and by doing so, explore a higher number of solutions to an optimisation problem as compared to other algorithms (Shankar & Bandyopadhyay, 2004). The optimum formulations of RTD green tea

beverages based on the eight flavour keys for both models are shown in Figure 5.8.

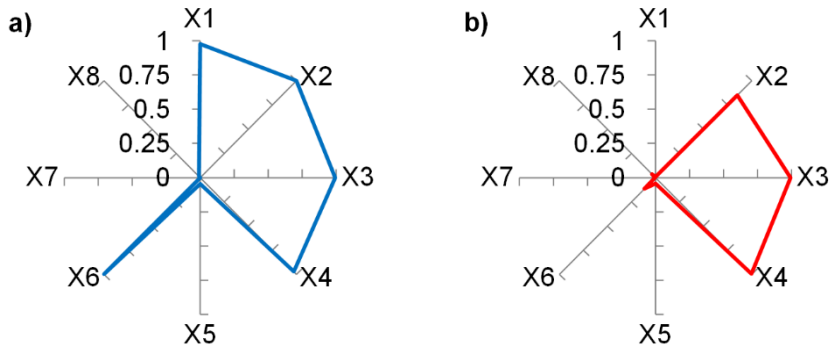

Figure 5.8: Optimum RTD green tea formulation based on (a) two-component PLS model; and (b) PLS-ANN hybrid regression model. Axes represent concentrations of flavour keys scaled to a range of zero to one.

The optimum consumer liking scores obtained through model optimisation were 5.87, 5.97, and 6.07 for the PLS model, PLS-ANN hybrid model, and ANN model, respectively. It should be noted that the optimal consumer liking score based on the PLS model was within the observed range of consumer liking. This suggests that consumer liking may not be well-modelled by PLS regression. In general, X_2, X_3, and X_4 were found to be at the maximum concentrations in both optimum formulations, while X_5, X_7, and X_8 were found to have the most positive impact on consumer liking when these flavour keys are at the lowest concentrations for both PLS and PLS-ANN hybrid models. The concentrations of X_1 and X_6 were found to be different in both formulations, suggesting that these two flavour keys are associated with some degree of higher order interactions with other flavour keys, or nonlinearity which was not modelled by

linear models. The relationship between flavour keys and consumer liking were subsequently studied in greater details using contour plots shown in Figure 5.9.

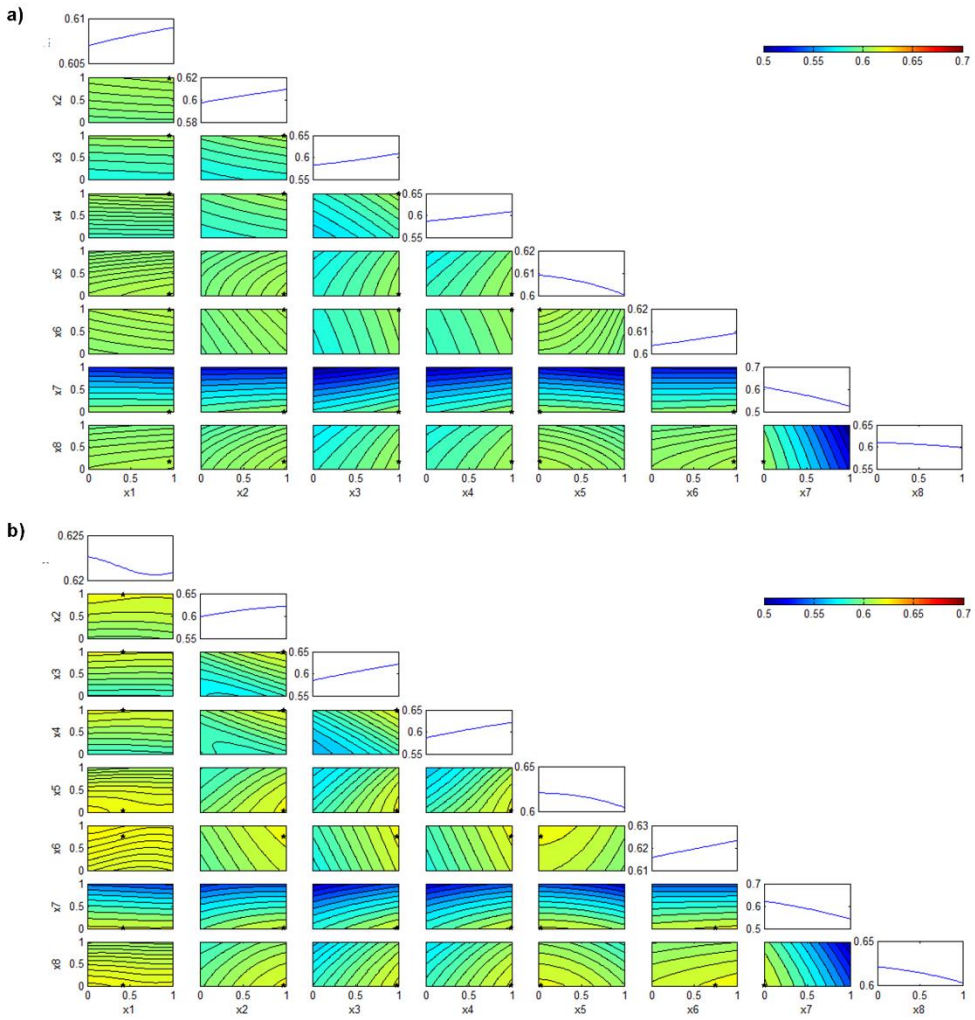

Figure 5.9: Contour plots showing effects of RTD green tea flavour keys (X_1 to X_8) on consumer liking for (a) PLS model; and (b) PLS-ANN hybrid model. Diagonal plots represent individual effect on consumer liking. Optimal points are indicated by ★. Baseline concentrations are equivalent to optimum formulations.

From Figure 5.9a, it can be seen that flavour keys X_1 to X_6 had limited effects on the overall consumer liking based on the two-component PLS model. A higher degree of complexity can be seen in Figure 5.9b, as observed by the

greater degree of curvature in the contours, suggesting that the PLS-ANN hybrid model was able to correlate nonlinearity more strongly, as compared to the PLS model. This is evident in the irregular contour lines in the X_1 flavour key and a nonlinear relationship between the X_1 flavour key and consumer liking in Figure 5.9b, which was not present in the corresponding plots in Figure 5.9a.

5.4. Conclusions

The relationship between chemical flavour keys and consumer liking has been established by mathematical modelling using linear PLS regression, nonlinear ANN, and a PLS-ANN hybrid model. Based on the PLS model, flavour keys X_3 and X_4 were found to be the main linear drivers of liking, while X_5, X_7, and X_8 were found to be the main linear drivers of dislike, suggesting that the consumer population preferred a bland-tasting green tea with floral and roasted odours. Among the three regression models established, the linear PLS model was found to have the lowest model quality, while both the ANN model and PLS-ANN hybrid models were found to be of similar qualities, based on the values of R^2 and RMSE, and residuals analyses. In-depth analysis using model optimisation revealed that the PLS model did not fully model the nonlinearity that may be present in the data. Between both ANN-based models, the PLS-ANN hybrid model has an added advantage of providing basic information regarding the RTD green tea in the form of drivers of liking and dislike, which was not readily derived from the ANN model.

CHAPTER 6

EVALUATION OF CONSENSUS PROFILING AND QUANTITATIVE FLAVOUR PROFILING IN THE OPTIMISATION OF A READY-TO-DRINK GREEN TEA BEVERAGE

Abstract

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.1. Introduction

Food sensory analysis and evaluation can be viewed as an information gathering process used to measure, analyse, and interpret behavioural responses to food products based on the five senses of sight, hearing, taste, smell, and touch, where human panellists are used as judges in measuring the attributes of a food product (Murray, Delahunty, & Baxter, 2001). It is comprised of a set of techniques that are used to measure human responses while minimising bias caused by potential confounding sources, which include branding and other information that may affect consumer perception (Mukhopadhyay, Majumdar, Goswami, & Mishra, 2013). Results obtained from food sensory studies provide

important information on the quality and characteristics of food product that can be used in several aspects such as new product development, consumer, flavour and taste profiling, and quality control.

Descriptive sensory analysis allows for understanding of the sensory profile, which is closely related to the chemical constituents, as well as the hedonic properties of a food product. Commonly used descriptive sensory analysis techniques include the Flavour Profile Method (FPM), Texture Profile Method, Quantitative Descriptive Analysis (QDA), and Quantitative Flavour Profiling (QFP), and have been reviewed previously (Lawless & Heymann, 2010; Murray et al., 2001). In this study, both FPM and QFP were used to collect sensory information for a series of RTD green tea beverages. FPM was first developed in the mid-20th century for evaluating new products in the then-developing food industry, and is a consensus profiling method utilising a small, highly trained panel consisting typically of four to six panellists. In other words, panellists of the trained sensory panel come to a consensus on various sensory characteristics of the evaluated product using descriptors developed during panel training. This process is often guided by a panel leader to ensure that the discussion is not dominated by a single panellist. The Profile Attribute Analysis (PAA) is a modern variation of FPM using numerical values allowing for results obtained to be analysed statistically, and has been used in recent studies (Bedini et al., 2013; Ömür-Özbek & Dietrich, 2008). QFP was first developed as a modification of QDA by Givaudan-Roure, and is used in the flavour and fragrance industry in sensory profiling. In QFP, a group of six to eight panellists are trained according to a set of sensory language developed by in-house experts and/or flavourists, resulting in a highly technical language that, although may

be of little consequence to the layperson or consumers, is useful in the development of new products for the food and fragrance industry (Murray et al., 2001). A key difference between consensus profiling and QFP is the individual evaluation of sensory characteristics of the tested samples by the trained sensory panel in QFP following panel discussion and training, contrary to FPM which only involves a consensus reached upon thorough discussion.

In the development and optimisation of new food products, it is necessary for a quick and efficient method to be used in all stages of the developmental process. This reduces the use of limited resources such as time and money. The development of an efficient data collection strategy encompassing sensory profiling and data analysis allows for this goal to be achieved. As consensus profiling methods require an arguably shorter time for data collection as compared to QFP-based methods, due to the additional individual profiling step present in QFP, it is of interest to investigate and compare the use of and results obtained from these two descriptive sensory analysis methods.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.2. Materials and methods

6.2.1. Materials and sample preparation

The materials and ingredients used in this research were the same as those described in Section 5.2.1 (Chapter 5). Sample preparation was conducted according to the method described in Section 5.2.2 (Chapter 5).

6.2.2. Sensory profiling

6.2.2.1. Sensory panel selection and training

A total of 10 female panellists (40 to 50 years of age) of Chinese ethnicity, with prior sensory evaluation experience, were recruited to be part of the trained sensory panel using the following criteria: normal colour vision, unimpeded sense of taste and smell. Panellists were not informed of the objectives and type of food product to be evaluated in this study.

6.2.2.1. Consensus profiling using flavour impression profiling

The sensory panel was trained over one session (three hours long) on key sensory descriptors of the 26 RTD green tea samples using volatile and non-volatile chemical standards that were representative of the corresponding odour or taste descriptor. Training on identification of sensory attributes in samples was conducted over a period of two weeks (eight three-hour sessions). The set of sensory descriptors used in this study was previously developed by in-house

flavourists, and are summarised in Table 6.1. The sensory panel was first trained to recognise the 13 sensory descriptors.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Panel training was conducted at Givaudan Singapore Pte Ltd in a well-lit room with an ambient temperature of 23.0 ± 1.0°C.

Table 6.1: Summary of sensory descriptors.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Flavour impression profiling (FIP), which is a profiling method based on some modifications of the Profile Attribute Analysis method, was used as a form of consensus profiling. Samples (50 mL) were presented to panellists in polypropylene cups (2 fl. oz.) coded with random three-digit codes at ambient temperature. Water, skimmed milk or diluted yoghurt (1:2, with water), and crackers were provided as palate cleansers, and panellists were instructed to consume small amounts whenever needed. Panellists were required to evaluate

the samples based on the 13 sensory descriptors using an intensity scale of zero to 100, and round table discussion was conducted, facilitated by a panel leader. Descriptor intensity was categorised as one of six levels (Table 6.2), before the sensory panel decided on an intensity score. Product evaluation was conducted over a period of four weeks.

Table 6.2: Intensity levels of sensory scores.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.2.2.2. Quantitative flavour profiling

For QFP of the RTD green tea samples, the same trained sensory panel from the previous section was used, along with the same set of sensory descriptors. Samples were first evaluated in a training phase, during which panellists were familiarised with the range of intensities of sensory descriptors present in the products. Subsequently, panellists were required to evaluate the RTD green tea samples on an individual basis in sensory evaluation booths. A random three-digit code was assigned to each sample, and presentation order of the samples was based on a Latin square to ensure a balanced and complete design. Panellists were provided with drinking water, skimmed milk or diluted yoghurt,

and crackers, and were instructed to consume small quantities after every sample to reduce the carry-over effects. Scoring of the descriptor intensities was done using FIZZ (Biosystèmes, Couternon, France) according to the intensity scale used in consensus profiling (Section 6.2.2.1). QFP of the green tea samples was repeated twice with the same sensory panel, and sensory scores were presented as the overall average.

6.2.3. Statistical analysis and mathematical modelling

Sensory scores obtained from consensus profiling were presented as a single number, while scores obtained from QFP were presented as mean ± standard deviation. Analysis of variance (ANOVA) was performed using MATLAB version 7.12 (The MathWorks, Inc, USA), with Tukey's Honestly Significant Difference as a post-hoc test. Differences were considered to be statistically significant at $p < 0.05$. The sensory profiles of the RTD green tea samples obtained from consensus profiling and QFP were correlated to the chemical formulation and consumer liking scores using a partial least squares-artificial neural network model, as discussed previously in Chapter 5. Sensory scores were normalised to a range of zero to one based on the original intensity range of zero to 100.

6.3. Results and discussion

6.3.1. Sensory profiling results

The scores obtained from QFP and FIP of the RTD green tea samples are shown in Tables 6.2 and 6.3, respectively.

6.3.1.1. QFP results

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Table 6.3: QFP scores of RTD green tea samples.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Table 6.4: FIP scores of RTD green tea samples.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.3.1.2. FIP results

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.3.2. Comparison of sensory profiling methods

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.3.2.1. Absolute sensory scores

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 6.1: This figure contains sensitive information, and has been omitted from the online version of this thesis.

6.3.2.2. PCA of sensory scores

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 6.2: This figure contains sensitive information, and has been omitted from the online version of this thesis.

6.3.2.3. k-means clustering

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 6.3: This figure contains sensitive information, and has been omitted from the online version of this thesis.

6.3.3. Development of mathematical model

Partial least squares-artificial neural network (PLS-ANN) hybrid models were trained to objectively predict consumer liking scores using sensory profiles of RTD green tea samples, according to Chapter 5. The use of a nonlinear hybrid

model in prediction of human responses is of interest due to its ability to model complex nonlinear relationships, which may be present in human sensory responses.

In developing a PLS-ANN hybrid model, a linear PLS model was first trained to be used as the 'backbone' of the hybrid model. Augmentation of the nonlinear ANN model was performed in the following manner:

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.3.4. PLS-ANN hybrid models

6.3.4.1. PLS model coefficients

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 6.4: This figure contains sensitive information, and has been omitted from the online version of this thesis.

6.3.4.2. Hybrid model quality

The two-component PLS models were augmented with ANN, as discussed in Section 6.3.3. The resulting PLS-ANN hybrid model for FIP results contained eight neurons in the ANN hidden layer, while that for QFP results contained three neurons. Modelling results obtained are shown in Figure 6.5.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 6.5: Plots of predicted liking scores against observed liking scores, based on (a) FIP results; and (b) QFP results. Training samples are indicated as ● while model validation samples are indicated as ●.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

6.4. Conclusions

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

CHAPTER 7

A COMPARISON OF EXPERIMENTAL DESIGNS IN THE DEVELOPMENT OF READY-TO-DRINK GREEN TEA BEVERAGES

Abstract

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.1. Introduction

Food product development is a critical process for the food industry, and is the culmination of advancements in knowledge of food preparation coupled with advancements in technology in the 20th century (Earle, 1997). The modern product development process is largely driven by the needs of consumers and market trends, and the nature of this process was summed up by Arteaga, Li-Chan, Vazquez-Arteaga, and Nakai (1994) as an optimisation problem, due to the identification and optimisation of factors (food ingredients and properties) in producing the best alternative (most well-accepted product) out of all possible alternatives (Arteaga, Li-Chan, Vazquez-Arteaga, & Nakai, 1994).

Design of Experiments (DOE) methodologies are efficient tools in studying the effects of variables or regressors, which include levels, number of factors, and number of observations, on a desired output (Arteaga et al., 1994). This allows for a deeper understanding of the interactive relationships between variables, for which simpler experimental designs such as the one-factor-at-a-time (OFAT) approach are unable to provide. Compared to OFAT approaches, classical methods such as factorial designs, Box-Behnken designs, and recent methods such as computer-generated optimal designs are considered to be much more efficient, and are able to provide more information for product and process development and optimisation.

Optimal designs are computer-generated, non-standard experimental designs used in place of classical experimental designs when certain design parameters are constrained, such as non-standard nonlinear models, or limitations on the number of experimental runs. Such designs are gradually gaining popularity in recent years, as seen by the increase in number of publications utilising optimal designs (Hewson, Hollowood, Chandra, & Hort, 2008; Hewson et al., 2009; Shiby et al., 2013). Although there are several optimality criteria in optimal designs, the D-optimality criterion is one of the most commonly used, due to its ease in computational requirements (Carlsson & Martinsson, 2003). D-optimal designs select a subset of design points from a candidate set in order to maximise the determinant of the Fisher information matrix, such that the variance of prediction of model coefficients is minimised. Compared to classical designs, D-optimal design allows for use of a lower number of samples, which in turn translates to a reduction in resources spent for collection of data.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.2. Materials and methods

7.2.1. Materials and sample preparation

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.2.2. Sensory evaluation

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.2.2.1. Consumer liking panel recruitment and selection

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.2.2.2. Consumer liking evaluation procedure

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.2.2.3. Trained panel selection and training

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

7.2.2.4. Quantitative flavour profiling

QFP was conducted according to the method described in Section 6.2.2.2 (Chapter 6).

7.2.3. Statistical analysis and mathematical modelling

Consumer liking scores were presented as mean ± standard deviation, while sensory scores obtained from consensus profiling were presented as a single number. The correlation between chemical formulation, sensory scores, and consumer liking was investigated using a partial least squares-artificial neural network (PLS-ANN) hybrid model, as discussed previously in Chapter 5. All variables were normalised to a range of zero to one based on the minimum and maximum values of each variable to ensure numerical consistency. Partial least squares regression models, artificial neural networks, and PLS-ANN hybrid models were constructed and trained using the Neural Network Toolbox in MATLAB version 7.12 (The MathWorks, Inc., USA).

7.3. Results and discussion

7.3.1. Comparison of experimental design quality

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Table 7.1: This figure contains sensitive information, and has been omitted from the online version of this thesis.

Table 7.2: This figure contains sensitive information, and has been omitted from the online version of this thesis.

Figure 7.1: This figure contains sensitive information, and has been omitted from the online version of this thesis.

7.3.2. Development of mathematical model for chemical and sensory data

Partial least squares-artificial neural network (PLS-ANN) hybrid models were developed according to the method described in Chapter 5 for each sensory attribute, which allowed for an objective prediction of the sensory profile of a product from its chemical formulation. In order to train a hybrid model of high prediction accuracy, the following algorithm was used:

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Figure 7.2: This figure contains sensitive information, and has been omitted from the online version of this thesis.

7.3.3. Effects on model quality

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Table 7.3: This figure contains sensitive information, and has been omitted from the online version of this thesis.

Figure 7.3: This figure contains sensitive information, and has been omitted from the online version of this thesis.

Figure 7.4: This figure contains sensitive information, and has been omitted from the online version of this thesis.

7.4. Conclusions

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

CHAPTER 8

IMPACTS OF STORAGE ON THE SENSORY PROFILE OF A GREEN TEA MODEL SYSTEM

Abstract

Ready-to-drink (RTD) green tea beverages are convenient green tea products, which are susceptible to changes in their sensory and chemical profiles over storage. These factors affect the eventual sensory characteristics and consumer liking of the product, and are of interest to the RTD beverages industry. The main objective of this study was to investigate the impacts of storage on the chemical and sensory profiles of a RTD green tea model system. Sensory data were correlated with chemical data obtained from instrumental analysis to allow for an objective prediction of the sensory profiles. Results obtained suggested that products stored at 25 °C, 35 °C, and 45 °C experienced a greater change in the chemical and sensory profiles compared to samples stored under refrigerated conditions. Furthermore, an ageing duration of at least two days was required in order for the flavour profile of samples to develop.

Keywords: green tea; storage; sensory; consensus profiling; partial least squares; artificial neural network

8.1. Introduction

Green tea, a beverage conventionally prepared by infusing the unfermented leaves of *Camellia sinensis* in hot water, is presently one of the most widely consumed drinks throughout the world, especially in East Asia. It is also known for its many health benefits, such as its anti-carcinogenic and anti-diabetic properties. Food manufacturers have responded to the growing popularity of green tea by increasing the availability and variety of RTD green tea products in the market, which supports the modern lifestyles of consumers such as young working adults, who value convenience.

Much of the substantial research on the odour and taste profiles of green tea has focused specifically on tea leaves and brews (Yang, Baldermann, & Watanabe, 2013; Kumazawa & Masuda, 2002). There are also a number of studies related to changes in the chemical and sensory profiles brought about by different processing methods; with thermal processing being the most commonly employed industrial method (Wang et al., 2000; Wang, Zhou, & Wen, 2006; Kim et al., 2007)

However, little is known about changes in the physicochemical and sensory profiles that may occur during storage of bottled RTD green tea prior to consumption, either when the product is stored at a warehouse, or in retail stores. These changes may be due to interactions taking place among the tea constituents, which may be dependent on storage conditions. This in turn has an impact on consumer acceptance, which has important implications for beverage manufacturers.

As a result, the objectives of this study were to investigate changes to the sensory and chemical profiles of an RTD green tea model system during storage, and to correlate sensory data to chemical constituents, in order to objectively predict the sensory profile of RTD green tea from analytical data.

8.2. Materials and Methods

8.2.1. Materials

The following materials were used in the preparation of samples: ascorbic acid and epigallocatechin gallate (EGCG)-enriched green tea extract (94% EGCG content) were supplied by DSM Nutritional Products Ltd (Basel, Switzerland), sodium bicarbonate by Merck KGaA (Darmstadt, Germany), caffeine by Givaudan International AG (Dübendorf, Switzerland), green tea extract by Damin Foodstuff (Zhangzhou) Co. (Fujian, China), and flavouring ingredients by Givaudan Singapore Pte Ltd (Singapore).

8.2.2. Sample preparation and storage

A green tea model system was designed based on prior analyses of several commercial RTD green tea samples. Green tea extract, a green tea flavour which was designed to mimic the aroma profile of a commercial RTD green tea product obtained from supermarkets in China, EGCG, ascorbic acid, sodium bicarbonate and caffeine in known amounts were mixed and dissolved in deionised water, and heat-treated by ultra-high temperature (UHT) processing

using an OMVE HT122-A HTST/UHT system (OMVE, Netherlands) at 138 ± 1 °C for a holding time of 15 s, then rapidly cooled to an outlet temperature of around 30 °C, before being aseptically filled into pre-sterilised polyethylene terephthalate (PET) bottles in an aseptic filling chamber (STAFtechco. Ltd, Thailand). After heat treatment, the bottled samples were kept at the following four storage temperatures: 4 °C in a chiller to simulate refrigeration, 25 °C in an air-conditioned room to mimic a typical environment of retailers of RTD products (e.g. a supermarket), and 35 °C and 45 °C to simulate storage during summertime or in tropical countries, as well as during transportation of products.

8.2.3. Chemical analyses

Quantitation of EGCG and caffeine was carried out by high performance liquid chromatography (HPLC) using an Agilent 1100 Series HPLC system coupled to a diode array detector, according to a previously described method (Section 4.2.2.2).

Quantitation of 44 volatile compounds was carried out by the standard addition method and analysed by gas chromatography (GC) on an Agilent 7890 Series GC system, equipped with an HP-INNOWax column (60 m × 0.25 mm, 0.25 μm), coupled to a flame ionisation detector (FID), and mass spectrometer (MS). Volatiles were first extracted by solid phase extraction using LiChrolut En 200 mg 3 mL (Merck Millipore, Germany), achieving a 150-fold concentration. Samples were injected in 1:10 split mode. The temperature programme used was 15 min at 50 °C, ramp of 3 °C/min to 250 °C, and holding time of 10 min. Nitrogen was used as a carrier gas at 1.87 mL/min.

8.2.4. Sensory evaluation

Sensory profiling was carried out by 10 to 12 panellists in duplicates, using the Flavour Profile Method (Murray et al., 2001). Samples were served at room temperature between 22 to 25 °C. The flavour descriptors used were as follows: bitter, astringent (mouth-drying, puckering), fermented nutty (herbal medicine), fermented hay (woody), fruity-floral (rose-like), tea-floral (jasmine-like), green pea (pea-like), fresh green (freshly cut leaves), leafy (Sencha), fatty (cucumber), roasted chestnut (roasted cashew), longjing (catty), and marine (salty, dried fish). The descriptors were rated on a 100-point scale at intervals of ten.

8.2.5. Statistical analysis

All HPLC and GC analyses were performed in triplicates. Analysis of variance (ANOVA) was performed using Excel 2010 (Microsoft Corporation, USA). Principal component analysis, partial least squares regression, and artificial neural network were performed using MATLAB version 7.12 (The MathWorks, Inc., USA).

8.3. Results and discussion

8.3.1. Changes in sensory profile

Results obtained from principal component analysis (PCA) of the average of two sensory evaluation sessions for the RTD green tea products are shown in Figure 8.1.

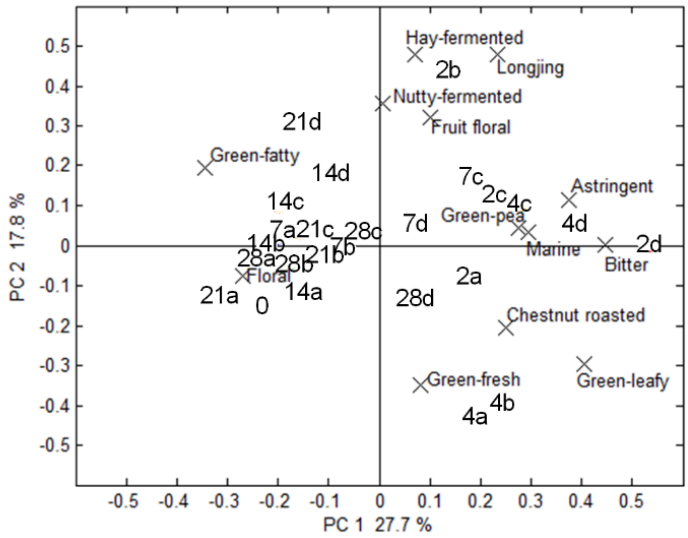

Figure 8.1: PCA biplot of sensory results as an average of two sessions. Sensory descriptors are represented by × as loadings on principal components. Samples are denoted as XY, where X refers to storage duration in days, and Y refers to storage temperature (a: 4 °C; b: 25 °C; c: 35 °C; d: 45 °C). Samples on day 0 is denoted by 0.

The first two principal components explained about 45% of the total variance. The first principal component (PC1) was positively correlated with marine, green pea, astringent, chestnut roasted, and bitter notes, which together, would reflect a more intense and roasted green tea profile; and was negatively correlated with the tea floral attribute, which would correspond to a lighter green tea profile. In general, most products stored at lower temperatures were found to have a higher intensity of the floral attribute, as compared to the other sensory attributes. Conversely, products that were stored at higher temperatures fell on the positive side of PC1, pointing to a more intense flavour and a stronger taste. This indicates that storage at or exposure to high temperatures may have changed the profile of RTD green tea products. However, it should be noted that the duration of storage plays an important role in the overall sensory profile of the samples. Products that were stored for two and four days were found to be

positively correlated to PC1, reflecting an increase in perception of astringency, bitterness, and roasted aromas over the first four days, but subsequent storage resulted in a decrease in the intensities of these attributes.

It was noted that after two days of storage, there was an increase in the intensities of most attributes, with the exception of the tea floral attribute. For green tea samples stored at 4 °C, there was in fact a large decrease in the floral intensity. The intensity of the floral attribute gradually increased again on prolonged storage, with a corresponding decrease in the other attributes. For samples stored at 45 °C, there was generally an increase in intensity of the sensory attributes after two days of storage, which resulted in a stronger tasting tea. These sensory attributes gradually decreased in intensity with an increase in storage duration, as seen by a smaller area shown on the spider web plots shown in Figure 8.2.

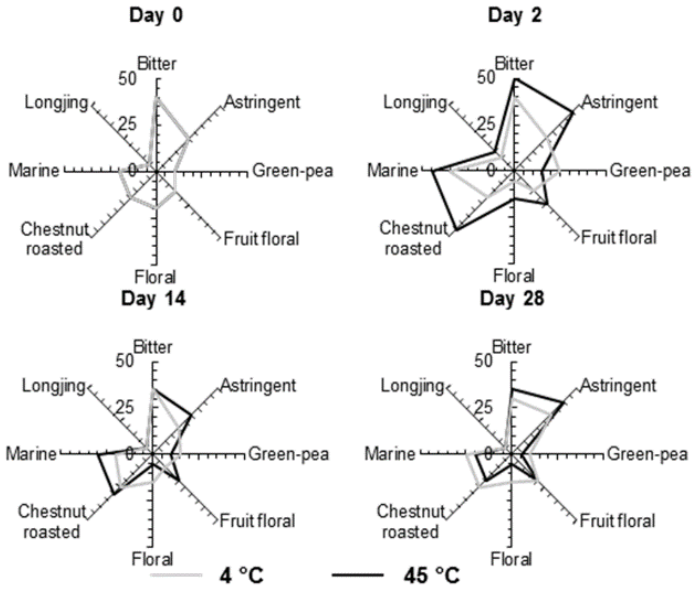

Figure 8.2: Spider web plots of RTD green tea samples stored at 4 °C and 45 °C.

8.3.2. Changes in chemical constituents

In order to provide insights into the observed changes in the sensory profiles during storage, the changes in selected volatile compounds (showing the greatest variation with storage time) are shown in Figure 3. During the first few days of storage at 4 °C (Figure 8.3a), the concentrations of compounds such as phenylacetaldehyde were relatively high, contributing to the predominantly floral note of the RTD samples. Phenylacetaldehyde degraded slowly over storage, and by mid-storage, the cis-3-hexenyl esters responsible for a green note became more prominent, resulting in an overall green note. Towards the end of storage, there was an increase in the concentrations of other floral compounds such as indole and methyl dihydrojasmonate, which led to a shift in sensory profile back to a more floral tasting green tea. Likewise, for samples stored at 45 °C (Figure 8.3b), there was a shift in the overall sensory profile with storage time. During early storage, compounds such as phenylacetaldehyde contributing to a floral profile, were dominating, but underwent degradation very quickly due to the elevated storage temperatures. Although the concentrations of pyrazines did not change greatly, the roasted nutty note still became the dominant one as perception of the overall floral note decreased.

This suggests that the changes in a perceived sensory cannot be explained solely by changes in the levels of compounds responsible for it, but also by changes in intensities of other attributes. Physical and chemical reactions such as adsorption of volatiles onto walls of the container (Marin, Acree, & Hotchkiss, 1993), hydrolysis of ester and glycosidic bonds (Yang et al., 2013), and

oxidation reactions may result in changes in the concentrations of volatile compounds within the samples, leading to changes in the sensory profiles as observed in this study.

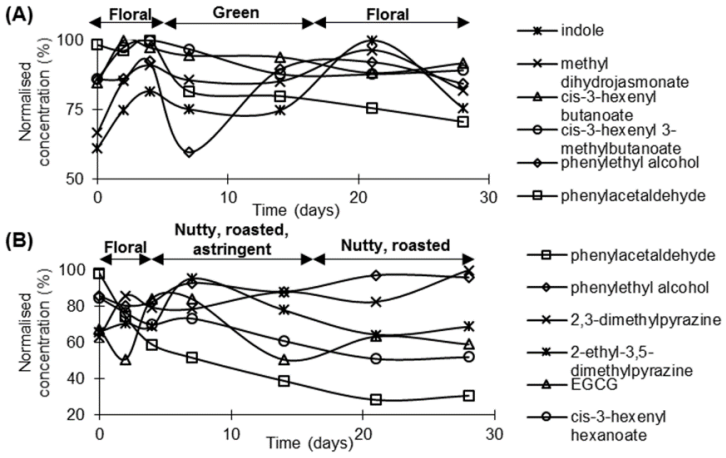

Figure 8.3: Changes in selected chemical compounds during storage for samples stored at (a) 4 °C and (b) 45 °C

8.3.3. Predictive modelling

It is known that the relationship between sensory perception and chemical stimuli is not straight forward, due to many possible interactions between different chemical compounds, and between chemical compounds and taste and odour receptors. Predictive modelling allowed for a better understanding of the system by correlating 46 chemical components to eight sensory attributes through regression models. In this study, partial least squares regression (PLSR) was used as a means of linear regression, and was compared against artificial neural networks (ANN), which was used as a form of nonlinear regression. It has been suggested that the relationship between food components and the

sensory attributes may not necessarily be linear, due to the manner in which humans perceive smells (Krishnamurthy et al., 2007). As such, ANN may be useful in modelling this nonlinearity.

ANN is based on a network of connected neuronal units, designed to mimic the biological nervous system. Input to individual units is determined by the sum of weighted outputs from neighbouring units, with a bias attached to each unit, and is processed by a neuronal transfer function. ANNs are able to learn by processing inputs and comparing against desired outputs. Errors are propagated back throughout the network, and weights of neural connections and neuron biases are adjusted accordingly to achieve the desired outcome. In this study, the input layer contained 46 neural units, each corresponding to a chemical compound. The hidden and output layers contained the sigmoidal and linear transfer functions, respectively. The network was trained by a back propagation algorithm to predict eight sensory attributes. The data were randomly divided into a training set and a validation set in a 75:25 ratio. The training set was used to develop the regression model using PLS or ANN, and the models were validated using the validation set. Model quality of the PLSR and ANN models is shown in Figure 8.4. A lower root-mean-squared error (RMSE) corresponds to a more accurate prediction by the model.

In general, both PLSR and ANN models were similar in terms of overall model quality, indicating that the linear PLSR model was able to perform on par with the nonlinear ANN model. This could be due to the high number of principal components selected (number of PCs = 9) for the PLSR model, which in turn, may suggest nonlinearity within the sensory and chemical data sets. Although

both the high dimensional PLSR and ANN model gave similar overall predictions, there were some differences within the individual sensory attributes.

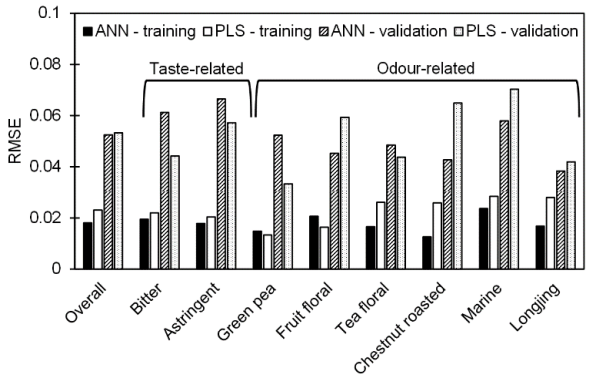

Figure 8.4: RMSE values of PLSR and ANN models for overall model quality and prediction qualities for eight individual sensory descriptors.

From Figure 8.4, it can be seen that the prediction quality of the ANN model for most of the odour-based attributes was better than that of the PLSR model, as shown by the lower validation RMSE values. For the taste-related sensory attributes, the opposite was true with the PLSR model providing a lower validation RMSE value. This suggest that the relationship between food components and odour attributes may contain a greater degree of nonlinearity, as compared to correlation between food components and taste attributes; but further work is required to ascertain this. Future work will focus on modelling the changes in the chemical composition of RTD green under different storage conditions by combining information from kinetic models and ANN to yield a hybrid grey-box model.

8.4. Conclusions

In conclusion, the sensory and chemical profiles of RTD green tea were affected by storage at different conditions. Storage under ambient conditions and higher temperatures resulted in a significant change in the sensory profile of the RTD green tea samples, which was caused by a change in the chemical constituents. This rate of change was reduced in samples stored under refrigerated conditions, indicating that there is a need for refrigeration of RTD green tea beverage products after the production stage, in order to minimalise the change in the overall sensory profile. Regression models were developed to objectively predict the sensory profile of RTD green tea based on its chemical composition.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

9.1. Conclusions

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

In Chapter 3, the key volatile flavour keys affecting consumer liking were identified using the MiniVAS. A linear stepwise regression model was developed to objectively predict hedonic properties of odour model systems of RTD green tea beverages. Fruity and floral smelling volatile flavour keys were found to be positive drivers of liking, indicating that RTD green tea beverages with stronger fruity and floral notes would be more popular with consumers. These findings were in line with results obtained in subsequent studies, highlighting the significance of the floral flavour key as a driver of liking, and the green pea flavour key as a driver of dislike. In order to reduce data dimensionality of the RTD green tea model system, the flavour keys with the least contribution to the overall consumer liking were identified and removed as independent variables, based on comparison between the initial regression model and the regression models that were developed from reduced experimental designs. Both the X_7 and X_8 flavour keys were found to have little

impact (i.e. neither positively nor negatively affecting liking), and were thus excluded from subsequent experimental designs.

A series of taste reconstruction and omission experiments were conducted in Chapter 4 to identify key non-volatile compounds affecting the overall taste profile of RTD green tea beverages. Bitter-tasting caffeine, bitter-tasting and astringent-causing EGCG, and umami-tasting glutamic acid were found to be important tastants in green tea following chemical and sensory analyses. Subsequent regression analysis using stepwise regression identified both caffeine and EGCG as the most significant non-volatile compounds in green tea, affecting bitterness and astringency respectively. The stepwise regression models that were developed also allowed for the prediction of intensities of bitterness and astringency based on the chemical profile of RTD green tea samples. Based on results obtained in Chapters 3 and 4, D-optimal experimental designs incorporating important volatile and non-volatile flavour keys were developed for use in Chapters 5, 6, and 7.

Mathematical models were developed using PLS regression, ANN, and PLS-ANN hybrid models to study the relationship between chemical flavour keys and consumer liking of RTD green tea model systems in Chapter 5. The floral and chestnut roasted flavour keys were found to positively drive consumer liking, while the green pea, bitter, and astringent flavour keys were found to be negative drivers based on the linear PLS model. Amongst the PLS, ANN, and PLS-ANN hybrid model, the linear PLS model was found to have the lowest model quality based on R^2, RMSE, and residuals analysis. Although both the ANN and PLS-ANN hybrid models were found to have comparable model

qualities, the PLS-ANN hybrid model contained an added advantage of providing basic information of the relationship between chemical and hedonic properties. Product optimisation was also performed using genetic algorithm to identify the chemical formulation of an RTD green tea model system corresponding to optimal consumer liking.

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Lastly, the effects of storage duration and temperature on the chemical and sensory profiles of RTD green tea was investigated in Chapter 8. A shift of sensory profile from floral to nutty, roasted attributes was observed in samples stored at higher temperatures. This corresponded to an overall decrease of compounds with floral attributes such as phenylacetaldehyde and phenylethyl alcohol, and an increase in pyrazines which were responsible for the nutty and roasted profile. For samples stored under refrigerated conditions, there was a slight change in the overall sensory profile from floral, to green, and then back to floral, due to changes in the chemical constituents over time. Results obtained from this study indicate that prolonged storage, even under refrigerated conditions, should be avoided due to potential changes in the chemical and sensory profile, which will in turn affect other sensory and hedonic properties.

9.2. Recommendations

9.2.1. Mathematical and statistical software for analysis

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

9.2.2. Choice of analytical procedure and comparison between methods

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

Table 9.1: Advantages and disadvantages of design of experiments methods.

Table 9.2: Advantages and disadvantages of regression methods.

9.2.3. Future work

****

This section contains confidential information, and has been omitted from the online version of this thesis.

****

BIBLIOGRAPHY

Abdi, H. (2003). Partial least squares (PLS) regression. – In: Lewis-Beck M. et al. (Eds.), *Encyclopaedia of social sciences research methods*, (pp. 792–795): Sage

Alam, F. M., McNaught, K. R., & Ringrose, T. J. (2004). A comparison of experimental designs in the development of a neural network simulation metamodel. *Simulation Modelling Practice and Theory*, *12*(7), 559-578.

Allais, I., Edoura-Gaena, R.-B., Gros, J.-B., & Trystram, G. (2007). How human expertise at industrial scale and experiments can be combined to improve food process knowledge and control. *Food Research International*, *40*(5), 585-602.

Ananingsih, V. K., Sharma, A., & Zhou, W. (2013). Green tea catechins during food processing and storage: a review on stability and detection. *Food Research International*, *50*(2), 469-479.

Angelino, S., & Gennaro, M. C. (2000). HPLC analysis of organic bases. In L. M. L. Nollet (Ed.), *Food Analysis by HPLC 2nd ed.*, (pp. 775-824): Marcel-Dekker, Inc.

Aparicio, R., Calvente, J. J., & Morales, M. T. (1996). Sensory Authentication of European Extra-Virgin Olive Oil Varieties by Mathematical Procedures. *Journal of the Science of Food and Agriculture*, *72*(4), 435-447.

Ares, G., Barrios, S., Lareo, C., & Lema, P. (2009). Development of a sensory quality index for strawberries based on correlation between sensory data and consumer perception. *Postharvest Biology and Technology*, *52*(1), 97-102.

Arteaga, G., Li-Chan, E., Vazquez-Arteaga, M., & Nakai, S. (1994). Systematic experimental designs for product formula optimization. *Trends in Food Science & Technology*, *5*(8), 243-254.

Bae, Y. W., Lee, S. M., & Kim, K. O. (2015). Age and gender differences in the influence of extrinsic product information on acceptability for RTD green tea beverages. *Journal of the Science of Food and Agriculture*, *96*(4), 1362-1372.

Bahamonde, A., Díez, J., Quevedo, J. R., Luaces, O., & del Coz, J. J. (2007). How to learn consumer preferences from the analysis of sensory data by means of support vector machines (SVM). *Trends in Food Science & Technology*, *18*(1), 20-28.

Bahramparvar, M., Salehi, F., & Razavi, S. M. A. (2014). Predicting Total Acceptance of Ice Cream Using Artificial Neural Network. *Journal of Food Processing and Preservation*, *38*(3), 1080-1088.

Baptista, J. A. B., Tavares, J. F. d. P., & Carvalho, R. C. B. (1998). Comparison of catechins and aromas among different green teas using HPLC/SPME-GC. *Food Research International*, *31*(10), 729-736.

Baryłko-Pikielna, N., & Kostyra, E. (2007). Sensory interaction of umami substances with model food matrices and its hedonic effect. *Food Quality and Preference*, *18*(5), 751-758.

Basheer, I. A., & Hajmeer, M. (2000). Artificial neural networks: fundamentals, computing, design, and application. *Journal of Microbiological Methods*, *43*(1), 3-31.

Bedini, A., Zanolli, V., Zanardi, S., Bersellini, U., Dalcanale, E., & Suman, M. (2013). Rapid and simultaneous analysis of xanthines and polyphenols as bitter taste markers in bakery products by FT-NIR spectroscopy. *Food Analytical Methods*, *6*(1), 17-27.

Benardos, P. G., & Vosniakos, G. C. (2007). Optimizing feedforward artificial neural network architecture. *Engineering Applications of Artificial Intelligence*, *20*(3), 365-382.

Bergstra, J., & Bengio, Y. (2012). Random search for hyper-parameter optimization. *Journal of Machine Learning Research*, *13*, 281-305.

Bindon, K., Holt, H., Williamson, P. O., Varela, C., Herderich, M., & Francis, I. L. (2014). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry*, *154*, 90-101.

Box, G. E. P., & Behnken, D. W. (1960). Some New Three Level Designs for the Study of Quantitative Variables. *Technometrics*, *2*(4), 455-475.

Brossaud, F., Cheynier, V., & Noble, A. C. (2001). Bitterness and astringency of grape and wine polyphenols. *Australian Journal of Grape and Wine Research*, *7*(1), 33-39.

Butt, M. S., & Sultan, M. T. (2009). Green tea: nature's defense against malignancies. *Critical Reviews in Food Science and Nutrition*, *49*(5), 463-473.

Cabrera, C., Artacho, R., & Gimenez, R. (2006). Beneficial effects of green tea - A review. *Journal of the American College of Nutrition*, *25*(2), 79-99.

Cabrera, C., Gimenez, R., & Lopez, M. C. (2003). Determination of tea components with antioxidant activity. *Journal of Agricultural and Food Chemistry*, *51*(15), 4427-4435.

Candioti, L. V., De Zan, M. M., Cámara, M. S., & Goicoechea, H. C. (2014). Experimental design and multiple response optimization. Using the desirability function in analytical methods development. *Talanta*, *124*, 123-138.

Carlsson, F., & Martinsson, P. (2003). Design techniques for stated preference methods in health economics. *Health Economics*, *12*(4), 281-294.

Cevoli, C., Cerretani, L., Gori, A., Caboni, M. F., Gallina Toschi, T., & Fabbri, A. (2011). Classification of Pecorino cheeses using electronic nose combined with artificial neural network and comparison with GC–MS analysis of volatile compounds. *Food Chemistry*, *129*(3), 1315-1319.

Chaturvedula, V. S. P., & Prakash, I. (2011). The aroma, taste, color and bioactive constituents of tea. *Journal of Medicinal Plants Research*, *5*(11), 2110-2124.

Chen, J., Sun, D. X., & Wu, C. F. J. (1993). A Catalogue of Two-Level and Three-Level Fractional Factorial Designs with Small Runs. *International Statistical Review / Revue Internationale de Statistique*, *61*(1), 131-145.

Chen, Q., Zhao, J., Chaitep, S., & Guo, Z. (2009). Simultaneous analysis of main catechins contents in green tea (*Camellia sinensis* (L.)) by Fourier transform near infrared reflectance (FT-NIR) spectroscopy. *Food Chemistry*, *113*(4), 1272-1277.

Chen, Q., Zhao, J., Chen, Z., Lin, H., & Zhao, D.-A. (2011). Discrimination of green tea quality using the electronic nose technique and the human panel test, comparison of linear and nonlinear classification tools. *Sensors and Actuators B: Chemical*, *159*(1), 294-300.

Chen, Q., Zhao, J., Guo, Z., & Wang, X. (2010). Determination of caffeine content and main catechins contents in green tea (*Camellia sinensis* L.) using taste sensor technique and multivariate calibration. *Journal of Food Composition and Analysis*, *23*(4), 353-358.

Chen, Z.-Y., Zhu, Q. Y., Tsang, D., & Huang, Y. (2001). Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry*, *49*(1), 477-482.

Cheng, Y., Huynh-Ba, T., Blank, I., & Robert, F. (2008). Temporal changes in aroma release of Longjing tea infusion: interaction of volatile and nonvolatile tea components and formation of 2-butyl-2-octenal upon aging. *Journal of Agricultural and Food Chemistry*, *56*(6), 2160-2169.

Cheong, M. W., Liu, S. Q., Zhou, W., Curran, P., & Yu, B. (2012). Chemical composition and sensory profile of pomelo (*Citrus grandis* (L.) Osbeck) juice. *Food Chemistry*, *135*(4), 2505-2513.

Cook, R. D., & Nachtrheim, C. J. (1980). A comparison of algorithms for constructing exact D-optimal designs. *Technometrics, 22*(3), 315-324.

Corrêa, S. C., Pinheiro, A. C. M., Siqueira, H. E., Carvalho, E. M., Nunes, C. A., & Vilas Boas, E. V. d. B. (2014). Prediction of the sensory acceptance of fruits by physical and physical–chemical parameters using multivariate models. *LWT - Food Science and Technology, 59*(2, Part 1), 666-672.

Cozzolino, D., Cynkar, W. U., Dambergs, R. G., Mercurio, M. D., & Smith, P. A. (2008). Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. *Journal of Agricultural and Food Chemistry, 56*(17), 7631-7636.

Cruz, A. G., Cadena, R. S., Faria, J. A. F., Oliveira, C. A. F., Cavalcanti, R. N., Bona, E., Bolini, H. M. A., & Da Silva, M. A. A. P. (2011). Consumer acceptability and purchase intent of probiotic yoghurt with added glucose oxidase using sensometrics, artificial neural networks and logistic regression. *International Journal of Dairy Technology, 64*(4), 549-556.

de Aguiar, P. F., Bourguignon, B., Khots, M. S., Massart, D. L., & Phan-Than-Luu, R. (1995). D-optimal designs. *Chemometrics and Intelligent Laboratory Systems, 30*(2), 199-210.

de Jong, S. (1993). SIMPLS: An alternative approach to partial least squares regression. *Chemometrics and Intelligent Laboratory Systems, 18*(3), 251-263.

de Kermadec, F. H., Durand, J. F., & Sabatier, R. (1997). Comparison between linear and nonlinear PLS methods to explain overall liking from sensory characteristics. *Food Quality and Preference, 8*(5), 395-402.

Dejaegher, B., & Vander Heyden, Y. (2011). Experimental designs and their recent advances in set-up, data interpretation, and analytical applications. *Journal of Pharmaceutical and Biomedical Analysis, 56*(2), 141-158.

Del Rio, D., Stewart, A. J., Mullen, W., Burns, J., Lean, M. E. J., Brighenti, F., & Crozier, A. (2004). HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *Journal of Agricultural and Food Chemistry, 52*(10), 2807-2815.

Ding, Y., Yu, H., & Mou, S. (2002). Direct determination of free amino acids and sugars in green tea by anion-exchange chromatography with integrated pulsed amperometric detection. *Journal of Chromatography A, 982*(2), 237-244.

Dong, J.-J., Li, Q.-L., Yin, H., Zhong, C., Hao, J.-G., Yang, P.-F., Tian, Y.-H., & Jia, S.-R. (2014). Predictive analysis of beer quality by correlating sensory evaluation with higher alcohol and ester production using multivariate statistics methods. *Food Chemistry, 161*, 376-382.

Dror, H. A., & Steinberg, D. M. (2008). Sequential experimental designs for generalized linear models. *Journal of the American Statistical Association*, *103*(481), 288-298.

Earle, M. (1997). Changes in the food product development process. *Trends in Food Science & Technology, 8*(1), 19-24.

Ekborg-Ott, K. H., Taylor, A., & Armstrong, D. W. (1997). Varietal differences in the total and enantiomeric composition of theanine in tea. *Journal of Agricultural and Food Chemistry*, *45*(2), 353-363.

El-Shahawi, M. S., Hamza, A., Bahaffi, S. O., Al-Sibaai, A. A., & Abduljabbar, T. N. (2012). Analysis of some selected catechins and caffeine in green tea by high performance liquid chromatography. *Food Chemistry*, *134*(4), 2268-2275.

Ellekjær, M. R., Ilseng, M. A., & Næs, T. (1996). A case study of the use of experimental design and multivariate analysis in product improvement. *Food Quality and Preference*, *7*(1), 29-36.

Euromonitor International (2015). Packaged food in Singapore – Industry overview. Retrieved from http://www.euromonitor.com/

Fedorov, V. V. (1972). Properties and methods of construction for optimal discrete designs. In *Theory of optimal experiments* (pp. 155-170). New York: Academic Press.

Fernqvist, F., & Ekelund, L. (2014). Credence and the effect on consumer liking of food–A review. *Food Quality and Preference, 32*, 340-353.

Finger, A., Kuhr, S., & Engelhardt, U. H. (1992). Chromatography of tea constutuents. *Journal of Chromatography*, 624(1-2), 293-315.

Friedman, M., Levin, C. E., Choi, S.-H., Kozukue, E., & Kozukue, N. (2006). HPLC analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: Comparison of water and 80% ethanol/water extracts. *Journal of Food Science*, 71(6), C328-C337.

Gao, Y., Tian, Y., Liu, D., Li, Z., Zhang, X.-X., Li, J.-M., Huang, J.-H., Wang, J., & Pan, Q.-H. (2015). Evolution of phenolic compounds and sensory in bottled red wines and their co-development. *Food Chemistry*, *172*, 565-574.

Geladi, P. (1988). Notes on the history and nature of partial least squares (PLS) modelling. *Journal of Chemometrics*, *2*(4), 231-246.

Geladi, P., & Kowalski, B. R. (1986). Partial least-squares regression: a tutorial. *Analytica Chimica Acta*, *185*, 1-17.

González Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2011). Relationships between Godello white wine sensory properties and its aromatic fingerprinting obtained by GC–MS. *Food Chemistry*, *129*(3), 890-898.

Granato, D., de Araújo Calado, V. M., & Jarvis, B. (2014). Observations on the use of statistical methods in Food Science and Technology. *Food Research International*, *55*, 137-149.

Greenhoff, K., & MacFie, H. J. H. (1994). Preference mapping in practice. In H. J. H. MacFie & D. M. H. Thomson (Eds.), *Measurement of Food Preferences* (pp. 137-166): Springer US.

Hamby, D. (1994). A review of techniques for parameter sensitivity analysis of environmental models. *Environmental Monitoring and Assessment*, *32*(2), 135-154.

Heenan, S. P., Dufour, J.-P., Hamid, N., Harvey, W., & Delahunty, C. M. (2009). Characterisation of fresh bread flavour: Relationships between sensory characteristics and volatile composition. *Food Chemistry*, *116*(1), 249-257.

Hewson, L., Hollowood, T., Chandra, S., & Hort, J. (2008). Taste–aroma interactions in a citrus flavoured model beverage system: Similarities and differences between acid and sugar type. *Food Quality and Preference*, *19*(3), 323-334.

Hewson, L., Hollowood, T., Chandra, S., & Hort, J. (2009). Gustatory, Olfactory and Trigeminal Interactions in a Model Carbonated Beverage. *Chemosensory Perception*, *2*(2), 94-107.

Hibbert, D. B. (2012). Experimental design in chromatography: A tutorial review. *Journal of Chromatography B*, *910*, 2-13.

Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition*, *43*(1), 89-143.

Horie, H., & Kohata, K. (2000). Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis. *Journal of Chromatography A*, *881*(1–2), 425-438.

Horie, H., Mukai, T., & Kohata, K. (1997). Simultaneous determination of qualitatively important components in green tea infusions using capillary electrophoresis. *Journal of Chromatography A*, *758*(2), 332-335.

Höskuldsson, A. (1988). PLS regression methods. *Journal of Chemometrics*, *2*(3), 211-228.

Hsiao, H.-Y., Chen, R. L. C., & Cheng, T.-J. (2010). Determination of tea fermentation degree by a rapid micellar electrokinetic chromatography. *Food Chemistry, 120*(2), 632-636.

Huang, Y., Kangas, L. J., & Rasco, B. A. (2007). Applications of Artificial Neural Networks (ANNs) in Food Science. *Critical Reviews in Food Science and Nutrition, 47*(2), 113-126.

Huo, D., Wu, Y., Yang, M., Fa, H., Luo, X., & Hou, C. (2014). Discrimination of Chinese green tea according to varieties and grade levels using artificial nose and tongue based on colorimetric sensor arrays. *Food Chemistry*, *145*, 639-645.

Hurling, R., & Shepherd, R. (2003). Eating with your eyes: effect of appearance on expectations of liking. *Appetite, 41*(2), 167-174.

Ikeda, G., Nagai, H., & Sagara, Y. (2004). Development of Food Kansei Model and its Application for Designing Tastes and Flavors of Green Tea Beverage. *Food Science and Technology Research*, *10*(4), 396-404.

Jaeger, S. R., Axten, L. G., Wohlers, M. W., & Sun-Waterhouse, D. (2009). Polyphenol-rich beverages: insights from sensory and consumer science. *Journal of the Science of Food and Agriculture, 89*(14), 2356-2363.

Jang, M., Park, J., & Park, H. (2011). Formulation optimization of salad dressing added with Chinese quince (*Chaenomelis sinensis*) juice by mixture design. *Food Science and Biotechnology, 20*(2), 409-417.

Jankun, J., Selman, S. H., Swiercz, R., & Skrzypczak-Jankun, E. (1997). Why drinking green tea could prevent cancer. *Nature, 387*(6633), 561-561.

Jiang, H., Engelhardt, U. H., Thräne, C., Maiwald, B., & Stark, J. (2015). Determination of flavonol glycosides in green tea, oolong tea and black tea by UHPLC compared to HPLC. *Food Chemistry*, *183*, 30-35.

Jumtee, K., Komura, H., Bamba, T., & Fukusaki, E. (2011). Predication of Japanese green tea (*Sen-cha*) ranking by volatile profiling using gas chromatography mass spectrometry and multivariate analysis. *Journal of Bioscience and Bioengineering*, *112*(3), 252-255.

Juneja, L. R., Chu, D.-C., Okubo, T., Nagato, Y., & Yokogoshi, H. (1999). L-theanine—a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Science & Technology*, *10*(6), 199-204.

Kakuda, T. (2002). Neuroprotective effects of the green tea components theanine and catechins. *Biological and Pharmaceutical Bulletin*, *25*(12), 1513-1518.

Kaneko, S., Kumazawa, K., Masuda, H., Henze, A., & Hofmann, T. (2006). Molecular and sensory studies on the umami taste of Japanese green tea. Journal of Agricultural and *Food Chemistry*, *54*(7), 2688-2694.

Kato, M., & Shibamoto, T. (2001). Variation of major volatile constituents in various green teas from Southeast Asia. *Journal of Agricultural and Food Chemistry*, *49*(3), 1394-1396.

Keenan, D. F., Brunton, N. P., Mitchell, M., Gormley, R., & Butler, F. (2012). Flavour profiling of fresh and processed fruit smoothies by instrumental and sensory analysis. *Food Research International*, *45*(1), 17-25.

Kengpol, A., & Wangkananon, W. (2015). An Assessment of Customer Contentment for Ready-to-Drink Tea Flavor Notes Using Artificial Neural Networks. In V. Kachitvichynaukul, K. Sethanan & P. Golińska-Dawson (Eds.), *Toward Sustainable Operations of Supply Chain and Logistics Systems* (pp. 421-434). Springer International Publishing.

Kim, E., Liang, Y., Jin, J., Sun, Q., Lu, J., Du, Y., & Lin, C. (2007). Impact of heating on chemical compositions of green tea liquor. *Food Chemistry*, *103*(4), 1263-1267.

Kim, S.-H., & Lee, K.-A. (2003). Evaluation of taste compounds in water-soluble extract of a *doenjang* (soybean paste). *Food Chemistry*, *83*(3), 339-342.

Knoop, J., Sala, G., Smit, G., & Stieger, M. (2013). Combinatory Effects of Texture and Aroma Modification on Taste Perception of Model Gels. *Chemosensory Perception*, *6*(2), 60-69.

Koshiishi, C., Crozier, A., & Ashihara, H. (2001). Profiles of purine and pyrimidine nucleotides in fresh and manufactured tea leaves. *Journal of Agricultural and Food Chemistry*, *49*(9), 4378-4382.

Krishnamurthy, R., Srivastava, A. K., Paton, J. E., Bell, G. A., & Levy, D. C. (2007). Prediction of consumer liking from trained sensory panel information: Evaluation of neural networks. *Food Quality and Preference*, *18*(2), 275-285.

Kruzlicova, D., Mocak, J., Balla, B., Petka, J., Farkova, M., & Havel, J. (2009). Classification of Slovak white wines using artificial neural networks and discriminant techniques. *Food Chemistry*, *112*(4), 1046-1052.

Kumazawa, K., & Masuda, H. (1999). Identification of potent odorants in Japanese green tea (*Sen-cha*). *Journal of Agricultural and Food Chemistry*, *47*(12), 5169-5172.

Kumazawa, K., & Masuda, H. (2002). Identification of potent odorants in different green tea varieties using flavor dilution technique. *Journal of Agricultural and Food Chemistry, 50*(20), 5660-5663.

Lawless, T. H., & Heymann, H. (2010). Descriptive Analysis Sensory Evaluation of Food: Principles and Practices. In H. T. Lawless & H. Heymann (Eds.), *Sensory Evaluation of Food: Principles and Practices* (pp. 227-257). New York, NY: Springer New York.

Leclercq, S., & Blancher, G. (2012). Multimodal sensory integration during sequential eating—linking chewing activity, aroma release, and aroma perception over time. *Chemical Senses, 37*(8), 689-700.

Lee, J.-H., Shim, J. S., Lee, J. S., Kim, J. K., Yang, I. S., Chung, M.-S., & Kim, K. H. (2006). Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (*Camellia sinensis*). *Journal of Agricultural and Food Chemistry, 54*(23), 8717-8723.

Leung, F. H. F., Lam, H. K., Ling, S. H., & Tam, P. K. S. (2003). Tuning of the structure and parameters of a neural network using an improved genetic algorithm. *IEEE Transactions on Neural Networks, 14*(1), 79-88.

Li, Z., Zhang, T., & Zhang, R. (2013). Three-level regular designs with general minimum lower-order confounding. *Canadian Journal of Statistics, 41*(1), 192-210.

Liang, Y., Ye, Q., Jin, J., Liang, H., Lu, J., Du, Y., & Dong, J. (2008). Chemical and instrumental assessment of green tea sensory preference. *International Journal of Food Properties, 11*(2), 258-272.

Lin, L. Z., Chen, P., & Harnly, J. M. (2008). New phenolic components and chromatographic profiles of green and fermented teas. *Journal of Agricultural and Food Chemistry, 56*(17), 8130-8140.

Lin, J., Dai, Y., Guo, Y.-n., Xu, H.-r., & Wang, X.-c. (2012). Volatile profile analysis and quality prediction of Longjing tea (Camellia sinensis) by HS-SPME/GC-MS. *Journal of Zhejiang University Science B, 13*(12), 972-980.

Liu, C., Dong, J., Wang, J., Yin, X., & Li, Q. (2012). A comprehensive sensory evaluation of beers from the Chinese market. *Journal of the Institute of Brewing, 118*(3), 325-333.

Liu, J., Toldam-Andersen, T. B., Petersen, M. A., Zhang, S., Arneborg, N., & Bredie, W. L. P. (2015). Instrumental and sensory characterisation of Solaris white wines in Denmark. *Food Chemistry, 166*, 133-142.

Lombardo, R. (2011). The analysis of sensory and chemical–physical variables via multivariate additive PLS splines. *Food Quality and Preference, 22*(8), 714-724.

Lombardo, R., Amenta, P., Vivien, M., & Sabatier, R. (2012). Sensory analysis via multi-block multivariate additive PLS splines. *Journal of Applied Statistics, 39*(4), 731-743.

Lobato, L. P., Anibal, D., Lazaretti, M. M., & Grossmann, M. V. E. (2011). Extruded puffed functional ingredient with oat bran and soy flour. *LWT - Food Science and Technology, 44*(4), 933-939.

Loredo, A. G., Guerrero, S., & Alzamora, S. (2014). Relationships Between Texture and Rheological Properties in Blanched Apple Slices (var. Granny Smith) Studied by Partial Least Squares Regression. *Food and Bioprocess Technology*, *7*(10), 2840-2854.

Lotong, V., Chambers, D. H., Dus, C., Edgar Chambers, I., & Civille, G. V. (2002). Matching results of two independent highly trained sensory panels using different descriptive analysis methods. *Journal of Sensory Studies, 17*(5), 429-444.

Lozano, J., Santos, J. P., & Carmen Horrillo, M. (2008). Enrichment sampling methods for wine discrimination with gas sensors. *Journal of Food Composition and Analysis, 21*(8), 716-723.

Mandenius, C. F., & Brundin, A. (2008). Bioprocess optimization using design-of-experiments methodology. *Biotechnology Progress*, *24*(6), 1191-1203.

Marin, A. B., Acree, T. E., Hotchkiss, J. H., & Nagy, S. (1992). Gas chromatography-olfactometry of orange juice to assess the effects of plastic polymers on aroma character. *Journal of Agricultural and Food Chemistry*, *40*(4), 650-654.

Martens, H., & Martens, M. (2000). Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality and Preference, 11*(1), 5-16.

Martin, N., Molimard, P., Spinnler, H. E., & Schlich, P. (2000). Comparison of odour sensory profiles performed by two independent trained panels following the same descriptive analysis procedures. *Food Quality and Preference*, *11*(6), 487-495.

Maulik, U., & Bandyopadhyay, S. (2002). Performance evaluation of some clustering algorithms and validity indices. *IEEE Transactions on Pattern Analysis and Machine Intelligence, 24*(12), 1650-1654.

Mentré, F., Mallet, A., & Baccar, D. (1997). Optimal design in random-effects regression models. *Biometrika, 84*(2), 429-442.

Michishita, T., Akiyama, M., Hirano, Y., Ikeda, M., Sagara, Y., & Araki, T. (2010). Gas Chromatography/Olfactometry and Electronic Nose Analyses of Retronasal Aroma of Espresso and Correlation with Sensory Evaluation by an Artificial Neural Network. *Journal of Food Science, 75*(9), S477-S489.

Millin, D. J., Crispin, D. J., & Swaine, D. (1969). Nonvolatile components of black tea and their contribution to the character of the beverage. *Journal of Agricultural and Food Chemistry*, *17*(4), 717-722.

Misaka, S., Kawabe, K., Onoue, S., Werba, J. P., Giroli, M., Kimura, J., Hiroshi, W., & Yamada, S. (2013). Development of rapid and simultaneous quantitative method for green tea catechins on the bioanalytical study using UPLC/ESI‐MS. *Biomedical Chromatography*, *27*(1), 1-6.

Mitchell, T. J. (1974). An Algorithm for the Construction of "D-Optimal" Experimental Designs. *Technometrics*, *16*(2), 203-210.

Monobe, M., Ema, K., Kato, F., & Maeda-Yamamoto, M. (2008). Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *Journal of Agricultural and Food Chemistry*, *56*(4), 1423-1427.

Montgomery, D. C. (2008). *Design and analysis of experiments*: John Wiley & Sons.

Mukhopadhyay, S., Majumdar, G. C., Goswami, T. K., & Mishra, H. N. (2013). Fuzzy logic (similarity analysis) approach for sensory evaluation of *chhana podo*. *LWT - Food Science and Technology*, *53*(1), 204-210.

Murray, J., Delahunty, C., & Baxter, I. (2001). Descriptive sensory analysis: past, present and future. *Food Research International*, *34*(6), 461-471.

Næs, T., Tomic, O., Mevik, B. H., & Martens, H. (2011). Path modelling by sequential PLS regression. *Journal of Chemometrics*, *25*(1), 28-40.

Nakakuki, H., Horie, H., Yamauchi, Y., & Kohata, K. (1999). Rapid analysis of methylated xanthines in teas by an improved high-performance liquid chromatographic method using a polyvinylpolypyrroridone pre-column. *Journal of Chromatography A*, *848*(1–2), 523-527.

Narukawa, M., Kimata, H., Noga, C., & Watanabe, T. (2010). Taste characterisation of green tea catechins. *International Journal of Food Science & Technology*, *45*(8), 1579-1585.

Nasri, N., Beno, N., Septier, C., Salles, C., & Thomas-Danguin, T. (2011). Cross-modal interactions between taste and smell: Odour-induced saltiness enhancement depends on salt level. *Food Quality and Preference*, *22*(7), 678-682.

Nguyen, N.-K., & Miller, A. J. (1992). A review of some exchange algorithms for constructing discrete D-optimal designs. *Computational Statistics & Data Analysis*, *14*(4), 489-498.

Nie, S.-P., & Xie, M.-Y. (2011). A review on the isolation and structure of tea polysaccharides and their bioactivities. *Food Hydrocolloids*, *25*(2), 144-149.

Niimi, J., Eddy, A. I., Overington, A. R., Heenan, S. P., Silcock, P., Bremer, P. J., & Delahunty, C. M. (2014). Cheddar cheese taste can be reconstructed in solution using basic tastes. *International Dairy Journal*, *34*(1), 116-124.

Niimi, J., Overington, A. R., Silcock, P., Bremer, P. J., & Delahunty, C. M. (2016). Cross-modal taste and aroma interactions: Cheese flavour perception and changes in flavour character in multicomponent mixtures. *Food Quality and Preference*, *48*, 70-80.

Ninomiya, K. (2002). Umami: a universal taste. *Food Reviews International*, *18*(1), 23-38.

Noble, A. C., & Ebeler, S. E. (2002). Use of multivariate statistics in understanding wine flavor. *Food Reviews International*, *18*(1), 1-20.

Noguerol-Pato, R., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2009). Quantitative determination and characterisation of the main odourants of Mencía monovarietal red wines. *Food Chemistry*, *117*(3), 473-484.

Obanda, M., Owuor, P. O., & Mang'oka, R. (2001). Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food Chemistry*, *75*(4), 395-404.

Ogungbenro, K., Graham, G., Gueorguieva, I., & Aarons, L. (2005). The use of a modified Fedorov exchange algorithm to optimise sampling times for population pharmacokinetic experiments. *Computer Methods and Programs in Biomedicine*, *80*(2), 115-125.

Olsson, I.-M., Gottfries, J., & Wold, S. (2004). Controlling coverage of D-optimal onion designs and selections. *Journal of Chemometrics*, *18*(12), 548-557.

Ömür-Özbek, P., & Dietrich, A. M. (2008). Developing hexanal as an odor reference standard for sensory analysis of drinking water. *Water Research*, *42*(10), 2598-2604.

Pages, J., & Tenenhaus, M. (2001). Multiple factor analysis combined with PLS path modelling. Application to the analysis of relationships between physicochemical variables, sensory profiles and hedonic judgements. *Chemometrics and Intelligent Laboratory Systems*, *58*(2), 261-273.

Pang, X., Qin, Z., Zhao, L., Cheng, H., Hu, X., Song, H., & Wu, J. (2012). Development of regression model to differentiate quality of black tea (*Dianhong*): correlate aroma properties with instrumental data using multiple linear regression analysis. *International Journal of Food Science & Technology*, *47*(11), 2372-2379.

Peleg, H., Gacon, K., Schlich, P., & Noble, A. C. (1999). Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture*, *79*(8), 1123-1128.

Peng, L., Song, X., Shi, X., Li, J., & Ye, C. (2008). An improved HPLC method for simultaneous determination of phenolic compounds, purine alkaloids and theanine in *Camellia* species. *Journal of Food Composition and Analysis*, *21*(7), 559-563.

Peres, R. G., Tonin, F. G., Tavares, M. F. M., & Rodriguez-Amaya, D. B. (2011). Determination of catechins in green tea infusions by reduced flow micellar electrokinetic chromatography. *Food Chemistry*, *127*(2), 651-655.

Périnel, E., & Pagès, J. (2004). Optimal nested cross-over designs in sensory analysis. *Food Quality and Preference*, *15*(5), 439-446.

Perrot, N., Ioannou, I., Allais, I., Curt, C., Hossenlopp, J., & Trystram, G. (2006). Fuzzy concepts applied to food product quality control: A review. *Fuzzy Sets and Systems*, *157*(9), 1145-1154.

Piclin, N., Pintore, M., Lanza, C. M., Scacco, A., Guccione, S., Giurato, L., & Chrétien, J. R. (2008). Sensory analysis of red wines: Discrimination by adaptive fuzzy partition. *Journal of Sensory Studies*, *23*(4), 558-569.

Pierce, J., & Halpern, B. P. (1996). Orthonasal and retronasal odorant identification based upon vapor phase input from common substances. *Chemical Senses*, *21*(5), 529-543.

Piggott, J. R., Simpson, S. J., & Williams, S. A. R. (1998). Sensory analysis. *International Journal of Food Science & Technology*, *33*(1), 7-12.

Piñeiro-Sotelo, M., Rodríguez-Bernaldo de Quirós, A., López-Hernández, J., & Simal-Lozano, J. (2002). Determination of purine bases in sea urchin (*Paracentortus lividus*) gonads by high-performance liquid chromatography. *Food Chemistry*, *79*(1), 113-117.

Pongsuwan, W., Bamba, T., Harada, K., Yonetani, T., Kobayashi, A., & Fukusaki, E. (2008). High-throughput technique for comprehensive analysis of Japanese green tea quality assessment using ultra-performance liquid chromatography with time-of-flight mass spectrometry (UPLC/TOF MS). *Journal of Agricultural and Food Chemistry*, *56*(22), 10705-10708.

Puškaš, V., & Miljić, U. (2012). The application of D-optimal design for modelling the red wine ageing process. *Food Control*, *28*(2), 362-367.

Ramírez-Sucre, M. O., & Vélez-Ruiz, J. F. (2013). Physicochemical, rheological and stability characterization of a caramel flavored yogurt. *LWT - Food Science and Technology*, *51*(1), 233-241.

Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2012). Dynamic headspace/GC–MS to control the aroma fingerprint of extra-virgin olive oil from the same and different olive varieties. *Food Control*, *25*(2), 684-695.

Resano, H., Sanjuán, A. I., Cilla, I., Roncalés, P., & Albisu, L. M. (2010). Sensory attributes that drive consumer acceptability of dry-cured ham and convergence with trained sensory data. *Meat Science*, *84*(3), 344-351.

Roland, W. S., van Buren, L., Gruppen, H., Driesse, M., Gouka, R. J., Smit, G., & Vincken, J.-P. (2013). Bitter taste receptor activation by flavonoids and isoflavonoids: Modeled structural requirements for activation of hTAS2R14 and hTAS2R39. *Journal of Agricultural and Food Chemistry*, *61*(44), 10454-10466.

Rosenzweig, S., Yan, W., Dasso, M., & Spielman, A. I. (1999). Possible novel mechanism for bitter taste mediated through cGMP. *Journal of Neurophysiology*, *81*(4), 1661-1665.

Rosipal, R., & Krämer, N. (2006). Overview and Recent Advances in Partial Least Squares. In C. Saunders, M. Grobelnik, S. Gunn & J. Shawe-Taylor (Eds.), *Subspace, Latent Structure and Feature Selection* (Vol. 3940, pp. 34-51): Springer Berlin Heidelberg.

Rossetti, D., Bongaerts, J., Wantling, E., Stokes, J., & Williamson, A.-M. (2009). Astringency of tea catechins: more than an oral lubrication tactile percept. *Food Hydrocolloids*, *23*(7), 1984-1992.

Rostagno, M. A., Manchón, N., D'Arrigo, M., Guillamón, E., Villares, A., García-Lafuente, A., Ramos, A., & Martínez, J. A. (2011). Fast and simultaneous determination of phenolic compounds and caffeine in teas, mate, instant coffee, soft drink and energetic drink by high-performance liquid chromatography using a fused-core column. *Analytica Chimica Acta*, *685*(2), 204-211.

Routray, W., & Mishra, H. N. (2012). Sensory evaluation of different drinks formulated from *Dahi* (Indian yogurt) powder using fuzzy logic. *Journal of Food Processing and Preservation*, *36*(1), 1-10.

Rovio, S., Yli-Kauhaluoma, J., & Sirén, H. (2007). Determination of neutral carbohydrates by CZE with direct UV detection. *Electrophoresis*, *28*(17), 3129-3135.

Saeleaw, M., Dürrschmid, K., & Schleining, G. (2012). The effect of extrusion conditions on mechanical-sound and sensory evaluation of rye expanded snack. *Journal of Food Engineering*, *110*(4), 532-540.

Scharbert, S., & Hofmann, T. (2005). Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *Journal of Agricultural and Food Chemistry*, *53*(13), 5377-5384.

Scharbert, S., Holzmann, N., & Hofmann, T. (2004). Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *Journal of Agricultural and Food Chemistry*, *52*(11), 3498-3508.

Schiffman, S. S., Sennewald, K., & Gagnon, J. (1981). Comparison of taste qualities and thresholds of D-and L-amino acids. *Physiology & Behavior*, *27*(1), 51-59.

Seeram, N. P., Henning, S. M., Niu, Y., Lee, R., Scheuller, H. S., & Heber, D. (2006). Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *54*(5), 1599-1603.

Shankar, T. J., & Bandyopadhyay, S. (2004). Optimization of extrusion process variables using a genetic algorithm. *Food and Bioproducts Processing*, *82*(2), 143-150.

Shiby, V. K., Radhakrishna, K., & Bawa, A. S. (2013). Development of whey-fruit-based energy drink mixes using D-optimal mixture design. *International Journal of Food Science & Technology*, *48*(4), 742-748.

Shiby, V. K., Radhakrishna, K., & Bawa, A. S. (2013). Development of whey-fruit-based energy drink mixes using D-optimal mixture design. *International Journal of Food Science & Technology*, *48*(4), 742-748.

Shimoda, M., Shigematsu, H., Shiratsuchi, H., & Osajima, Y. (1995). Comparison of volatile compounds among different grades of green tea and their relations to odor attributes. *Journal of Agricultural and Food Chemistry*, *43*(6), 1621-1625.

Simon, S., Hall, W., & Schiffman, S. (1992). Astringent-tasting compounds alter ion transport across isolated canine lingual epithelia. *Pharmacology Biochemistry and Behavior*, *43*(1), 271-283.

Singh, R. R. B., Ruhil, A. P., Jain, D. K., Patel, A. A., & Patil, G. R. (2009). Prediction of sensory quality of UHT milk – A comparison of kinetic and neural network approaches. *Journal of Food Engineering*, *92*(2), 146-151.

Sinija, V. R., & Mishra, H. N. (2011). Fuzzy Analysis of Sensory Data for Quality Evaluation and Ranking of Instant Green Tea Powder and Granules. *Food and Bioprocess Technology*, *4*(3), 408-416.

Small, D. M., Gerber, J. C., Mak, Y. E., & Hummel, T. (2005). Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron*, *47*(4), 593-605.

Song, W., Pabbisetty, D., Groeber, E. A., Steenwyk, R. C., & Fast, D. M. (2009). Comparison of fused-core and conventional particle size columns by LC–MS/MS and UV: Application to pharmacokinetic study. *Journal of Pharmaceutical and Biomedical Analysis*, *50*(3), 491-500.

Soukoulis, C., Lyroni, E., & Tzia, C. (2010). Sensory profiling and hedonic judgement of probiotic ice cream as a function of hydrocolloids, yogurt and milk fat content. *LWT - Food Science and Technology*, *43*(9), 1351-1358.

Syu, K.-Y., Lin, C.-L., Huang, H.-C., & Lin, J.-K. (2008). Determination of theanine, GABA, and other amino acids in green, oolong, black, and *pu-erh* teas with dabsylation and high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *56*(17), 7637-7643.

Tamamoto, L. C., Schmidt, S. J., & Lee, S.-Y. (2010). Sensory Profile of a Model Energy Drink with Varying Levels of Functional Ingredients—Caffeine, Ginseng, and Taurine. *Journal of Food Science*, *75*(6), S271-S278.

Therdthai, N., & Zhou, W. (2002). Hybrid neural modeling of the electrical conductivity property of recombined milk. *International Journal of Food Properties*, *5*(1), 49-61.

Tomlins, K., Owori, C., Bechoff, A., Menya, G., & Westby, A. (2012). Relationship among the carotenoid content, dry matter content and sensory attributes of sweet potato. *Food Chemistry*, *131*(1), 14-21.

Valentin, D., Chollet, S., Lelievre, M., & Abdi, H. (2012). Quick and dirty but still pretty good: A review of new descriptive methods in food science. *International Journal of Food Science & Technology*, *47*(8), 1563-1578.

Vieira, H., Sanchez, S. M., Kienitz, K. H., & Belderrain, M. C. N. (2011, December). Improved efficient, nearly orthogonal, nearly balanced mixed designs. In *Proceedings of the 2011 Winter Simulation Conference (WSC)* (pp. 3600-3611). IEEE.

Viljanen, K., Heiniö, R.-L., Juvonen, R., Kössö, T., & Puupponen-Pimiä, R. (2014). Relation of sensory perception with chemical composition of bioprocessed lingonberry. *Food Chemistry*, *157*, 148-156.

Wang, D., Lu, J., Miao, A., Xie, Z., & Yang, D. (2008). HPLC-DAD-ESI-MS/MS analysis of polyphenols and purine alkaloids in leaves of 22 tea cultivars in China. *Journal of Food Composition and Analysis*, *21*(5), 361-369.

Wang, H., & Helliwell, K. (2001). Determination of flavonols in green and black tea leaves and green tea infusions by high-performance liquid chromatography. *Food Research International*, *34*(2–3), 223-227.

Wang, H., Helliwell, K., & You, X. (2000). Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. *Food Chemistry*, *68*(1), 115-121.

Wang, K., & Ruan, J. (2009). Analysis of chemical components in green tea in relation with perceived quality, a case study with *Longjing* teas. *International Journal of Food Science & Technology*, *44*(12), 2476-2484.

Wang, K., Liu, F., Liu, Z., Huang, J., Xu, Z., Li, Y., Chen, J, Gong, Y, & Yang, X. (2010). Analysis of chemical components in oolong tea in relation to perceived quality. *International Journal of Food Science & Technology*, *45*(5), 913-920.

Wang, L.-F., Kim, D.-M., & Lee, C. Y. (2000). Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. *Journal of Agricultural and Food Chemistry*, *48*(9), 4227-4232.

Wang, L.-F., Lee, J.-Y., Chung, J.-O., Baik, J.-H., So, S., & Park, S.-K. (2008). Discrimination of teas with different degrees of fermentation by SPME–GC analysis of the characteristic volatile flavour compounds. *Food Chemistry*, *109*(1), 196-206.

Wang, L., Xu, R., Hu, B., Li, W., Sun, Y., Tu, Y., & Zeng, X. (2010). Analysis of free amino acids in Chinese teas and flower of tea plant by high performance liquid chromatography combined with solid-phase extraction. *Food Chemistry*, *123*(4), 1259-1266.

Wang, R., & Zhou, W. (2004). Stability of tea catechins in the breadmaking process. *Journal of Agricultural and Food Chemistry*, *52*(26), 8224-8229.

Wang, R., Zhou, W., & Wen, R. A. H. (2006). Kinetic study of the thermal stability of tea catechins in aqueous systems using a microwave reactor. *Journal of Agricultural and Food Chemistry*, *54*(16), 5924-5932.

Wang, Y., Yang, X., Li, K., Li, C., Li, L., Li, J., Huang, H., He, Y., Ye, C., & Song, X. (2010). Simultaneous determination of theanine, gallic acid, purine alkaloids, catechins, and theaflavins in black tea using HPLC. *International Journal of Food Science & Technology*, *45*(6), 1263-1269.

Warmke, R., & Belitz, H.-D. (1993). Influence of glutamic acid on the bitter taste of various compounds. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, *197*(2), 132-133.

Wei, X., Xi, X., Wu, M., & Wang, Y. (2011). A novel method for quantitative determination of tea polysaccharide by resonance light scattering. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *79*(5), 928-933.

Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems*, *58*(2), 109-130.

Wu, C. Y., Xu, H. R., Heritier, J., & Andlauer, W. (2012). Determination of catechins and flavonol glycosides in Chinese tea varieties. *Food Chemistry*, *132*(1), 144-149.

Wythoff, B. J. (1993). Backpropagation neural networks: a tutorial. *Chemometrics and Intelligent Laboratory Systems*, *18*(2), 115-155.

Xu, L., Li, Y., Xu, N., Hu, Y., Wang, C., He, J., Cao, Y., Chen, S., & Li, D. (2014). Soy Sauce Classification by Geographic Region and Fermentation Based on Artificial Neural Network and Genetic Algorithm. *Journal of Agricultural and Food Chemistry*, *62*(51), 12294-12298.

Yamaguchi, S. (1998). Basic properties of umami and its effects on food flavor. *Food Reviews International*, *14*(2-3), 139-176.

Yamaguchi, S., & Ninomiya, K. (2000). Umami and food palatability. *Journal of Nutrition*, *130*(4), 921S-926S.

Yamazaki, T., Narukawa, M., Mochizuki, M., Misaka, T., & Watanabe, T. (2013). Activation of the hTAS2R14 human bitter-taste receptor by (−)-epigallocatechin gallate and (−)-epicatechin gallate. *Bioscience, Biotechnology, and Biochemistry*, *77*(9), 1981-1983.

Yang, Z., Baldermann, S., & Watanabe, N. (2013). Recent studies of the volatile compounds in tea. *Food Research International*, *53*(2), 585-599.

Ying, Y., Ho, J. W., Chen, Z. Y., & Wang, J. (2005). Analysis of theanine in tea leaves by HPLC with fluorescence detection. *Journal of Liquid Chromatography & Related Technologies*, *28*(5), 727-737.

Yu, H., Wang, J., Yao, C., Zhang, H., & Yu, Y. (2008). Quality grade identification of green tea using E-nose by CA and ANN. *LWT-Food Science and Technology*, *41*(7), 1268-1273.

Zegler, J. (2013). Tea and RTD Teas - US - July 2013. London, England: Mintel Group Ltd.

Zhang, H., Wang, J., & Ye, S. (2008). Prediction of soluble solids content, firmness and pH of pear by signals of electronic nose sensors. *Analytica Chimica Acta, 606*(1), 112-118.

Zhou, X., Wang, D., Sun, P., Bucheli, P., Li, L., Hou, Y., & Wang, J. (2007). Effects of soluble tea polysaccharides on hyperglycemia in alloxan-diabetic mice. *Journal of Agricultural and Food Chemistry, 55*(14), 5523-5528.

Zielinski, A. A. F., Haminiuk, C. W. I., Nunes, C. A., Schnitzler, E., van Ruth, S. M., & Granato, D. (2014). Chemical Composition, Sensory Properties, Provenance, and Bioactivity of Fruit Juices as Assessed by Chemometrics: A Critical Review and Guideline. *Comprehensive Reviews in Food Science and Food Safety, 13*(3), 300-316.

Zuo, Y., Chen, H., & Deng, Y. (2002). Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and *pu-erh* teas using HPLC with a photodiode array detector. *Talanta, 57*(2), 307-316.