Development of a novel functional yogurt containing anti-inflammatory bioactive compounds

Akshay Bisht

2019

Development of a novel functional yogurt containing anti-inflammatory bioactive compounds

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Food Technology

Massey University

Albany, New Zealand

Akshay Bisht

June 2019

Abstract

The consumption of bioactive compounds is increasingly becoming popular due to their beneficial effects on health and wellbeing. The anti-inflammatory properties of bioactives such as curcumin are well established. However, curcumin has low bioavailability, hence it is frequently consumed in capsules to enable the delivery of the required dosage to achieve optimum health benefits. Synergistic effects may be achieved by combining curcumin with other anti-inflammatory bioactive compounds. Recent investigations on lupeol and chlorogenic acid (CGA) have reported that these bioactive compounds show similar therapeutic benefits to curcumin. Furthermore, delivery of bioactives *via* a food matrix, such as fermented coconut yogurt, may improve bioavailability. Thus, this research investigated the potential of an anti-inflammatory combination of curcumin with CGA or lupeol with the objective of developing coconut yogurt to deliver the combined bioactives to humans.

This research was performed in two parts. In part 1, the anti-inflammatory potential of three bioactive compounds (curcumin, CGA and lupeol), individually and in combination, was investigated using an *in vitro* model of human THP-1 macrophages stimulated with LPS. Differentiated THP-1 cells were treated with variable concentrations of curcumin, CGA and lupeol and their effects on the production of TNF-α, a pro-inflammatory cytokine, and cell viability was measured using ELISA and MTT assays, respectively. Curcumin alone significantly ($p \leq 0.05$) suppressed TNF-α production in a dose dependent manner. Curcumin in combination with lupeol gave an additional 15-35 % reduction in TNF-α level. However, the reduction in TNF-α production by curcumin + lupeol was accompanied by cell death. In contrast, treatment with CGA appeared to protect the THP-1 cells from LPS toxicity and its co-administration with curcumin at a 1:1 ratio reduced TNF-α production without impacting cell viability. Further, it is proposed that the latter combination showed anti-inflammatory activity by reducing mRNA expression of pro-inflammatory cytokines and COX-2 enzyme *via* suppressing NF-κB, IκB-β-kinase and TLR-4 receptor. Thus, a 1:1 combination of curcumin with CGA was selected to be delivered in coconut yogurt.

In part 2, coconut yogurt enriched with turmeric and coffee to deliver the benefits of curcumin and CGA, respectively, was developed. Addition of 100 mg of each bioactive compound to 150 g coconut cream did not have any significant ($p \leq 0.05$) effect on the viable cell counts of the yogurt culture, pH and titratable acidity during fermentation. However, slight changes in pH, titratable acidity, viable cell counts and colour were noted during

refrigerated storage of the yogurt for 15 days; no changes in syneresis was observed in the control and bioactive added samples. By the end of the storage period, 63.31±3.20 % and 84.81±3.17 % of curcumin and CGA, respectively, were retained in the yogurt samples. The yogurt samples with added bioactive compounds were well accepted by consumer sensory evaluation panellists. Thus, from the obtained data it can be concluded that coconut yogurt may be a potential delivery medium for health promoting curcumin and CGA to consumers.

Acknowledgement

I am indebted to many individuals for their help and encouragement rendered while conducting this research work. Firstly, I would like to express my gratitude to the Riddet Institute, Palmerston North, New Zealand for giving me the opportunity to work on this project by providing financial support. I express my profound thanks to **Dr. Harjinder Singh** (Director, Riddet Institute) for his valuable comments during conceptualizing of the project.

This work would not have been possible without the constant guidance of all my thesis advisors. I am especially indebted to my supervisor, **Dr. Tony N Mutukumira** (Senior Lecturer, School of Food and Nutrition, College of Sciences, Massey University) for believing in me and proving his valuable comments, guidance, and encouragement starting from project conceptualization, conducting trials and to submission of the final thesis. I am also grateful to Dr. Mutukumira's open door policy that makes him more accessible and allowed me to run into his office whenever I was into trouble. Thank you Dr. Mutukumira for letting me freely explore research environment and identify my niche in that.

I would also like to express my gratitude to **Dr. Kay Rutherfurd-Markwick** (Associate Professor, School of Health Sciences, College of Health, Massey University) and **Dr. Martin Dickens** (Senior Lecturer, School of Health Sciences, College of Health, Massey University) for their help while drafting the project and working with cell culture. Dr. Rutherfurd-Markwick and Dr. Dickens are also thanked for helping with the interpretation of the results and sharing their valuable knowledge. **Dr. Rohith Thota** (Research Officer, Riddet Institute, Massey University) is thanked for his guidance in conceptualizing the work.

Furthermore, I want to thank **PC Tong** (Human Nutrition Laboratory Manager, School of Sport, Exercise and Nutrition, Massey University) for always being there to help me and solving all my childish problems while handling cells. I am also grateful to **Rachel Liu** (Food Microbiology Laboratory Manager, Institute of Food Science and Technology, Massey University) and **Negah Nikanjam** (Institute of Food Science and Technology, Massey University) for helping with the procurement of the required materials and making me acquaint with various equipment's available in their respective labs. I also express my profound thanks to my **Dr. Kaio Vitzel** (Lecturer, School of Health Sciences, College of Health, Massey University) for his inputs during qRT-PCR.

I would also like to thank all my friends for their moral support and encouragement during the tough times. All the members of G Gang and Sevenzzz are deeply acknowledged as they had been a motivational force at some or the other time in my journey. Special thanks to **Ghoda, Saummya, Jaini, Sanju, Yathu,** and **Kunal** for bearing all my tantrums and helping me during all thick and thin. You people are the safety net that gave me enough strength to aim high. I am grateful to **Saummya, Gautam** and **Preetha** for helping me with editing my work despite the fact that they didn't understand it most of the time. I am also thankful to my batch-mates **Arthur Jonathan, Lisa Ning** and **Doris** for always finding time to engage with me in random discussions. I am thankful to **Nitesh, Aditya,** and **Sanjay** for making a home away from home.

Lastly, I would like to thank my sister **Diksha** for always being my spine.

Dedicated to all the sacrifices made by my parents.
Thank you for believing in my dreams and letting me fly.

मेरे माता पिता के प्रति समर्पित जिन्होंने मेरे लिए अनेक त्याग किए!
शुक्रिया आपने मेरे सपनों पर भरोसा किया और मुझे उड़ने का हौसला दिया!

Translated by Neelabh Utkarsh

Table of Content

List of Figures

List of Tables

Abbreviation and Terminology

Abbreviation	Terminology
ANOVA	Analysis of variance
Cfu/g	Colony forming unit per gram
CGA	Chlorogenic acid
CO_2	Carbon dioxide
COX	Cyclooxygenase
ºC	Degree Celsius
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FAO	Food and agriculture organisation
g	Gram
h	Hour
iNOS	Inducible nitric oxide synthase
IL	Interleukin
L. bulgaricus	*Lactobacillus bulgaricus*
LPS	Lipopolysaccharide
LOX	Lipoxygenase
L	Litre
mRNA	Messenger ribonucleic acid
µM	Micromoles
ml	Millilitre
mg	Milligram
min	Minute
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor
nm	Nanometre
NO	Nitric oxide
NF-κB	Nuclear transcription factor kappa-B
%	Percentage

PBS	Phosphate buffered saline
pH	Potential of hydrogen
PMA	Phorbol 12-myristate 13-acetate
PGE-2	Protagladin-E2
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
rcf	Relative centrifugal force
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
SPSS	Statistical package for the social sciences
S. thermophilus	*Streptococcus thermophilus*
TNF- α	Tumor necrosis factor-alpha
UV-vis	Ultraviolet–visible
WHO	World health organisation

CHAPTER 1
Introduction

1.1 Background

Today, food is not only consumed to curtail hunger and provide necessary macro- and micro-nutrients but also to achieve additional mental and physiological health benefits (Betoret, Betoret, Vidal, & Fito, 2011; Galanakis, 2017; Sun-Waterhouse, 2011; Szakály, Szente, Kövér, Polereczki, & Szigeti, 2012). Traditionally, therapeutic effects were derived naturally from a diet composed of fruits, vegetables, cereals, dairy, and meat. However, today's busy lifestyle has led to an increase in the consumption of processed food over fresh food (Mozaffarian, Hao, Rimm, Willett, & Hu, 2011). To compensate for inadequate intake of nutrients and therapeutic non-nutritive compounds from processed food, there is an increase in the intake of dietary supplements (Gahche et al., 2011). Hence, there is a demand from the modern health-conscious consumer who wants food with balanced calories that can improve their health, leading to the development of contemporary so-called "functional foods" (Bech-Larsen & Scholderer, 2007; Galanakis, 2017). The increasing demand is the result of a number of factors such as urbanisation, busy lifestyle, increasing healthcare cost, growing awareness, surge in life expectancy and the desire to improve the quality of life (Kaur & Das, 2011; Roberfroid, 2000; Siro, Kápolna, Kápolna, & Lugasi, 2008). Therefore, the development of functional foods is one of the most growing research area (Silva, Barreira, & Oliveira, 2016).

The term "functional food" was first introduced in Japan in the mid-1980s and was referred to as "food for specified health uses (FOSHU)" (Ohama, Ikeda, & Moriyama, 2008). The American Dietetic Association defines functional food as "foods that are in forms of whole, fortified, enriched or enhanced foods that provide a functional advantage and/or health benefits beyond basic nutrition, when consumed at an effective level on a regular basis" (Thomson et al., 1999). In Europe, there is no legal definition but a working definition explains functional food as "a food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of diseases, and it is consumed as part

of a normal food pattern (not a pill, a capsule or any form of dietary supplement)" (European Commission, 2010).

Despite disparities in the legal definition of function food, its market worth is increasing. The functional food market was worth approximately USD 200 billion in 2013 and is expected to increase above USD 300 billion by 2020 (Santeramo et al., 2018). A large number of functional foods are commercially available such as vitamin and mineral fortified fruit juice, vitamin D and calcium fortified milk, vitamin and mineral fortified bread, catechin enriched green tea, cereals with soluble fibres, Omega-3 enriched eggs and yogurt with probiotics and prebiotics.

The health of an individual is greatly affected by their diet and lifestyle. The World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) have reported several dietary patterns and lifestyle habits that can lead to the development of chronic diseases (WHO, 2003). Chronic diseases were the leading cause of about 60 % mortality across the globe in 2005 which further increased to approximate 68 % in 2012 (Tsai, Lin, & Wu, 2016; WHO, 2014). Chronic diseases are also an economic burden on society and are estimated to cost about USD 7 trillion during 2011-25 in low- and middle-income countries (WHO, 2014). Chronic diseases such as obesity, type-2 diabetes, arthritis, asthma, bronchitis, pancreatitis, cardiovascular, neurodegenerative, colitis, multiple sclerosis, and metabolic diseases as well as some types of cancer are the onsets lead by long-term uncontrolled or chronic inflammation (He et al., 2015; Hewlings & Kalman, 2017; Panahi et al., 2016).

During chronic inflammatory responses several complex cellular signalling pathways are activated in the body, resulting in increased levels of inflammatory biomarkers including transcription factors such as nuclear transcription factor kappa-B (NF-κB); inflammatory cytokines and chemokines such as tumour necrosis factor-alpha (TNF-α), and interleukin (IL-6); and inflammatory enzymes such as cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) (Franceschi & Campisi, 2014; He et al., 2015; Lin & Tang, 2008; Prasad & Aggarwal, 2014).

To control inflammatory responses, one needs to combat the stimulating pathogen and/or reduce the production of cell mediator's (Liang & Kitts, 2015). Several anti-inflammatory drugs have been developed to control chronic inflammation but long-term consumption of these drugs may result in side effects such as bleeding in the stomach and predisposition to

ulcers (Laine, 2001). Therefore, there is a growing interest in alternative treatments that are more natural and can be a part of the diet (Khan, Grigor, Winger, & Win, 2013). Traditionally plant-based diets and its derivatives have been used to prevent inflammation and thereby related chronic diseases (Mueller, Hobiger, & Jungbauer, 2010). The therapeutic effect of food is primarily associated with the presence of bioactive compounds (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005; Day, Seymour, Pitts, Konczak, & Lundin, 2009; Herrero, Plaza, Cifuentes, & Ibáñez, 2010; Vicentini, Liberatore, & Mastrocola, 2016). Bioactive compounds are present in fruits, vegetables, and whole grains and can exert physiological effects on consumer's body (Astley & Finglas, 2016; Galanakis, 2017). Certain bioactive compounds can suppress inflammatory biomarkers and reduce oxidative stress in cells, thereby help in averting chronic inflammation-related diseases (Astley & Finglas, 2016; Calixto, Otuki, & Santos, 2003). For example, a study by Ha, Park, Eom, Kim, and Choi (2012), reported a decrease in expression of iNOS and COX-2 following administration of the narirutin fraction of citrus peels in lipopolysaccharide (LPS) stimulated macrophages. Similar results have been reported in other *in vitro* and *in vivo* studies testing a variety of different bioactive compounds (Joseph, Edirisinghe, & Burton-Freeman, 2016; Zhang & Tsao, 2016; Zhu, Du, & Xu, 2018).

Curcumin is one such bioactive that has been extensively studied for its health benefits. It is the main phenolic compound that is extracted from the rhizome of the turmeric plant (Wilken, Veena, Wang, & Srivatsan, 2011). Its therapeutic benefits include anti-oxidant, antiseptic, anti-tumour, anti-malarial, analgesic, anti-obesity and anti-inflammatory properties (Amalraj, Pius, Gopi, & Gopi, 2017). It can exert anti-inflammatory properties by acting on multiple biomarkers including transcription factors, enzymes, pro-inflammatory cytokines and chemokines, and free radical (Lozada-García et al., 2017; Yunes Panahi et al., 2015). However, the low bioavailability of curcumin has been a challenge. Due to the low bioavailability of curcumin high dose is required to deliver its intended benefits in humans. Previous studies have reported a minimum dosage of 3.6 g/day of curcumin is required to achieve measurable plasma levels in humans (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Cui et al. 2009). It is possible that this high dose requirement may be compensated by co-administrating curcumin with other bioactive compounds that may result in a synergistic effect.

Although less studied than curcumin, chlorogenic acid (CGA) and lupeol are two bioactive compounds obtained primarily from coffee and several fruits, respectively, which have been reported to have similar therapeutic effects. Initial studies reported that CGA and lupeol can downregulate multiple inflammatory pathways, similar to curcumin, principally by suppressing oxidative stress and the activation of NF-κB (Liang & Kitts, 2015; Salminen, Lehtonen, Suuronen, Kaarniranta, & Huuskonen, 2008). It is hypothesised that administration of curcumin with CGA or lupeol may result in a synergistic anti-inflammatory effect.

High dose requirement can also be reduced by enhancing the bioavailability of bioactive compounds. Presently, most of the bioactive compounds are consumed as dietary supplements in the form of pills and capsules but bioavailability can be improved by delivering these compounds *via* appropriate food matrices (Rodríguez-Roque et al., 2016). Milk and milk products may form a suitable delivery vehicle as they can solubilise hydrophobic bioactive compounds (such as curcumin, CGA and lupeol) in their oil phase (Cuomo et al., 2011; Jakobek, 2015; Rege & Momin, 2017). In addition, the acidity of the delivery medium can improve the stability of bioactive compounds. Hence, coconut milk yogurt, a fermented product with a final pH ≈4.5-4.6 (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018), may be an effective vehicle to deliver bioactive compounds for human consumption.

1.2 Aim and objectives

The aim of this work was to identify the most promising anti-inflammatory combination of curcumin with CGA and/or lupeol and thereby develop a coconut cream yogurt to deliver the combination of bioactive compounds for human consumption. To achieve the targeted aim, the experimental investigations were divided into three major objectives:

- *Objective 1:* To investigate the effective synergistic combination of curcumin with CGA and lupeol on reducing TNF-α secretion;

The anti-inflammatory effects of curcumin in combination with different amounts of CGA and lupeol were studied using an *in vitro* model of human TPH-1 monocyte cell line by assessing changes in the production of the pro-inflammatory cytokine TNF-α.

- *Objective 2:* To investigate the effect of the most promising synergistic combination of bioactive compounds on other inflammatory pathways;

The synergistic combination was studied for its effect on mRNA expression of inflammatory biomarkers such as IL-6, IL-10, TNF-α, NF-κB, iNOS, and COX-2.

- *Objective 3:* To develop a coconut cream yogurt containing the synergistic combination of bioactive compounds suitable for human consumption.

The effective concentration of bioactives were added to coconut cream yogurt and their effect on the fermentation process was studied. The resulting yogurt was analysed for its sensory attributes, physical-chemical and microbiological stability.

CHAPTER 2

Literature Review

2.1 Introduction

Lifestyle changes have increased the occurrence of "modern life disorders" including chronic inflammation, which is occurring over a wide range from children to adults. Chronic inflammation is important as it may play a key role in triggering other deadly diseases such as asthma, arthritis, type-2 diabetes, heart disease and several forms of cancers (Franceschi & Campisi, 2014; Meirow & Baniyash, 2017).

Many food-derived bioactive compounds such as epigallocatechin-3-gallate (EGCG), gingerol, naringenin, quercetin, resveratrol, silymarin, tocopherol, β-carotene, genistein, chrysin, curcumin, lupeol and chlorogenic acid (CGA) have been documented for their anti-inflammatory properties (Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012; Gil-Cardoso et al., 2016; Prabhala, Pai, & Prabhala, 2013; Yu, Bi, Yu, & Chen, 2016; Zhu, Du, & Xu, 2018). Therefore, recent research has focused on the potential of these naturally occurring bioactive compounds to aid in the prevention or treatment of chronic inflammation. This review focuses on outlining the events that occur during inflammation and the mechanisms of bioactive compounds such as curcumin, lupeol, and CGA in suppressing uncontrolled inflammatory responses.

2.2 What is inflammation?

Inflammation is the first complex biological response by the host to any stimuli such as invasion by a foreign body like microbes, toxins, dirt or burns or cuts or tissue necrosis or radiation (Cavaillon, 2017a; Prabhala et al., 2013). According to Kumar, Abbas, and Aster (2012c) page 29:

"Inflammation is a protective response involving host cells, blood vessels, and proteins and other mediators that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues resulting from the original insult, and to initiate the process of repair"

The inflammatory response can be: (i) rapid and short-term called acute inflammation, (ii) or be for longer duration called chronic inflammation (Ward, 2010). Acute inflammation can last for a few minutes to a few days and is predominantly the response of neutrophilic leukocytes to mild and self-limiting tissue injuries resulting in prominent local and systemic signs (Ward, 2010).

Chronic inflammation is the result of severe and progressive injury, predominantly due to macrophages responses that can last for several days (Ward, 2010). Chronic inflammation can be caused by: (i) continuous infection caused by microbes that are difficult to eliminate, such as *Mycobacterium tuberculosis*, fungi and viruses; (ii) auto-immune responses resulting in diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease; (iii) allergies like bronchial asthma; and (iv) persistent contact with toxic elements e.g. inhalation of silica causing silicosis: a chronic inflammatory response in the lungs (Hnizdo & Vallyathan, 2003; Kumar et al., 2012c; Shacter & Weitzman, 2002). Uncontrolled chronic inflammation may also contribute to the onset of diseases such as Alzheimer, type-2 diabetes, metabolic syndrome and several forms of cancer that traditionally were not considered to be related to inflammation (Franceschi & Campisi, 2014; Kumar et al., 2012c).

2.3 Events during inflammation

Any stimuli entering beyond the first line of defence (the skin and mucus layer) triggers multiple events at the cellular level. The immune response is the combined result of a number of different cells including monocytes, mast cells, dendritic cells, and T, B and NKT lymphocytes and is not limited to only one cell type (Prabhala et al., 2013). The cellular events during inflammation can be summarised as shown in Figure 2.1. Stimuli such as bacteria express a certain molecular pattern of proteins on their surface, called the pathogen-associated molecular pattern (PAMP), which can be detected by the receptors such as toll-like receptors (TLR) present on the cells, particularly on mast cells and macrophages (Cavaillon, 2017b; Kumar et al., 2012c). On recognising the pathogen, the mast cell releases histamine stored in its granules (Amin, 2012), while macrophages produce cytokines such as TNF-α and IL-6 which as a result initiate the events in blood vessels (Dunster, 2016).

The main reason for changes in vessels is to increase the blood flow at the site of action. Histamine and cytokines leads to vasodilation and increased blood flow (Benly, 2015). This allows more blood cells and protein to be delivered to the place of injury. Histamine also

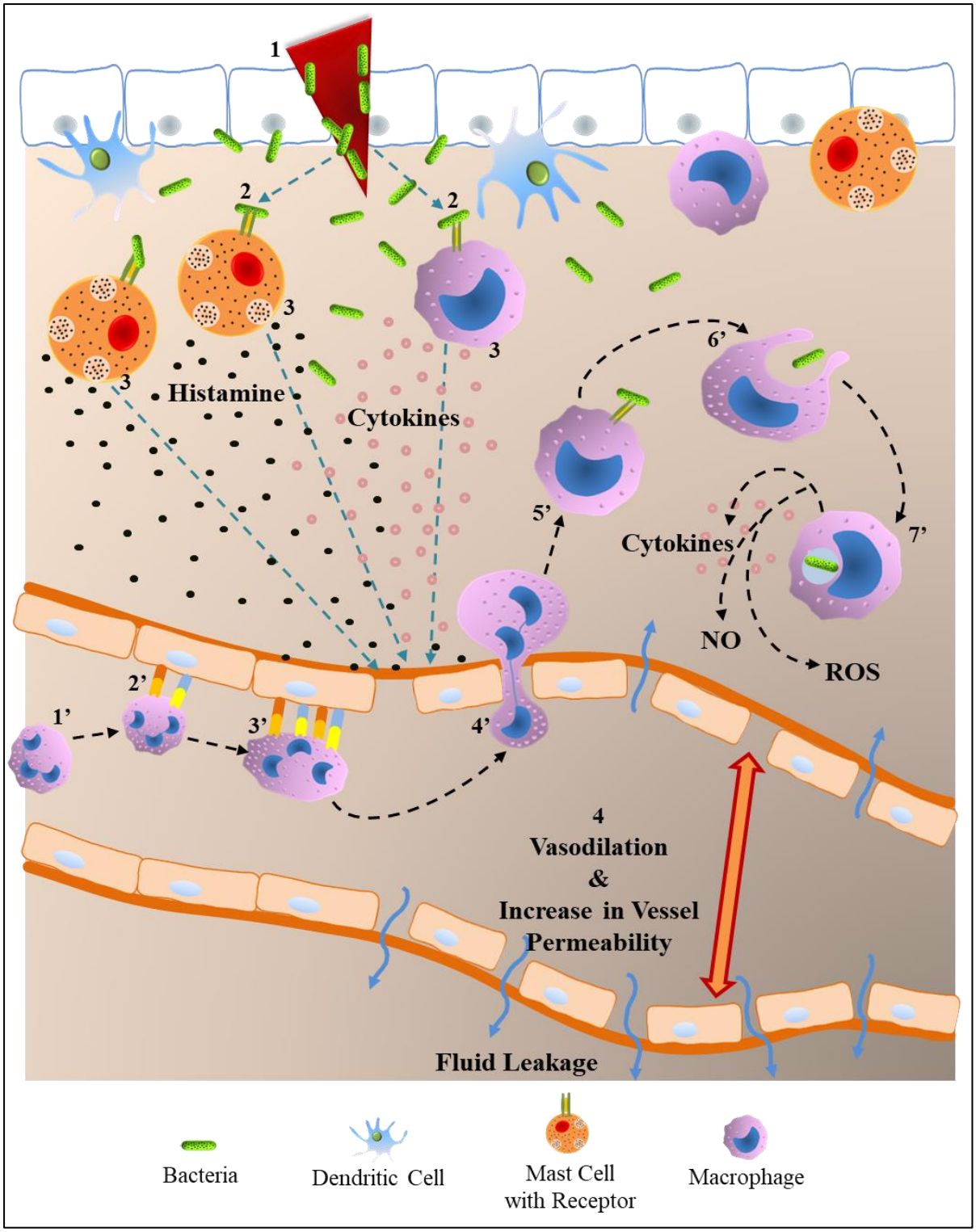


Figure 2.1: Key events during inflammatory response of body and migration of leukocytes. 1: Invasion of bacteria in body; 2: mast cells and macrophages detect bacteria; 3: secretion of histamine and cytokines from their respective cells; 4: vasodilation and increase in blood vessel permeability resulting in fluid leakage. 1': leukocyte moving towards blood vessel wall; 2': rolling of leukocyte to form weak adhesion; 3': strong adhesion of leukocyte with endothelial cells; 4': leukocyte squeezes between endothelial cells; 5': leukocyte migrates towards the site of action; 6': bacteria engulfed by leukocyte; and 7': bacteria ingested by leukocyte and release of cytokines, reactive oxygen species (ROS) and nitric oxide (NO) (Created using Microsoft PowerPoint, 2016)

causes contraction of the endothelial cells which increases vessel permeability (Ashina et al., 2015), and aids in moving the fluid carrying proteins and blood cells into extra-vascular tissues so that repair mechanisms can be established (Benly, 2015). During vasodilation, cytokines and histamine also activate the adhesion molecules on the endothelial cells that facilitate the migration of leukocytes out from the vessel (Shalova, Saha, & Biswas, 2017).

The type of leukocyte leaving the blood vessels will depend on the duration of the inflammatory response. During acute inflammation neutrophils are dominant because these cells are more abundant in the blood than monocytes, hence they respond more rapidly to mediators (Kumar et al., 2012c). However, after entering the tissues, neutrophils die within 24-48 h while macrophages (developed from monocytes) can survive longer (Kumar et al., 2012c). Hence macrophages dominate during chronic inflammation.

The leukocytes move towards the site of action by following a chemical gradient by a process called chemo-taxis (Shalova et al., 2017). At the site of infection, the leukocytes can bind to microbes, damaged cells, and foreign bodies and results in phagocytosis (Shalova et al., 2017). Leukocytes then produce substances like reactive oxygen species (ROS), nitric oxide (NO) and liposomal enzyme to degrade the engulfed material.

2.4 Cell mediators of inflammation

The complex cascades of cellular events during inflammation involve the production of enzymes, pro-inflammatory cytokines and chemokine, and other chemical mediators that eventually eliminates the pathogen and heal the injured tissues. Macrophages, mast cells and other cell involved in inflammatory response can produce a number of cell mediators as described below.

2.4.1 Cytokines

Cytokines are small proteins which are synthesised and secreted by immune cells (principally from macrophages and lymphocytes) to commence, amplifies, prolong and proliferate inflammation process (Holdsworth & Gan, 2015; Prabhala et al., 2013). Cytokines can act on their producer cells (autocrine) or neighbouring cells (paracrine) or on cells away from their production site (endocrine) (Kumar et al., 2012b; Zhang & An, 2007). Broadly, cytokines are classified as pro-inflammatory and anti-inflammatory (Table 2.1). Pro-inflammatory cytokines such as TNF-α and IL-6 upregulate inflammatory responses while anti-

inflammatory cytokines such as IL-10 downregulate and have a negative feedback on pro-inflammatory cytokine responses (Corwin, 2000). An imbalance between productions of pro- and anti-inflammatory cytokines may be responsible for chronic inflammatory responses in the body resulting in the damage to host cells (Holdsworth & Gan, 2015).

2.4.2 Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

After macrophage engulf bacteria, two independent antibacterial pathways are stimulated involving (i) nicotinamide adenine dinucleotide phosphate (NADPH) phagocyte oxidase (or NADPH oxidase) and (ii) inducible nitric oxide synthase (iNOS), which results in the production of ROS and NO respectively (Mittal, Siddiqui, Tran, Reddy, & Malik, 2014; Swindle & Metcalfe, 2007). Both ROS and NO are free radicals that can react with organic and inorganic chemicals to kill the bacteria (Kumar, Abbas, & Aster, 2012a). However, during chronic inflammation high amounts of ROS and NO are produced that may damage the host cells (Soufli, Toumi, Rafa, & Touil-Boukoffa, 2016).

During inflammation, NADPH phagocyte oxidase can produce ROS in the cytoplasm and phagosome of macrophage. NADPH phagocyte oxidase once activated by pro-inflammatory cytokines like TNF-α, will utilise NADPH and oxygen (O_2) to produce superoxide ($O_2^{\bullet-}$) by the process called the oxidative bust or respiratory bust (Mittal et al., 2014). $O_2^{\bullet-}$ has very low half-life, therefore it rapidly converts to hydrogen peroxide (H_2O_2), which is then converted to highly reactive hypochlorous acid (HOCl) by the enzyme myeloperoxidase (MPO) or the eosinophil peroxidase (EPO) (Swindle & Metcalfe, 2007). In the presence of metal ions such as iron (Fe^{2+}) and copper (Cu^{2+}), H_2O_2 can undergo the Fenton or Haber Weiss reaction to produce hydroxyl radical ($OH^{\bullet}$) and hydroxyl anion (OH^-) (Mittal et al., 2014).

NO is the principle reactive specie of nitrogen produced *de novo* by oxidation of an amino acid L-arginine in the presence of NADPH (Kumar et al., 2012a). During inflammation, this reaction is carried out in macrophages or other inflammatory cells by the iNOS (Fang, 2004; Forrester, Kikuchi, Hernandes, Xu, & Griendling, 2018; Soufli et al., 2016). NO can further react with O_2 to produce nitrogen dioxide ($NO_2^{\bullet}$), which can then react with itself to yield one molecule of dinitrogen tetraoxide (N_2O_4) (Swindle & Metcalfe, 2007). The presence of PAMP and cytokines such as TNF-α, and IL-6 can trigger cascades like NF-κB and Janus-activated kinase-signal transducer and activator of transcription (JAK-STAT) leading to transcription of iNOS (Fang, 2004).

Table 2.1: Major pro- and anti-inflammatory cytokines produced during inflammation.

Cytokine	Principal Cell Source	Major Activity
	Pro-inflammatory	
TNF-α	Macrophages, mast cells and dendritic cells	Stimulate adhesion of leukocytes to endothelial cells and the production of other pro-inflammatory cytokines e.g. IL-1, IL-2 and IL-6, induces death of host cells resulting in pain and fever
IFN-γ	T cells and NK cells	Activates macrophages, influence B cell proliferation, increases expression of antigens and induce death of host cells
MIP-1α	Macrophages	Chemo-taxis
TGF-β	T cells and monocytes	Chemo-taxis and synthesis of IL-1 and IgA
IL-1α and IL-1β	Macrophages, neutrophils, B cells, endothelial cells and other cell types	Recruitment of macrophages and neutrophils to inflammation site, stimulates the production of IL-6, increases vessel permeability and promotes tumor development
IL-5	Mast cells and T cells	Stimulates growth and functioning of B cells and eosinophils
IL-6	Macrophages, T cells and fibroblasts	Stimulates proliferation of B cells, works synergistically with TNF and IL-1, activates and recruits macrophages to inflammation site, activates acute-phase proteins, acts on hypothalamus to induce sickness behaviors e.g. fever and anorexia
IL-8	Macrophages	Increases vessel permeability and recruits neutrophils to inflammation site via chemo-taxis
IL-12	Macrophages, B cells and dendritic cells	Induces proliferation of NK cells, stimulates IFN production
	Anti-inflammatory	
IL-4	Mast cells, T cells and NK cells	Stimulates growth and functioning of B cells, suppresses production of IL-1 and TNF
IL-10	T cells, B cells and macrophages	Stimulates proliferation of B cells, suppresses IL-1 synthesis in macrophages
IL-11	Bone marrow	Promotes growth of bone marrow cells, activates acute-phase proteins
IL-13	T cells	Down-regulates the production of pro-inflammatory cytokines similar to IL-4

TNF: tumour necrosis factor; IFN: interferon; NK: natural killer; MIP: macrophage inflammatory proteins; TGF: transforming growth factor; IL: interleukin; IgA: immunoglobulin A. Adapted from: (Corwin, 2000; Neurath, 2014; Zhang & An, 2007)

2.4.3 Arachidonic acid metabolites

Arachidonic acid is a polyunsaturated fatty acid which is present in the human body as an esterified cell membrane phospholipid. Production of cytokines such as TNF-α and IL-6 and ROS and NO triggers the release of free arachidonic acid from the membrane phospholipids by activating enzyme phospholipase A_2 (Li, Gao, Du, Cheng, & Mao, 2018). Free arachidonic is metabolised by two enzymatic pathways: (i) cyclooxygenase (COX) or (ii) lipoxygenase (LOX) to synthesise various classes of eicosanoids (Figure 2.2) (Meirer, Steinhilber, & Proschak, 2014; Prabhala et al., 2013), that result in an increase in vascular permeability, vasodilation, vasocontraction and bronchoconstriction (Prabhala et al., 2013).

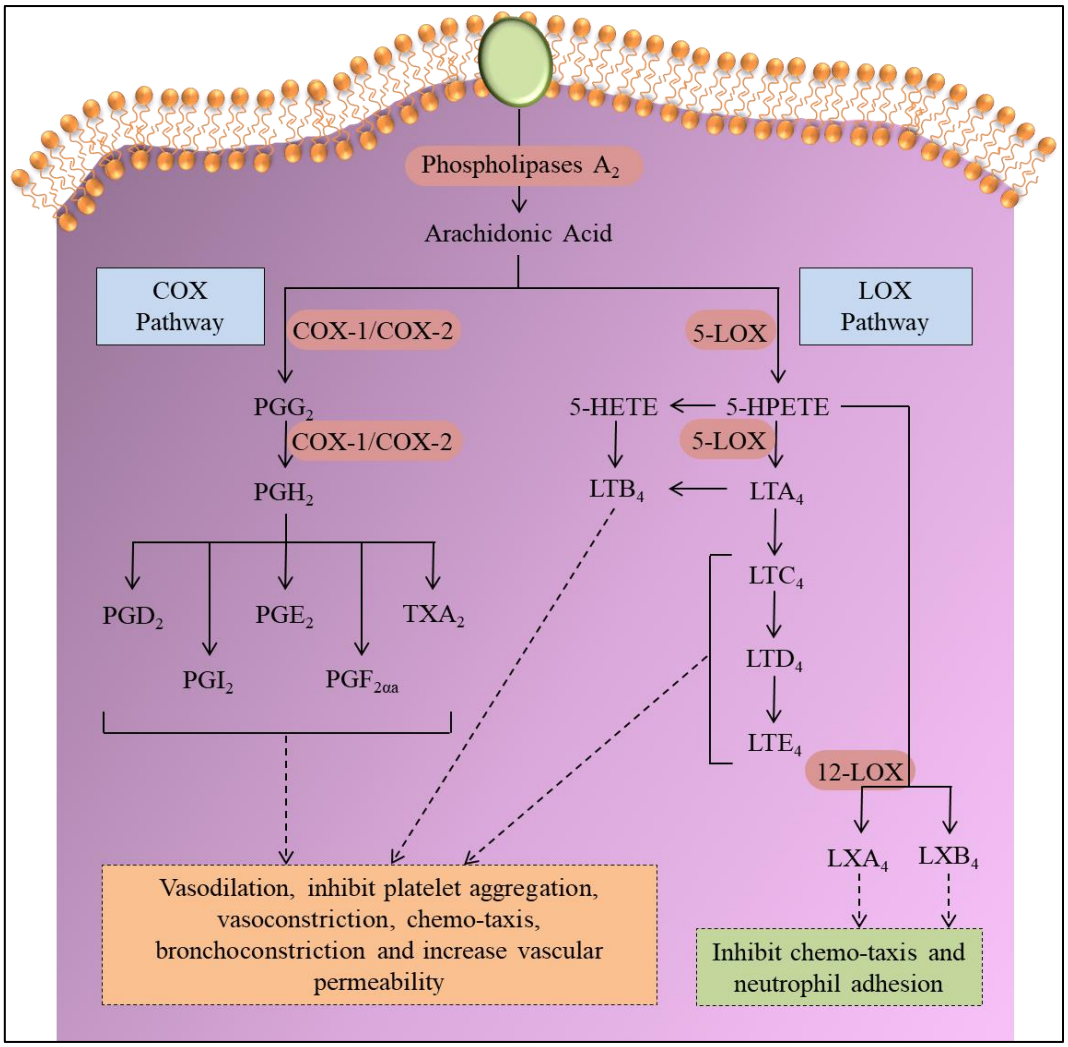


Figure 2.2: Cyclooxygenase (COX) and lipoxygenase (LOX) pathways for production of arachidonic acid metabolites.

PG(G_2, H_2, D_2, I_2, $F_{2\alpha a}$): prostaglandin (G_2, H_2, D_2, I_2, $F_{2\alpha a}$); TXA_2: thromboxane A_2; 5-HPETE: 5-hydroperoxyeicosatetraenoic acid; 5-HETE: 5-hydroxyeicosatetraenoic acid; LT(A_4, B_4, C_4, D_4, E_4): leukotriene (A_4, B_4, C_4, D_4, E_4); LX(A_4, B_4) : lipoxin (A_4, B_4). Adapted from: (Kumar et al., 2012c; Meirer et al., 2014) (Created using Microsoft PowerPoint, 2016)

COX initially downregulate arachidonic acid to produce unstable prostaglandin G_2 (PGG_2) which is further transformed into more stable prostaglandin H_2 (PGH_2) (Meirer et al., 2014). COX has two isoforms, of which COX-1 is always expressed to maintain prostaglandin homeostasis while COX-2 on other hand is expressed as a result of pro-inflammatory cytokines, bacteria or growth factors (Funk, 2001; Li et al., 2018; Prabhala et al., 2013). Both COX-1 and COX-2 function similarly to produce PGH_2. Further downregulation of PGH_2, produces various prostaglandins and thromboxane (Meirer et al., 2014). Of all the prostaglandins produced, prostaglandin E_2 (PGE_2) is the most critical during inflammation as it is responsible for inflammatory symptoms of pain and fever (Funk, 2001; Hwang, Wecksler, Wagner, & Hammock, 2013).

LOX-5 is the key enzyme involved in arachidonic acid metabolism in neutrophils. LOX-5 oxidises arachidonic acid to produce intermediary 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is then oxidised to produce a series of leukotriene's resulting in inflammatory responses. 5-HPETE can also be transformed by 12-LOX to produce lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4). Both the lipoxins are anti-inflammatory in nature and can inhibit neutrophil chemo-taxis and adhesion to the endothelial surface (Kumar et al., 2012c; Meirer et al., 2014).

2.5 Bioactive compounds and inflammation

In addition to the macro- and micro- nutrients, food also contain bioactive compounds which are significant for maintaining the well-being of humans (Galanakis, 2017). Bioactive compounds are naturally occurring extra-nutritional compounds that can affect the metabolic process by targeting whole body or specific tissues or cells, thus, impacting on the function and wellness of the body (Astley & Finglas, 2016; Galanakis, 2017; Torres-Fuentes, Schellekens, Dinan, & Cryan, 2015). Table 2.2 summarises the sources and health benefits of some bioactive compounds. These compounds can have positive biological effects such as anticancer, antibacterial, antifungal, antioxidant, anticoagulant, anti-obesity and anti-inflammatory (Astley & Finglas, 2016; D'Orazio et al., 2012; Elias, Kellerby, & Decker, 2008; Gooda Sahib et al., 2012; Korhonen & Pihlanto, 2003; Phelan & Kerins, 2011; Torres-Fuentes et al., 2015; Wang et al., 2014).

Currently, the role of bioactive compounds are being investigated to develop a novel strategy for the alleviation of chronic inflammation. Some of the bioactive compounds with reported

anti-inflammatory effect are shown in Table 2.3. Bioactives can reduce the stress signals produced by cells in response to stimuli thereby control inflammatory responses in the body (Prabhala et al., 2013).

Table 2.2: Bioactive compounds and their associated health benefits.

Class	Bioactive Compounds	Major Food Source	Health Benefits
Bioactive peptide	Various peptides	Milk, meat and fish	Antihypertensive, antioxidant properties or immunomodulatory activities
Carotenoids	Curcumin, lutein and β-carotenr	Carrots, tomato, spinach, apricot, pepper and citrus	Prevention against cardio vascular disease, cancer and age related degeneracy
Essential Oil	Cardamom oleoresin, eugenol and eugenyl acetate	Citrus fruit skin, cardamom, marjoram, clove oil and basil oil	Antidepressant, antispasmodic and anti-inflammatory properties Prevention against cardio vascular disease, cancer
Fatty acid	Omega 3 and omega 6	Flex seeds, vegetable oils, fish oil, nuts and egg	Anti-inflammatory, anti-carcinogenic and immunomodulatory properties
Phenol	Caffeine, catechins, chlorogenic acid, ellagic acid, gallic acid, isoflavone, mangiferin, naringenin, quercetin, vanillin, resveratrol, rutin and anthocyanins	Fruits like apple, cherries, berries, plums and grapes, legumes, green coffee extract, green tea, olive oil, broccoli and onion	Prevention against cancer, neurodegenerative, cardio vascular and metabolic disease Reduce oxidative stress
Protein	Albumin, hirudin and papain	Egg, peas, grains and papaya	Antioxidant, age related degeneracy, immunity booster and weight and blood pressure management
Organic acid	Citric acid and hydroxycitric acid	Citrus fruits, malabar tamarind and hibiscus sabdariffa	Antioxidant, anti-ageing and weight management
Vitamin and mineral	Water and fat soluble vitamins, iron, zinc, magnesium, calcium	Majorly in all fruits and vegetables, milk and meat	Benefits ranging from improved vision and gums to development of bones, and teeth's They even play important role as co-factors and co-enzymes in metabolic reactions

Adapted from: (Augustin & Sanguansri, 2015; Dias, Ferreira, & Barreiro, 2015; Onwulata, 2013)

Table 2.3: In vitro and animal evidences of anti-inflammatory properties of bioactive compounds.

Name	Food sources	Evidences of anti-inflammatory effects (dose used)				Safety for human consumption
		In vitro trial		Animal trial		
		Experiment model	Result(s)	Experiment model	Result(s)	
Ascorbic acid	Papaya, broccoli, kiwi fruit, mango, cauliflower, strawberries, pineapple	Inflammation induced in female BALB/c mice by LPS and effect of ascorbic acid on collected cells (60 % lymphocytes and 40 % macrophages) (0.001-2.5 mM) (Victor, Guayerbas, Puerto, Medina, & Fuente, 2000)	↓ TNF-α ↓ Superoxide anion	$K_2Cr_2O_7$ induced adult male wistar rats (250 mg/kg) (Al Jameil et al., 2017)	↓ IL-6 ↓ IL-8 ↓ IL-10 ↓ IL-18 ↓ TNF-α	GRAS (FDA, 2013)
β-carotene	Carrots, sweet potatoes, pumpkin, spinach, apricots	Inflammation induced in RAW264.7 cells stimulated with LPS (10-50 mM) (Bai et al., 2005)	↓ TNF-α ↓ NO ↓ iNOS ↓ PGE_2 ↓ NF-κB ↓ IL-1 β	162 Sprague-Dawley (SD) male rats fed with high fat diet (0.05 %) (Choia et al., 2006)	↓ COX-2	GRAS (FDA, 2018)
Capsaicin	Jalapeño peppers, cayenne peppers	LPS stimulated peritoneal macrophages (10– 50 µM) (Kima et al., 2003)	↓ iNOS ↓ COX-2 ↓ PGE_2 ↓ NO	Septic female Sprague-Dawley rats (Demirbilek et al., 2004)	↓ TNF-α ↓ IL-6 ↑ IL-10 ↓ NO	Debatable (Bode & Dong, 2011a)
Chlorogenic acid	Coffee, tea, eggplant, potatoes	Inflammation induced in RAW264.7 cells stimulated by LPS (2-20 µM) (Hwang, Kim, Park, Lee, & Kim, 2014)	↓ TNF-α ↓ IL-6 ↓ iNOS ↓ COX-2 ↓ NO ↓ NF-κB	LPS induced male ICR mice (5-50 mg/kg) (Zhang et al., 2010)	↓ NO ↓ iNOS	Reported safe but lack sufficient data to conclude safe (Onakpoya, Spencer, Thompson, & Heneghan, 2014)

Table 2.3 (cont.)

Chrysin	Blue passion flower, propolis, honey	Inflammation induced in RAW264.7 cells stimulated by LPS (0.01-100 µM) (Cho, 2004)	↓ COX-2 ↓ NO ↓ iNOS	Adult wistar rats with spinal cord injury (30-100 mg/kg) (Jiang, Gong, Zhao, & Li, 2014)	↓ TNF-α ↓ IL-1β ↓ IL-6 ↓ iNOS ↓ NO ↓ NF-κB p65	Lack sufficient data
Epigallocatec-hin gallate (EGCG)	Green tea	Inflammation induced in HCEpiC cells by IL-1β (0.3–30 µM) (Cavet, Harrington, Vollmer, Ward, & Zhang, 2011)	↓ IL-6 ↓ IL-8 ↓ MCP-1 ↓ G-CSF ↓ GM-CSF ↓ ROS ↓ NFκB ↓ AP-1	Male adult Spargue-Dawley rats with spinal cord injury (50 mg/kg) (Khalatbary & Ahmadvand, 2011)	↓ TNF-α ↓ IL-1β ↓ iNOS ↓ COX-2	Safe (European Food Safety Authority et al., 2018)
Ellagic acid	Raspberries, pomegranate, blackberries, cherries, pecans, walnuts	Inflammation induced in human umbilical vein endothelial cells by oxLDL (oxidized low-density lipoprotein) (5-20 µM) (Lee et al., 2010)	↑ LOX-1 ↓ ROS ↓ iNOS ↓ NO	Carrageenan-induced adult male wistar rats paw edema model (1-30 mg/kg) (Mansouri et al., 2015)	↓ COX-2 ↓ iNOS ↓ PGE_2 ↓ TNF-α ↓ IL-1 β	Lack sufficient data (Kang, Buckner, Shay, Gu, & Chung, 2016)
Evodiamine	Evodia plant	Hypoxia induced inflammation in RAW264.7 cells (0.3-3 µM) (Liu et al., 2009)	↓ COX-2 ↓ PGE_2 ↓ iNOS	Zymosan-induced female C57BL/6J mice (10-15 mg/kg) (Fan et al., 2017)	↓ TNF-α ↓ IL-6 ↓ NF-κB	Lack sufficient data
Gingerol	Ginger	LPS induced inflammation in RAW246.7 cells (50-300 µg/ml) (Liang, Sang, Liu, Yu, & Wang, 2018)	↓ NO ↓ TNF-α ↓ IL-1β ↓ IL-6 ↓ PGE_2 ↓ iNOS	1 % tween 80 induced male wistar rats (25 mg/kg) (Algandaby et al., 2016)	↓ TNF-α ↓ NF-κB ↓ iNOS ↓ COX-2	GRAS (Bode & Dong., 2011b)

Table 2.3 (cont.)

Genistein	Soy beans, fava beans	Homocysteine induced inflammation in endothelial cells (ECV-304) (10-100 µM) (Han, Wu, Li, & Gao, 2015)	↓ IL-6 ↓ NF-κB ↓ ROS ↓ ICAM-1	LPS induced male albino wistar rats (10-100 mg/kg) (Mirahmadi et al., 2018)	↓ IL-6 ↓ TNF-α ↓ iNOS ↓ COX-2 ↓ NF-κB ↓ TLR4 ↓ ROS ↓ Nrf2	Reported safe but lack sufficient data to conclude safe (Marini et al., 2012)
α-linolenic acid	Flaxseed oil, walnuts, rapeseed oil, soybean oil	Inflammation induced in THP-1 by LPS (0.5-100 µM) (Zhao et al., 2005)	↓ IL-6 ↓ TNF-α ↓ IL-1β ↓ NF-κB ↓ PPARγ	2-4-6-trinitrobenzen sulfonic acid (TNBS) induced Sprague-Dawley male rats (450 mg/kg) (Hassan et al., 2010)	↓ TNF-α ↓ NF-κB ↓ COX-2	Reported safe but lack sufficient data to conclude safe (Takeuchi et al., 2007)
Lupeol	Mango, carrot, cucumber	Inflammation induced in RAW 264.7 by LPS (1-8 µM) (Chen et al., 2012)	↓ PGE_2 ↓ COX-2 ↓ iNOS	Carrageenan-induced male Swiss mice paw edema model (10-50 mg/kg) (Lucetti, Lucetti, Bandeira, Veras, Silva, Leal, Lopes, Alves, Silva, Brito, et al., 2010)	↓ NF-κB ↓ iNOS ↓ TNF-α	Reported safe but lack sufficient data to conclude safe (Siddique & Saleem, 2011)
Lycopene	Tomatoes, guavas, papaya, grapefruit	LPS induced inflammation in RAW246.7 cells (2.5-10 µM) (Rafi, Yadav, & Reyes, 2007)	↓ NO ↓ iNOS ↓ COX-2	Endotoxin-induced adult Sprague-Dawley rats (10 mg/kg) (Goncu et al., 2016)	↓ NO ↓ TNF-α ↓ IL-6	GRAS (if from tomatoes) (Devaraj et al., 2008; Trumbo, 2005)
Melatonin	Tomatoes, ginger, pomegranate, rice, olives, almonds	Inflammation induced in CHON-001 by H_2O_2 (0.1-100 ng) (Lim et al., 2012)	↓ NO ↓ PGE_2 ↓ TNF-α ↓ IL-1β ↓ IL-8 ↓ iNOS ↓ COX-2 ↓ SIRT1	Adult male Sprague–Dawley rats l ischemia/reperfusion injury (5 mg/ml) (Pei & Cheung, 2004)	↓ nNOS ↓ NO ↓ COX-2 ↓ MPO	Lack sufficient data

Table 2.3 (cont.)

Naringenin	Oranges, lemons, grapefruit	Inflammation induced in co-cultured RAW264.7 cells and 3T3-L1 preadipocyte stimulated by LPS (25-200 µM) (Hirai et al., 2007)	↓ TNF-α ↓ NO ↓ MCP-1	Adult male albino wistar rats with ethanol induced liver injury (50 mg/ml) (Jayaraman, Jesudoss, Menon, & Namasivayam, 2012)	↓ TNF-α ↓ IL-6 ↓ NF-κB ↓ COX-2 ↓ MIP-2 ↓ iNOS	Reported safe but lack sufficient data to conclude safe (Nguyen, Staubach, Tamai, & Langguth, 2015)
Omega-3 fatty acids	Fish oil, flaxseed, walnuts	LPS induced inflammation in THP-1 cells (100 µM) (Weldon, Mullen, Loscher, Hurley, & Roche, 2007)	↓ TNF ↓ IL-1β ↓ IL-6 ↓ NF-κB	Hyperthyroidism-induced hepatic dysfunction adult male wistar Albino rats (3 g/kg) (Gomaa & Abd El-Aziz, 2016)	↓ TNF-α ↓ IL-6 ↓ IL-1β ↓ oxidative stress	Safe (adequate intakes ≈ 1g/day) (National Institutes of Health USA, 2018)
Resveratrol	Peanuts, red wine, pistachios, blueberries, grapes	LPS induced inflammation in macrophages from murine. (0.3-30 µM) (Martineza & Morenoa, 2000)	↓ COX-2 ↓ ROS	Dextran sulfate sodium (DSS) induced male Fischer F344 rats (1 mg/kg) (Larrosa et al., 2009)	↓ COX-2 ↓ PGE_2	GRAS (if derived from *Saccharomyces cerevisiae)* otherwise reported safe but lack sufficient data to conclude safe (Mei, Liu, Wang, Wang, & Dai, 2015; Tomé-Carneiro et al., 2013)
Sulforaphane	Broccoli, cauliflower, cabbage	Inflammation induced in murine microglia cell line BV_2 stimulated by LPS (0.5-2 µM) (Brandenburg, Kipp, Lucius, Pufe, & Wruck, 2010)	↓ NO ↓ ERK1/2 ↓ JNK-phosphorylation ↓ NF-κB ↓ AP-1	LPS induced male BALB/c mice (50 mg/kg) (Qi, Xu, Yan, Li, & Li, 2016)	↓ TNF-α ↓ IL-6 ↓ COX-2 ↓ PGE_2 ↓ NF-κB	Lack sufficient data on long term studies at high dose

Table 2.3 (cont.)

α-Tocopherol	Sunflower oil, almonds, spinach	Inflammation induced in Kupffer cells by LPS (50 µM) (Fox, Brower, Bellezzo, & Leingang, 1997)	↓ TNF-α ↓ NF-κB	LPS induced adult male BALB/c mice (20 mg) (Godbout, Berg, Krzyszton, & Johnson, 2005)	↓ TNF-α ↓ IL-6 ↓ IL-1β ↓ NF-κB	GARS (Aggarwal & Nesaretnam, 2012)
Quercetin	Tomatoes, apple skins, onions, grapes, red wine	Inflammation induced in RAW264.7 cells by *Porphyromonas gingivalis* fimbriae (12.5-100 µM) (Murakami, Kawata, Ito, Katayama, & Fujisawa, 2015)	↓ TNF-α ↓ COX-2 ↓ NOS-2	Carrageenan induced male wistar rats weighing (10 mg/ml) (Morikawa et al., 2003)	↓ COX-2 ↓ PGE_2 ↓ TNF-α ↓ MIP-2	GRAS (highly purified form, up to 200 mg/day). (Andres et al., 2018)

TNF-α: tumor necrosis factor-alpha; IL: interleukin; NO: nitric oxide; COX: cyclooxygenase; NOS: nitric oxide synthase; PGE_2: protagladin-E2; NF-κB: nuclear transcription factor kappa-B; ROS: reactive oxygen species; Nrf2: nuclear factor (erythroid-derived 2)-like 2; MCP: monocyte chemotactic protein; G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; AP: activator protein; LOX: lipoxygenase; PPARγ: peroxisome proliferator-activated receptor gamma; MPO: myeloperoxidase; SIRT: sirtuin; MIP: macrophage inflammatory protein; ERK: extracellular signal-regulated kinase; JNK: Jun N-terminal kinase; LPS: lipopolysaccharide; GRAS: generally recognized as safe

2.5.1 Curcumin

Turmeric, a member of the ginger family (*Zingiberaceae*), is obtained from the rhizome of the *Curcuma longa* plant (Alappat & Awad, 2010; Nelson et al., 2017). Turmeric is a popular spice and a key ingredient in traditional Chinese and India Ayurvedic medicines for wound healing, scabbing of pox, reducing discomfort from insect bites, and treatment of colds, coughs, stomach disorders and cancers (Kim, Lee, & Shin, 2015; Nelson et al., 2017). Turmeric has been used in the food industry for its natural yellow colour and distinct flavour due to the essential oils and oleoresins. Nutritionally, turmeric typically contains 60-70 % carbohydrate, 5-10 % fat, 6-8 % protein, 3-7 % mineral, 2-7 % fiber, 3-7 % essential oil and 6-13 % moisture (Nelson et al., 2017; Prasad, Gupta, Tyagi, & Aggarwal, 2014).

Today turmeric is increasingly being used as a therapeutic agent, with its benefits primarily due to the presence of curcuminoids (Raina, Srivastava, & Syamsundar, 2005; Ramirez et al., 2018; Zeng et al., 2015). The term curcuminoids refer to a group of compounds, namely curcumin (accounting for ≈ 60-70 %), demethoxycurcumin and bis-demethoxycurcumin and a trace amount of secondary metabolites (Nelson et al., 2017; Priyadarsini, 2014). Together, curcuminoids make-up approximately 2-9 % of turmeric on a dry matter basis (Tsuda, 2018).

Structurally, curcumin (also known as diferuloyl methane) is $C_{12}H_{20}O_6$ with IUPAC name 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Kocaadam & Şanlier, 2017; PubChem Open Chemistry Data Base, 2018). The presence of a β-diketone moiety is responsible for keto-enol isomers of curcumin (Figure 2.3), however, the enol isoform is reported to be more stable (Payton, Sandusky, & Alworth, 2007; Shen & Ji, 2007).

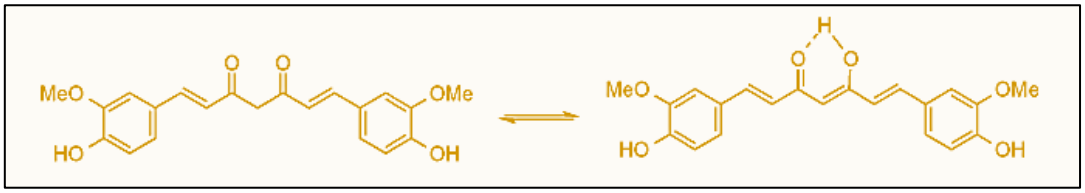


Figure 2.3: K*eto-enol isomers of curcumin.*
Source: (Wanninger et al., 2015) *(Reproduced from an open access article)*

Curcumin is a hydrophobic molecule, therefore, it is insoluble in water but soluble in solvents like ethanol, methanol, chloroform and dimethyl sulfoxide (DMSO) (Hatcher, Planalp, Cho, Torti, & Torti, 2008; Priyadarsini, 2014). Its solubility can further be improved by mixing

solvent with serum such as fetal calf serum (FCS) or bovine serum albumin (BSA). This technique is commonly used for *in vitro* studies (Klawitter et al., 2012; Quitschke, 2008). However, Wang et al. (1997) reported 50 % degradation of curcumin dissolved in 10 % FBS for 8 h. Although the solubility of curcumin is enhanced at alkaline pH (up to pH ≈ 10.2), it is relatively unstable and degrades quickly (about 90 % in 30 min) in neutral or alkaline pH to produce compounds like vanillin and ferulic acid (Figure 2.4a) (Nelson et al., 2017). In aqueous solution curcumin is mainly degraded by autoxidation. Free radical species initiate a series of degradation steps, producing several unstable intermediary products, resulting in a stable cyclic compound called bicyclopentadione (Figure 2.4b) (Gordon, Luis, Sintim, & Schneider, 2015).

Curcumin is also sensitive to light and degradation occurs rapidly in both crystalline and aqueous states. Powdered curcumin exposed to sunlight converts into vanillin, vanillic acid

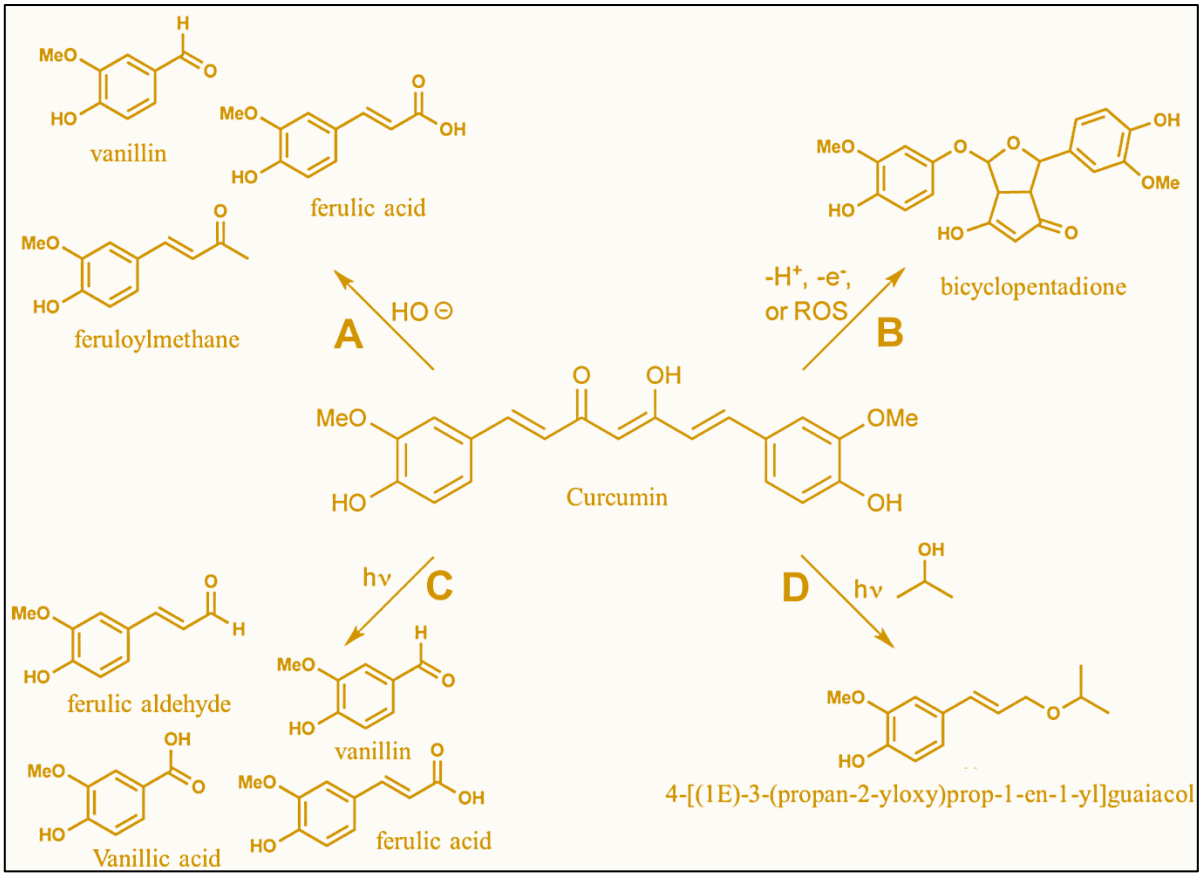


Figure 2.4: Degradation of curcumin (a) at alkaline pH; (b) autoxidation in solvent; (c) photo-oxidation when in crystalline or aqueous state; and (d) photo-oxidation when in specific solvent like isopropanol.

Source: (Nelson et al., 2017) *(Reproduced with permission)*

and ferulic aldehyde like compounds (Figure 2.4c) (Griesser et al., 2011). Curcumin dissolved in a solvent and exposed to light produces several solvent-depended products, e.g. production of guaiacol derivative when dissolved in isopropanol (Figure 2.4d) (Nelson et al., 2017). Curcumin is relatively stable at high temperature, but above 90ºC the β-diketone linkage break and leads to degradation (Peram, Jalalpure, Palkar, & Diwan, 2017).

Despite poor water solubility and low stability, curcumin has found wide applications as a therapeutic agent. The α and β-diketo moiety is a good chelating agent, therefore curcumin can reduce the toxicity of heavy metals such as lead and cadmium by forming strong complexes with them (Akram et al., 2010; Priyadarsini, 2014). The phenolic group of curcumin can scavenge free radicals by trapping the radical and thereby inhibiting the chain reaction, hence act as an anti-oxidant (Barzegar, 2012; Chen, Xue, & Mu, 2014). It can act on a wide spectrum of microorganism showing antibacterial, antifungal, anti-parasitic and anti-HIV activities (Nelson et al., 2017). Curcumin is also reported to be effective against several infections such as malaria, sexually transmitted disease and chronic inflammation-related diseases such as arthritis, type-2 diabetes, osteoporosis, psoriasis, bronchitis, depression, obesity and several cancers (Hewlings & Kalman, 2017; Kocaadam & Şanlier, 2017; Mullaicharam & Maheswaran, 2012).

2.5.1.1 Curcumin and inflammation

Curcumin has been extensively studied for its anti-inflammatory properties, which helps to explain some of its multiple therapeutic benefits (Hewlings & Kalman, 2017; Marchiani, Rozzo, Fadda, Delogu, & Ruzza, 2014). Curcumin can influence the activity of multiple pathways by blocking certain enzymes (e.g. COX-2, 5-LOX and iNOS), growth factors (e.g. transforming growth factor-β1: TGF-β1), receptors (e.g. integrin receptor: IR and interleukin 8-receptor: IL-8-R), kinases (e.g. mitogen-activated protein kinase: MAPK and Janus kinase: JAK), inflammatory cytokines (e.g. TNF-α, IL-6 and IL-8) and transcriptional factors (e.g. NF-κB and nuclear factor 2-related factor: Nrf-2) (Anand, Sundaram, Jhurani, Kunnumakkara, & Aggarwal, 2008; Jurenka, 2009; Lozada-García et al., 2017). The different molecular targets reported to be affected by curcumin are illustrated in Figure 2.5. Of these targets, the suppression of inflammatory cytokines and transcriptional factors are key contributors to the anti-inflammatory property of curcumin.

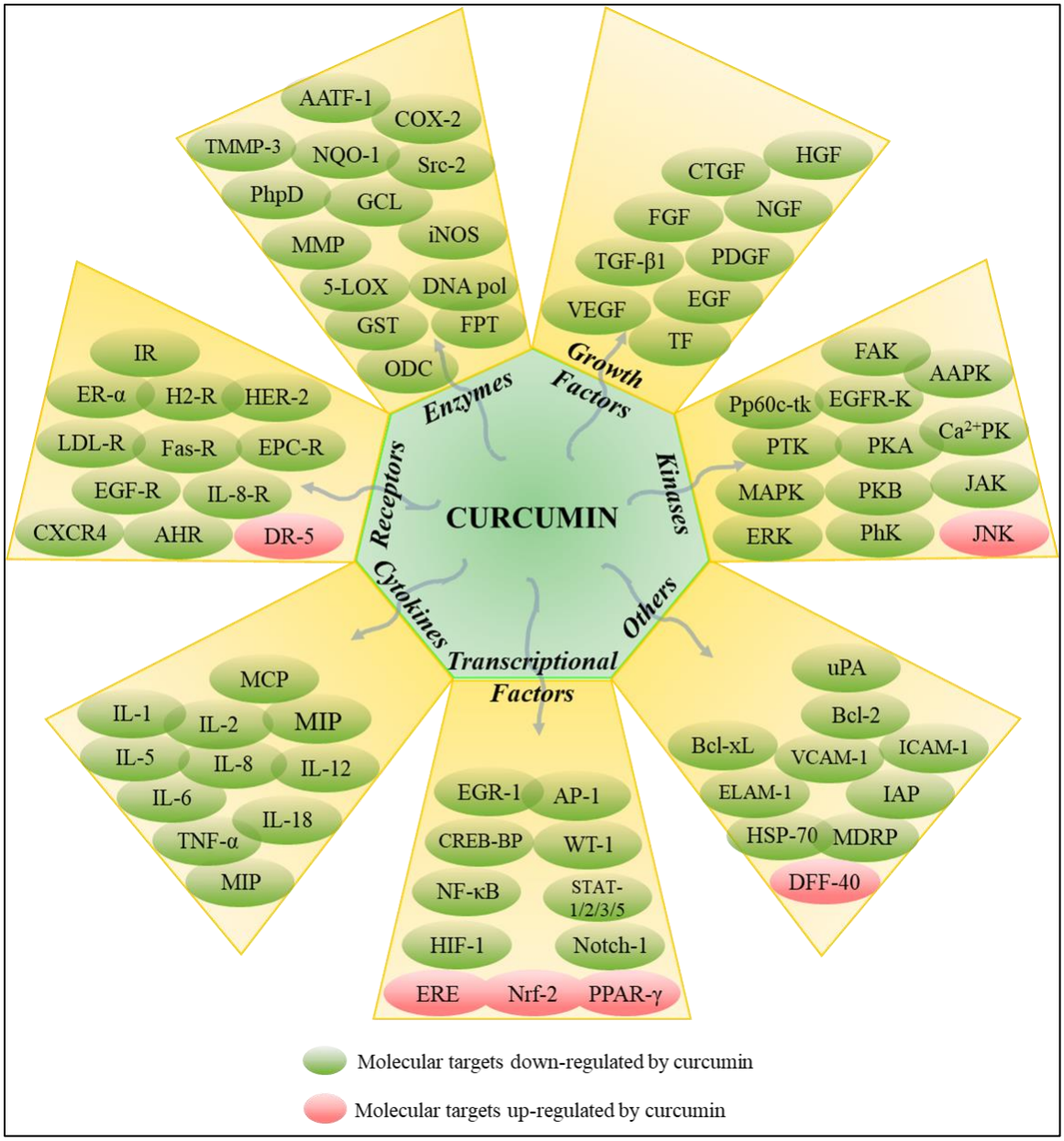


Figure 2.5: Various molecules targeted by curcumin.

EGR-1: early growth response gene-1; AP-1: activating protein1; CREB-BP: CREB-binding protein; WT-1: Wilms' tumour gene-1; NF-κB: nuclear transcription factor kappa-B; STAT: signal transducers and activators of transcription; HIF-1: hypoxia inducible factor-1; ERE: electrophile response element; Nrf-2: nuclear factor 2-related factor; PPAR-γ: peroxisome proliferator-activated receptor-gamma; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; IL: interleukin; TNF-α: tumour necrosis factor alpha; MaIP: macrophage inflammatory protein; IR: integrin receptor; ER-α: estrogen receptor alpha; H2-R: histamine (2)- receptor; HER-2: human epidermal growth factor-2; LDL-R: low density lipoprotein receptor; Fas-R: Fas receptor; EPC-R: endothelial protein C-receptor; AR: androgen receptor; EGF-R: epidermal growth factor-receptor; IL-8-R: interleukin 8-receptor; CXCR4: alpha-chemokine receptor; AHR: aryl hydrocarbon receptor; DR-5: death receptor-5; AATF-1: arylamine N-acetyltransferases-1; COX-2: cyclooxygenase-2; NQO-1: NADPH-quinoneoxidoreductase-1; TMMP-3: tissue inhibitor of metalloproteinase-3; Src-2: src homology-2 domain containing tyrosine phosphatase 2; PhpD: phospholipase D; GCL: glutamate cysteine ligase; MMP: matrix metalloproteinase; iNOS: inducible nitric oxide

oxidase; LOX, lipoxygenase; DNA pol: DNA polymerase; GST: glutathione S-transferase; FPT: farnesyl protein transferase; ODC: ornithine decarboxylase; HGF: hepatocyte growth factor; CTGF: connective tissue growth factor; FGF: fibroblast growth factor; NGF: nerve growth factor; PDGF: platelet-derived growth factor; TGF-β1: transforming growth factor-β1; EGF: epidermal growth factor; VEGF: vascular endothelial growth factor; TF: tissue factor; FAK: focal adhesion kinase; AAPK: autophosphorylation-activated protein kinase; Pp60c-tk: a non-receptor protein tyrosine kinase c-Src; EGFR-K: EGF receptor-kinase; Ca^{2+}PK, Ca^{2+}-dependent protein kinase; PTK: protein tyrosine kinase; MAPK: mitogen activated protein kinase; PKB: protein kinase B; PKA: protein kinase A; JAK: janus kinase; ERK: extracellular receptor kinase; PhK: phosphorylase kinase; JNK: c-jun N-terminal kinase; uPA: urokinase-type plasminogen activator; Bcl-2: beta-cell lymphoma protein; Bcl-xL: beta-cell lymphoma extra-large; VCAM-1: vascular cell adhesion molecule- 1; ICAM-1: intracellular adhesion molecule-1; ELAM-1: endothelial leukocyte adhesion molecule-1; IAP: inhibitory apoptosis protein; HSP-70: heat-shock protein 70; MDRP: multi-drug resistance protein; DFF-40: DNA fragmentation factor 40-kd subunit. Adapted from: (Anand et al., 2008; Noorafshan & Ashkani-Esfahani, 2013) and created using Microsoft PowerPoint.

NF-κB is the principal regulator influencing the expression of more than 500 inflammation-related genes (Buhrmann et al., 2011). NF-κB protein is present in the cytoplasm of the cell which on activation is translocated into the nucleus (Jobin et al., 1999). In its in-activated form, the heterodimer structure of NF-κB is sequestered by binding with IκB, an NF-κB inhibitory protein (Surh et al., 2001). When cells are exposed to an external stimuli e.g. bacteria, inflammatory cytokines, ROS, ultraviolet radiation or viral proteins, NF-κB is functionally activated (Hatcher et al., 2008; Jobin et al., 1999; Surh et al., 2001). The exposure to the external stimulus will activate various kinases such as NF-κB inducing kinase (NIK) and IκBα kinase (IKK) resulting in phosphorylation and degradation of inhibitor κB (IκB), thereby activating NF-κB (Surh et al., 2001). The exposure to the external stimulus can also activate mitogen-activated protein kinases (MAPK, a family of serine/threonine kinase) signalling pathways leading to phosphorylation and activation of NF-κB (Hatcher et al., 2008; Kaminska, 2005; Liang et al., 2015). Activated NF-κB travel into the nucleus where it binds to the promoter region on DNA and result in increased transcription of various inflammation-related genes responsible for enhancing the expression of cytokines e.g. TNF-α and IL-6 and enzymes e.g. COX-2 and iNOS (Li, Suwanwela, & Patumraj, 2017; Surh et al., 2001). The pro-inflammatory cytokines, free radicals and products formed during arachidonic acid metabolism can further promote activation of NF-κB and result in prolonging inflammatory response (He et al., 2015). Curcumin can suppress the secretion of pro-inflammatory cytokines, quenches ROS and RNS and inhibits TNF-α mediated phosphorylation and degradation of the IκB signalling pathway. Thus, it can help control

prolonged inflammation (Alappat & Awad, 2010; Hatcher et al., 2008; Jobin et al., 1999; Panahi et al., 2015; Singh & Aggarwal, 1995; Surh et al., 2001).

Evidence for the anti-inflammatory properties of curcumin

Studies from many *in vitro*, animal and clinical trials have reported the anti-inflammatory effect of curcumin, some of which are summarised in Table 2.4.

Evidence for in vitro studies

As previously discussed, NF-κB inhibition is an important pathway involved in the anti-inflammatory effects of curcumin. Several studies (Table 2.4) have shown that curcumin can suppress NF-κB activation and NF-κB-regulated gene expression in both animal and human cell lines.

Pan, Lin-Shiau, and Lin (2000) reported that curcumin could down-regulate iNOS secretion and transcription in LPS induced inflammation in RAW264.7 cell. Further, these authors reported that curcumin decreases the activity of IκB kinase-1/2 (IKK-1/2 or IKK-α/β), thereby inhibiting NF-κB activation. Ben et al. (2011) confirmed that curcumin suppresses iNOS secretion and transcription, and proposed that curcumin inhibits extracellular signal-regulated kinase-1/2 (ERK-1/2) activation and subsequently iNOS secretion. Other studies on LPS treated RAW 264.7 cells showed that treatment with curcumin decreased secretion of pro-inflammatory cytokines (e.g. TNF-α and IL-6) and enzymes (e.g. COX-2 and iNOS) (Murakami et al., 2008; Sun et al., 2010). Curcumin treatment of LPS induced inflammation in BV2 cell resulted in a decrease in the secretion of NO, PGE_2, TNF-α, IL-6 and IL-1β and downregulation of expression of iNOS and COX-2 (Jin, Lee, Park, Choi, & Kim, 2007). In another study, 3T3-L1 preadipocyte, isolated from male C57BL/6 mice fed with high-fat diet for 3 months, were cultured along with RAW 264.7 and a decrease in monocyte chemoattractant protein-1 (MCP-1, responsible for chemo-taxis in adipose tissues) was reported on treatment with 10 μM curcumin for 6 h (Woo et al., 2007).

Curcumin has elicited similar effects on human cells. For example, administration of curcumin to U937 and A293 cells stimulated with TNF-α to induce inflammation, resulted in a dose-dependent decrease in the expression of NF-κB-regulated genes like COX-2, IκB-α and IKK (Aggarwal et al., 2006). Vascular smooth muscle cells, administrated with curcumin, suppressed expression of TNF-α, NO, MCP-1 and iNOS by reducing NF-κB activation *via* downregulation of MAPK pathways (Meng, Yan, Deng, Gao, & Niu, 2013).

Curcumin incubated with human umbilical vein endothelial cells led to a decrease in high mobility group box 1 (HMGB-1) (Kim, Lee, & Bae, 2011), which is responsible for the secretion of pro-inflammatory cytokines and neutrophil adhesion and migration.

Evidence for animal studies

The molecules shown to be targeted by curcumin in *in vitro* studies are also reported to be affected in animal trials (Table 2.4). Curcumin administered for 6 h at a rate of 35 mg/kg/h decreased the level of TNF-α and IL-6 in Sprague-Dawley rats with induced pancreatitis by blocking IκB mediated NF-κB activation (Gukovsky, Reyes, Vaquero, Gukovskaya, & Pandol, 2003). In another study, 100 mg/kg curcumin administered for 20 days prior to inducing acute pancreatitis in male Wistar-Albino rats decreased TNF-α, IL-6, iNOS and NO, (Gulcubuk et al., 2013).

Curcumin is also effective in reducing obesity-induced inflammation as shown by Weisberg, Leibel, and Tortoriello (2008) and Shao et al. (2012). In these two studies, dietary curcumin was administrated *via* high-fat diet in male C57BL/6J mice and reported inhibition of NF-κB activation resulting in a decrease in oxidative stress and macrophage infiltration in white adipose tissues. Incubation of mice with curcumin (200 µl of 0.2 mg/ml/day for 12 days) reduces TNF-α level and also speeds up the wound healing process by increasing collagen level (Yen et al., 2018). Similar results were reported by Kant et al. (2014) in wounded diabetic adult male Wistar rats on treatment with curcumin for 19 days. Kant et al. (2014) also reported an increase in anti-inflammatory cytokine IL-10 and enzyme superoxide dismutase (SOD) on exposure with curcumin. Curcumin downregulates pro-inflammatory cytokines e.g. IL-1β, TNF-α and IL-6, and upregulates anti-inflammatory cytokines e.g. TGF-α and IL-10 in hippocampus, cortex, hypothalamus and spleen of Wistar rats affected with chronic mild stress (You et al., 2011).

Evidence for clinical trials

A clinical trial is the best way to understand the effectiveness of a compound in performing the intended function in the human body. However, due to high cost, longer duration, withdrawal of participants and high precision requirement there is limited clinical evidence supporting the anti-inflammatory effects of curcumin. Some of the clinical trials are summarised in Table 2.4.

Table 2.4: Pre-clinical and clinical studies showing the anti-inflammatory effects of curcumin.

Researcher(s) ***(reference)***	**Experiment Design**	**Dose and Duration**	**Key Observations**	**Mechanism Purposed/ Comment** ***(if any)***
		In vitro studies		
Pan et al. (2000)	LPS (100 ng/ml) induced inflammation in RAW 264.7 (mouse monocyte) cells	10 µM for 6 h	Decrease in iNOS secretion and transcription Inhibition of IKK1 and IKK2 activity	Curcumin down-regulate NF-κB activation by inhibiting IKK
Aggarwal et al. (2006)	TNF-α (0.1 and 1 nM) induced inflammation in U937 (human myeloid leukemia) and A293 (human embryonic kidney) cells	10-50 µM for 2-24 h	Downregulate COX-2, cyclin D1, IAP-1, IAP-2, Bcl-2, Bcl-xL, Bfl-1/A1, p65, IκB-α, IKK and Akt in dose dependent manner	Curcumin suppress TNF-α induced NF-κB activation and NF-κB-regulated gene expression by inhibiting IKK and Akt activation
Bachmeier et al. (2007)	TNF-α (10 ng/ml) induced inflammation in MDA-MB-231 (human breast cancer) cell line	25 µM for 2-24 h	Reduces IκB, p65 phosphorylation, MMP and AP-1	Curcumin silences NF-κB functioning
Woo et al. (2007)	3T3-L1 preadipocyte were isolated from mice fed with high-fat diet and were cultures along with RAW 264.7	10 µM for 6 h	Decrease in TNF-α, NO and MCP-1	Curcumin can suppress obesity-induced inflammatory responses by suppressing MCP-1 release from adipocytes
Jin et al. (2007)	LPS (0.5 µg/mL) induced inflammation in BV2 (mouse murine microglial) cell line	5-20 µM for 24 h	Decrease in secretion of NO, PGE_2, TNF-α, IL-6 and IL-1β Downregulate expression of iNOS and COX-2	Curcumin reduce pro-inflammatory cytokines by suppressing NF-κB
Murakami et al. (2008)	LPS (100 ng/ml) induced inflammation in RAW 264.7 cells	0.2-20 µM for 3 h	Decrease in COX-2 and NF-κB expression	
Chen, Nie, Fan, and Bian (2008)	LPS (1 µg/ml) induced inflammation in RAW 264.7 cells	5-30 µmol/l for 8 h	Decrease in TNF-α and IL-1β	Curcumin downregulate NF-κB dependent transcription factors
Sun et al. (2010)	LPS (50 ng/ml) induced inflammation in RAW 264.7 cells	20 µmol/l for 7 h	Decrease in TNF-α and IL-6	
Kim et al.	LPS (100 ng/ml)	2.5-100	Decrease in HMGB-1	Curcumin inhibit

(2011)	induced inflammation in human umbilical vein endothelial cells (HUVEC)	µM for 6 h	release and neutrophil adhesion and migration	HMGB-1-mediated pro-inflammatory response
Ben et al. (2011)	LPS (100 ng/ml) induced inflammation in RAW 264.7 cells	20 µM for 12 h	Decrease in iNOS secretion and transcription	Curcumin inhibits ERK 1/2 activation and subsequently suppressed iNOS enzyme activity
Meng et al. (2013)	LPS (1 µg/ml) induced inflammation in vascular smooth muscle cells (VSMCs) of rats	5-30 µmol/l for 24 h	Decrease in TNF-α, NO, MCP-1, iNOS, IκBα and NF-κB	Curcumin suppresses MAPK and NF-κB pathways
Youn, Kwon, Ju, Choi, and Park (2013)	THP-1 (human monocyte) cells were cultured alone and in combination with HaCaT (immortalized human keratinocyte) cells in presence of TNF-α	1-30 µM for 1-24 h	Decrease in ICAM-1, Nrf2 and HO-1	Curcumin suppress the TNF-α-induced ICAM-1 expression and subsequent THP-1 adhesion via expression of HO-1 in the HaCaT
Animal trials				
Gukovsky et al. (2003)	Ethanol and CCK-8 induce pancreatitis in Sprague-Dawley rats	200 mg/kg for 6 h	Downregulate TNF-α, IL-6, IκB and AP-1	Curcumin blocks NF-κB and AP-1 activation
Banerjee, Tripathi, Srivastava, Puri, and Shukla (2003)	Arthritis induced in male Sprague Dawley rats via Freund's adjuvant (10 mg/ml)	100 mg/kg every day for 35 days	Decrease in TNF-α, IL-1β and NO	
Weisberg et al. (2008)	Wild-type and ob/ob male obese C57BL/6J mice	3 % dietary curcumin per day for 35 days	Decrease in macrophage infiltration of white adipose tissue	Curcumin downregulate NF-κB activity
Gupta et al. (2011)	Streptozotocin induced diabetic Wistar albino rats	1 g/kg every day for 16 weeks	Downregulate SOD, TNF-α and VEGF	
Shao et al. (2012)	Male C57BL/6J mice fed with high fat diet	Dietary curcumin for 28 weeks	Decrease in oxidative stress	Curcumin inhibit NF-κB or pJNK levels
Jiang et al. (2013)	Chronic mild stress induced in male Wistar	10 mg/kg per day for	Inhibit TNF-α and IL-6 at both mRNA and protein	Curcumin reduced the activation of NF-κB

	rats	21 days	level	
Gulcubuk et al. (2013)	Sodium taurocholate induced acute pancreatitis in male Wistar-Albino rats	100 mg/kg for 20 days before pancreatitis	Downregulation of TNF-α, IL-6 and iNOS and NO (but not at all time points)	Curcumin inhibit NF-κB and AP-1
Kant et al. (2014)	Wounded diabetic adult male Wistar rats	400 µl applied on wounds every day for 19 days	Decrease in pro-inflammatory cytokine TNF-α and IL-1β Upregulation of anti-inflammatory cytokine IL-10 and enzyme SOD	Curcumin have thicker collagen deposition at wounded area
Wang et al. (2014)	LPS (0.83 mg/kg) induced depression in adult male Kun-Ming mice	50 mg/kg per day for 7 days	Decrease in TNF-α, IL-1β, iNOS and COX-2	Curcumin have anti-depressive effect by inhibiting NF-κB pathway
Zhang, Li, Jia, and He (2015)	Ovalbumin (40 µg/kg) induced allergy in female BALB/c and C57BL/6 mice	100 and 200 mg/kg per day for 3 days	Downregulate TNF-α, IL-1β, IL-6, IL-8, ERK, p38, JNK, IκBα and NF-κB	Curcumin have anti-allergic effect by inhibiting MAPK/NF-κB pathway
Kaur, Patro, Tikoo, and Sandhir (2015)	PTZ induced chronic epilepsy in adult male Wistar rats	100 mg/kg every day for 30 days	Decrease in secretion and transcription of IL-1β, IL-6, TNF-α and MCP-1	
Zhong et al. (2016)	LPS (5 mg/kg) injected male wild-type C57BL/6 mice	20-80 mg/kg every day for 28 days	Decrease in serum level of TNF-α, IL-1β and IL-18 Downregulate $O_2^{\bullet-}$, H_2O_2, ROS and NO in liver	Curcumin inhibit PI3K/Akt and CYP2E/Nrf2/ROS signaling
Li et al. (2017)	Stroke-induced male Wistar rats	300 mg/kg 30 min after operation	Decrease in NF-κB, ICAM-1 and MMP-9	
Yang et al. (2018)	DSS induced inflammatory bowel disease in male ICR mice	0.1 or 0.25 mmol/kg for 7 days	Decrease in NF-κB, COX-2 and iNOS	Curcumin inhibit STAT-3 pathway
Clinical trials				
Dhillon et al. (2008)	Nonrandomized, open-label, phase II trial with patients confirmed adenocarcinoma of the pancreas (n=21)	8 g/day until disease progression	Downregulate NF-κB and COX-2 peripheral blood mononuclear cells from patients	No toxicity observed
Khajehdehi et al. (2011)	Randomized double-	500 mg turmeric	Decrease in serum level of TGF-β and IL-8	No adverse effects related to the turmeric

	blind and placebo-controlled trail with patients suffering from type 2 diabetic nephropathy (n=20)	capsule trice/day for 2 months		supplementation Each capsule contain 22.1 mg of the active ingredient curcumin
Ganjali et al. (2014)	Randomized double-blind crossover trial with obese patients (n=28)	500 mg capsules twice/day for 30 days (each capsule contains 5 mg bioperine)	Downregulate IL-1β, IL-4 and VEGF No effect on serum level of IL-1α, IL-2, IL-6, IL-8, IL-10, IFNγ, EGF, MCP-1, and TNFα	
Panahi et al. (2015)	Randomized double-blind placebo-controlled trial with male participants suffering from chronic sulfur mustard (n=40)	500 mg capsules /day for 4 weeks	Decrease in TNF-α, IL-6, TGF-β, hs-CRP, CGRP and MCP-1	No toxicity observed
Sciberras et al. (2015)	Effect on inflammatory cytokine after 2 h exercise in randomized double-blind cross-over trail with male athletes (n=11)	500 mg capsule with midday meal for 3 days and 1 capsule just before exercise	Statistically insignificant decrease in serum level of IL-1, IL-6 and IL-10	
Panahi et al. (2016)	Randomized double-blind placebo-controlled trial with a parallel-group design having males and females diagnosed with metabolic syndrome (n=50)	500 mg capsules twice/day for 8 weeks (each capsule contains 5 mg piperine)	Decrease in serum level of TNF-α, IL-6, TGF-β and MCP-1	No toxicity observed

LPS: lipopolysaccharide; iNOS: inducible nitric oxide synthases; IKK: IκB kinase; NF-κB: nuclear transcription factor kappa-B; TNF-α: tumour necrosis factor alpha; COX-2: cyclooxygenase-2; IAP: inhibitor of apoptosis protein; Bcl-2: beta-cell lymphoma protein; Bcl-xL: beta-cell lymphoma extra-large; MMP: matrix metalloproteinases; AP-1: activating-protein-1; NO: nitric oxide; MCP-1: monocyte chemoattractant protein-1; PGE_2: prostaglandin E_2; IL: interleukin; VCAM-1: vascular cell adhesion molecule- 1; ICAM-1: intracellular adhesion molecule-1; HMGB-1: high mobility group box 1; ERK: extracellular signal-regulated kinase; MAPK: mitogen activated protein kinase; Nrf2: nuclear factor 2-related factor 2; HO-1: heme oxygenase-1; mRNA: messenger ribonucleic acid; SOD: superoxide dismutase; VEGF: vascular endothelial growth factor; JNK: c-jun N-terminal kinase; PTZ: pentylenetetrazole; $O_2^{\bullet-}$: superoxide; H_2O_2: hydrogen peroxide; ROS: reactive oxygen species; NO: nitric oxide; PI3K/Akt: phosphatidylinositol 3-kinase/protein kinase B; CYP2E: cytochrome P450- 2E1; DSS: dextran sulfate sodium; IFNγ: interferon γ; EGF: epidermal growth

factor; TGF-β: transforming growth factor-β; hs-CRP: high-sensitivity C-reactive protein; CGRP: calcitonin gene related peptide.

Panahi, Ghanei, Bashiri, Hajihashemi, and Sahebkar (2015) conducted a randomized double-blind placebo-controlled trial on male participants suffering from chronic sulfur mustard. Participants were given 500 mg capsules of curcuminoids per day for 4 weeks. 40 individuals completed the trial and authors reported a decrease in TNF-α, IL-6, MCP-1, transforming growth factor-β (TGF-β), high-sensitivity C-reactive protein (hs-CRP) and calcitonin gene-related peptide (CGRP). Similar results have been reported in another randomized double-blind placebo-controlled trial in people diagnosed with metabolic syndrome (Panahi et al., 2016). A decrease in serum levels of TNF-α, IL-6, TGF-β and MCP-1 was reported following administration of 500 mg curcumin consumed twice a day for 8 weeks. In contrast, consumption of 500 mg curcuminoids twice a day for 30 days did not downregulate IL-1α, IL-2, IL-6, IL-8, IL-10, interferon γ (IFNγ), epidermal growth factor (EGF), MCP-1, and TNF-α in obese individuals (Ganjali et al., (2014).

2.5.1.2 Bioavailability and metabolism of curcumin

Curcumin is known to have poor bioavailability in both humans and animals. The small portion of curcumin that is absorbed in the intestine quickly degrades in the blood and liver (Liu et al., 2016), leading to the formation of curcumin glucuronide and curcumin sulphate *via* two different pathways (Mahran, Hagras, Sun, & Brenner, 2017). Curcumin can undergo a series of reduction reactions, where four bonds of curcumin are broken to form octa-hydro-curcumin. During this conversion, intermediates such as hexahydrocurcimin glucuronide and hexahydrocurcimin sulphate are produced (Hassaninasab, Hashimoto, Tomita-Yokotani, & Kobayashi, 2011; Mahran et al., 2017). Curcumin, *via* the second pathway, can form conjugates with monoglucuronide and monosulfate to produce curcumin glucuronide and curcumin sulphate which are more prominent in the blood (Cuomo et al., 2011; Ghosh, Banerjee, & Sil, 2015). The unabsorbed fraction of curcumin which may be as high as 75 % is excreted directly in faeces (Yadav, Sah, Jha, Sah, & Shah, 2013).

2.5.1.3 Safety of curcumin

Turmeric is listed as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) (Nelson et al., 2017) and curcumin has been reported safe for human

consumption. Curcumin dose of 0-3 mg/kg body weight have been reported as adequate daily intake (ADI) by the Joint FAO/WHO Expert Committee on Food Additives (2004) (JECFA) and European Food Safety Authority (2014) (EFSA). However, some studies have used curcumin doses of up to 8,000-12,000 mg/day and reported adverse effects such as headache, diarrhoea, rash, nausea and yellow stool (Hewlings & Kalman, 2017; Kocaadam & Şanlier, 2017; Nelson et al., 2017). Such high doses are used because of poor bioavailability of curcumin in the human body as its therapeutic effects are limited at lower doses.

2.5.1.4 Co-administration of curcumin with other bioactives

Different methods are being considered to improve the bioavailability of curcumin such as the development of nanoparticles, nanoemulsions and liposomes (Cuomo et al., 2011; Jin, Lu, & Jiang, 2016; Mahran et al., 2017; Prasad et al., 2014). Curcumin is also being investigated in combination with other bioactive compounds that may enhance its activity and thereby its therapeutic effects at lower doses (Mahran et al., 2017). Some of the bioactive compounds listed in Table 2.2 have been studied in combination with curcumin and their synergistic effects reported. Piperine, a bioactive compound from black pepper, has been extensively studied in combination with curcumin and it is reported to improve the absorption of curcumin in the body by 2000 % (Hewlings & Kalman, 2017). In a phase III randomized double-blind placebo-controlled trial with a parallel-group design on 59 subjects diagnosed with metabolic syndrome treated with a daily dose of 1 g curcuminoids mixed with 10 mg of piperine for 8 weeks, the authors (Panahi et al., 2015) reported a significant increase in serum concentration of SOD in comparison with the control. Curcumin was also studied in combination with resveratrol (a bioactive compound from grapes, berries and wine). In combination, curcumin (10 µM) and resveratrol (10 µM) synergistically downregulated MAPK pathway in human articular chondrocytes (Shakibaei, Mobasheri, & Buhrmann, 2011). However, in a double-blind, crossover, randomized study in obese adults treatment with a combination of curcumin (100 mg) and resveratrol (200 mg) was not effective in significantly reducing serum concentration of IL-6, IL-8, MCP-1 or VCAM-1 (Vors et al., 2018). Furthermore, there was no difference in NF-κB gene expression in comparison with the control.

2.5.2 Chlorogenic acid

Chlorogenic acid (CGA) is a less studied bioactive compound. It is one of the principle polyphenols in the human diet as it is abundant in many food sources such as fruits (e.g. apples, grapes, pears, peaches, plums, kiwi fruit, mangoes, blueberries, and blackberries) and vegetables (e.g. cabbage, eggplants, carrots, potatoes, tomatoes and coriander) (Clifford, 2000; Furrer, Cladis, Kurilich, Manoharan, & Ferruzzi, 2017; Liang & Kitts, 2015; Naso et al., 2014; Santana-Gálvez, Cisneros-Zevallos, & Jacobo-Velázquez, 2017; Upadhyay & Mohan Rao, 2013). However, the amount available varies according to the maturity, storage conditions and processing steps applied to different food sources. For example, CGA in potato is reported to increase slowly during storage in the presence of light (Onyeneho & Hettiarachchy, 1993). CGA is distributed throughout the plant including roots, seeds, leaves, flowers, and tubes, as well as products developed from them, such as coffee, juice and even wine (Gil & Wianowska, 2017). Green coffee beans are key source of CGA containing about 6-12 % CGA on a dry weight basis (Gil & Wianowska, 2017).

Chemically, CGA is a group of polyphenols derived from esterification reactions between cinnamic acids derivatives and quinic acid (QA) (Tajik, Tajik, Mack, & Enck, 2017). CGA is the whole hydroxyl-cinnamic acid family containing caffic acid (CA), ferulic acid (FA) and *p*-coumaric acid with quinic acid (Naveed et al., 2018). Further, these subgroups have several isomeric forms which are distributed in food sources. The chemical structures of CGA which are dominant in food are shown in Figure 2.6. Coffee beans for instance consist of several CGA isoforms, including 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic) (Liang & Kitts, 2015; Matei, Jaiswal, & Kuhnert, 2012). In coffee beans, 5-CQA is present in the largest quantity accounting for 76-84 % of all CGA which makes up approximately 10 g / 100 g of beans on dry weight basis (Farah & Donangelo, 2006; Perrone, Donangelo, Donangelo, & Farah, 2010). Caution is required when reading documents related to CGA as in most of the publications 5-CQA is referred to as CGA. In the present study, CGA is referred to 5-CQA, unless specified.

CGA is poorly soluble in water but has good solubility in organic solvents like methanol, ethanol, acetone and DMSO. Depending on the solvent, CGA may hydrolyse to QA and cinnamic acids derivatives or decarboxylation of cinnamic acids derivatives or lactones formation can occur (Jaiswal, Matei, Golon, Witt, & Kuhnert, 2012).

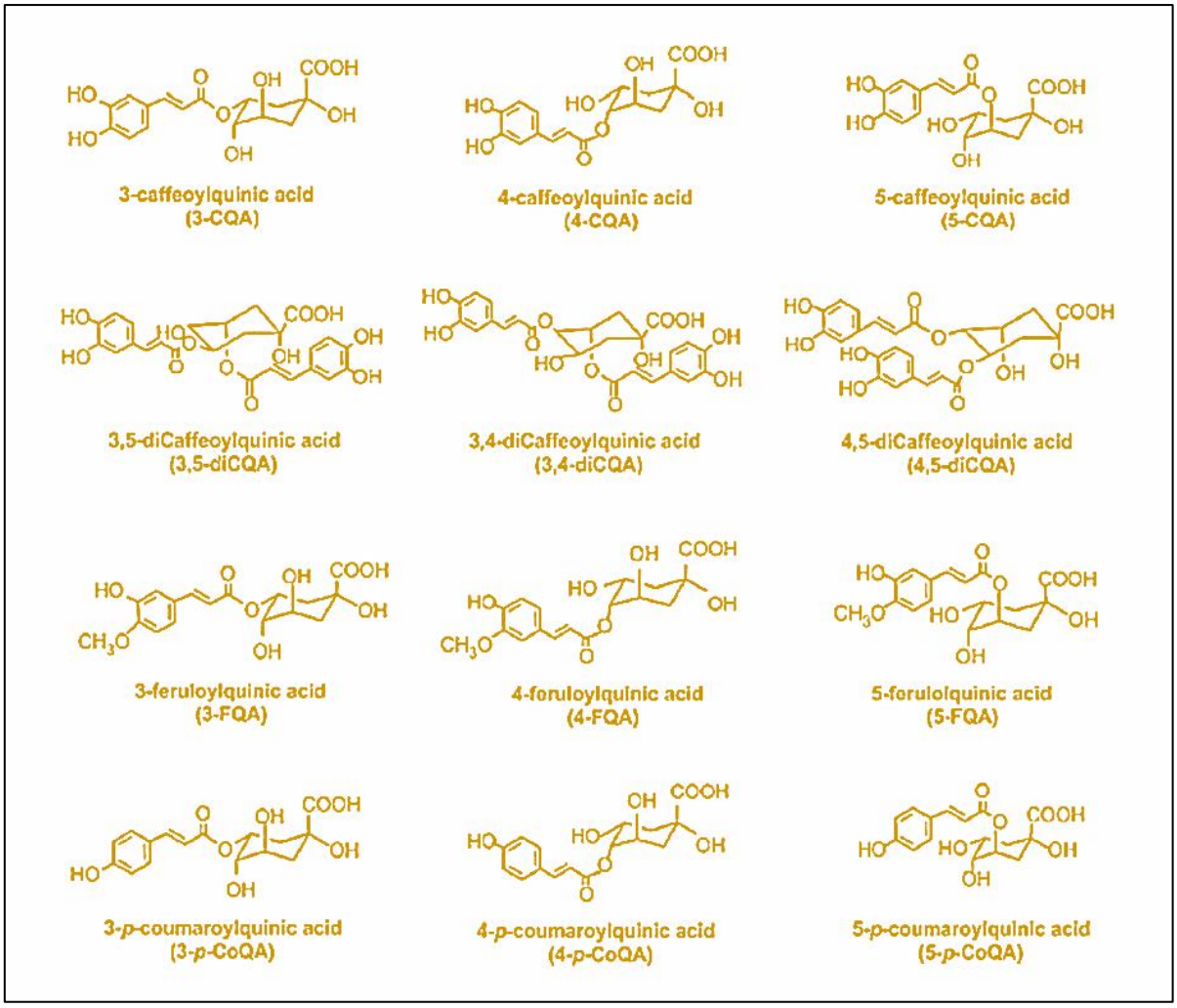


Figure 2.6: Chemical structure of different isomers of chlorogenic acid.
Source: (Liang & Kitts, 2015) *(Reproduced from an open access article)*

Under basic pH conditions, the rate of degradation of CGA increases, escalating hydrolysis and acyl migration (Deshpande, Jaiswal, Matei, & Kuhnert, 2014). pH-dependent degradation can be reduced by adding ascorbic acid and epigallocatechin-3-gallate (EGCG) to the CGA solution (Narita & Inouye, 2013). An increase in temperature increases the degradation of CGA *via* intermolecular isomerization. Effect of temperature intensity (time and temperature) on CGA in coffee beans revealed that mild roasting (230℃ for 12 min) of beans reduces CGA by 45-54 % while 99 % reduction was reported in high roasting (250℃ for 17 min) (Moon, Yoo, & Shibamoto, 2009). CGA is photosensitive and on exposure to UV or visible light, it may undergo cis-trans isomerization (Jaiswal, Kiprotich, & Kuhnert, 2011). CGA mixed in methanol is reported to degrade more rapidly on exposure to light when compared with the light-protected solution (Xue et al., 2016).

There are evidences that CGA have therapeutic effects in humans. CGA is reported to exhibit anti-oxidant and anti-inflammatory properties which are associated with its effectiveness in reducing obesity, type-2 diabetes, hypertension, metabolic syndrome, stroke, blood pressure and some forms of cancer (Naveed et al., 2018; Santana-Gálvez et al., 2017; Tajik et al., 2017; Zhao, Wang, Ballevre, Luo, & Zhang, 2012).

2.5.2.1 Chlorogenic acid and inflammation

CGA is an anti-oxidant (Cha et al., 2014; Hao, Pan, Zhang, & Wang, 2015; Karthikesan, Pari, & Menon, 2010; Koriem & Soliman, 2014; Sato et al., 2011; Xu, Hu, & Liu, 2012) and exhibits its anti-inflammatory effects predominantly by scavenging ROS and RNS, thereby reducing oxidative stress in the cell (Liang & Kitts, 2015; Tajik et al., 2017). CGA can donate an H^+ ion to ROS and inhibits the chain reaction. After donating H^+, CGA is oxidised to phenoxyl radical and stabilize itself by resonance (Tošović, Marković, Marković, Mojović, & Milenković, 2017). A decrease in ROS within the cell will reduce the activation of the NF-κB signalling pathway which in result will reduce the production of various pro-inflammatory cytokines such as TNF-α and IL-6, and other cell mediators; ultimately reducing chronic inflammation in the host (Naveed et al., 2018).

Evidence for the anti-inflammatory properties of chlorogenic acid

In vitro, animal and clinical trials demonstrating the anti-inflammatory effect of CGA are summarised in Table 2.5.

Evidence for in vitro studies

Krakauer (2002) studied the effect of CGA (0.2-200 µg/ml) on staphylococcal exotoxin (SEB) induced inflammation in human peripheral blood mononuclear cells and reported a dose-dependent decrease in IL-1β, TNF-α, IL-6, IFNγ, MCP-1, MIP-1α and MIP-1β. Similar results have been reported by others in mouse murine microglial BV2 (Guo et al., 2015) and porcine intestinal epithelial cell line (Palócz, Pászti-Gere, Gálfi, & Farkas, 2016). Guo et al. (2015) also reported suppression of NF-κBp65 pathway by CGA. Administration of CGA (12.5-37.5 µg/ml) to LPS induced inflammation in RAW 264.7 cells resulted in downregulation of COX-2 and PGE_2 at protein and mRNA level by suppressing the NF-κB and JNK/AP-1 signalling pathway (Shan et al., 2009). However, there was no effect on iNOS or NO expression. In contrast, several authors have reported a decrease in NO and iNOS levels in inflammation-induced cells (Shen et al., 2012; Hwang et al., 2014; Chen and Wu,

2014). Incubation of MC3T3-E1 and Caco-2 cells with CGA reduced ROS in cell and therefore decreased pro-inflammatory cytokines (Han et al., 2017; Liang & Kitts, 2018).

Evidence for animal studies

The molecular targets reported to be suppressed by CGA in *in vitro* studies are also downregulated in animal models. Treatment of LPS induced inflammation in the left knee joint of Wistar rats with 40 mg/kg CGA significantly decreases TNF-α and IL-1β levels (Chauhan et al., 2012). Yun, Kang, and Lee (2012) reported a decrease in TNF-α, iNOS, COX-2 and Nrf2 levels in hepatic ischemia and reperfusion injured male Sprague Dawley rats. Administration of 40 mg/kg CGA in acetaminophen-induced hepatotoxicity in mice suppresses IκB/p65-NF-κB activation pathway (Zheng, Sheng, Lu, & Ji, 2015). On contrary, treatment with 1 mM CGA (for 15 days) had no effect on TNF-α expression of dextran sulphate sodium (DSS) induced colitis in female C57BL/6 mice (Shin et al., 2015). Of all the studies summarised in Table 2.5, Bagdas et al. (2015) reported cytotoxicity and genotoxicity in wounded male diabetic Sprague Dawley rats treated with 50 mg/kg/day of CGA for 15 days. In a recent study, a decrease in pro-inflammatory cytokine (e.g. TNF-α, IL-6 and IL-1β) was observed in pigs administrated with 1000 mg/kg/ day of CGA for 14 days (Chen et al., 2018b).

Evidence for clinical trials

Clinical evidences demonstrating the anti-inflammatory effects of CGA are limited (Table 2.5). A trial conducted over 15 years, where 41836 post-menopausal women were followed, reported that consumption of coffee reduced inflammation and cardiovascular diseases, thereby reducing the risk of death (Andersen, Jacobs Jr, Carlsen, & Blomhoff, 2006). In some cross-section surveys, participants were asked to fill a food-frequency questionnaire (FFQ) and blood samples were analysed to see the effect on inflammatory cytokines. These studies showed that coffee consumption (≥1 cup per day) reduced IL-6 and TNF-α in participant's serum (Lopez-Garcia et al., 2006; Yamashita et al., 2012; Zampelas et al., 2004). Kempf et al. (2010) conducted a single-blind study where participants were asked to consume 4 cups of coffee per day for the 1st month and 8 cups per day for the 2nd month and reported that coffee consumption could significantly reduce IL-8 in serum. Based on information search, there is no published clinical trial where the amount of CGA in coffee was quantified during administration in human.

Table 2.5: Pre-clinical and clinical studies showing the anti-inflammatory effects of chlorogenic acid (CGA).

Researcher(s) ***(reference)***	**Experiment Design**	**Dose and Duration**	**Key Observations**	**Mechanism Purposed/ Comment** ***(if any)***
		In vitro studies		
Krakauer (2002)	SEB (150 ng/ml) induced inflammation in human peripheral blood mononuclear cells (PBMC)	0.2-200 µg/ml for 16 h	Decrease the production of IL-1β, TNF-α, IL-6, IFNγ, MCP-1, MIP-1α and MIP-1β	
Zhao, Shin, Satsu, Totsuka, and Shimizu (2008)	LPS (2mM) induced inflammation in Caco-2 (human epithelial cells) cell line	0.25-2 mM/l for 48 h	Decrease in IL-8 secretion and mRNA expression	
Shan et al. (2009)	LPS (100 ng/ml) induced inflammation in RAW 264.7 (mouse monocyte) cells	12.5, 25.0 and 37.5 µg/ml for 2 h	Decrease in COX-2 and PGE_2 secretion and expression No effect on iNOS and NO	CGA suppress NF-κB and JNK/AP-1 signaling pathway
Qin et al. (2010)	Anti-DNP IgE (100 ng/ml) treated RBL-2H3 (rat basophil) cell lines	2.5-10 µg/ml for 30 min	Decrease in histamine and TNF-α release Increase in cAMP	
Shen et al. (2012)	LPS (500 ng/ml) induced inflammation in mice microglia	1-4 mM for 2 h	Decrease in NO and TNF-α production	CGA inhibit IκBα degradation hence suppress NF-κB activation pathway
Hwang et al. (2014)	LPS (1 µg/ml) induced inflammation in RAW 264.7 and BV2 (mouse murine microglial) cells	5-20 µM for 24 h	Decrease in NO, COX-2, iNOS, TNF-α, IL-1β, and IL-6	CGA suppress NF-κB signaling pathway
Chen and Wu (2014)	IL-1β (10 ng/ml) induced inflammation in human chondrocytes	5-20 µM for 1 h	Decrease in PGE_2, NO, iNOS and COX-2	
Shin et al. (2015)	TNF-α (10 ng/ml) and H_2O_2 (2 mM) induced inflammation in Caco-2 cells	0.5-2 mM/l for 3 h	Decrease in IL-8 secretion	
Guo et al. (2015)	HSV-1 induced response in BV2 cells	25-100 ng/ml for 24 h	Decrease in TNF-α and IL-6 secretion and mRNA expression	CGA suppress NF-κBp65 pathway

Palócz et al. (2016)	LPS (10 µg/ml) induced inflammation in porcine intestinal epithelial cell line (IPEC-J2)	25-50 µM for 6-24 h	Lower IL-6, IL-8, TNF-α, COX-2 and ROS	
Han et al. (2017)	H_2O_2 (400 µM) induced oxidative stress in MC3T3-E1 (mouse preosteoblast) cell line	25-100 µM for 1-6 h	Decrease ROS, NO and apoptosis Upregulate Nrf2 pathway	
Liang and Kitts (2018)	PMA (0.1 µg/µl)-IFNγ (8000 U/ml) induced oxidative stress in Caco-2 cell line	0.2-2 mM for 24 h	Decrease in ROS and IL-8	CGA activate Nrf2 signaling pathway
Animal trials				
Zhang et al. (2010)	LPS (0.5 µg/µl) induced acute lung injury in male ICR mice	5-50 mg/kg for 3 h	Decrease in iNOS and NO	
Chauhan et al. (2012)	LPS (100 µl) induced inflammation in left knee joint of Wistar rats	2.5-40 mg/kg for 4 h	Downregulate TNF-α and IL-1β (most significant at 40 mg/kg)	
Yun et al. (2012)	Hepatic ischemia and reperfusion injury in male Sprague Dawley rats	2.5-10 mg/kg for 5 h	Decrease in TNF-α, iNOS, COX-2 and Nrf2	
Shen et al. (2012)	LPS (1 µg/µl) induced inflammation in C57BL/6 mice	100 mg/ml per day for 7 days	Downregulate TNF-α and IL-1β	
Shi et al. (2013)	CCl_4 (3 ml/kg in olive oil) induced liver fibrosis in male Sprague Dawley rats	60 mg/kg twice weekly for 8 weeks	Downregulate iNOS, COX-2, TNF-α, IL-6 and IL-1β	CGA suppress NF-κB pathway
Shin et al. (2015)	DSS (3 %) induced colitis in female C57BL/6 mice	1 mM per day for 15 days	Decrease in mRNA expression of MCP-1 and IL-1β No significant change in TNF-α	
Zheng et al. (2015)	Acetaminophen (400 mg/kg) induced hepatotoxicity in mice	10-40 mg/kg for 4 h	Decrease in mRNA level of TNF-α, IL-6, IL-1β and MCP-1	CGA suppress IκB/p65-NF-κB activation pathway

Bagdas et al. (2015)	Wounded male diabetic Sprague Dawley rats	50 mg/kg per day for 15 days	Decrease in NO No effect on SOD enzyme	Side effect: cyto/genotoxicity was observed
Zatorski et al. (2015)	TNBS (4 mg) induced colitis in male balbC mice	20 mg/kg twice a day for 3-6 days	Decrease in MPO and H_2O_2	CGA inhibit NF-κB dependent pathways
Zheng et al. (2016)	Monocrotaline (90 mg/kg) induced sinusoidal obstruction syndrome in male Sprague Dawley rats	20 mg/kg every day for 6 days	Decrease MPO, TNF-α and IL-1β	CGA downregulate MAPK/ NF-κB pathway
Ali et al. (2017)	MTX (20 mg/kg) induced hepatotoxicity in male Wistar rats	50 and 100 mg/kg for 20 days	Inhibition of COX-2, iNOS, Bax, Bcl-2 and apoptosis	
Ohkawara, Takeda, and Nishihira (2017)	Arginine (5 g/kg) induced pancreatitis in male C57B6 mice	20 and 40 mg/kg for 1 h	Decrease in IL-6, MIP-2 and MIF after 72 h	
Shi et al. (2018)	CCl_4 (3 ml/kg in olive oil) induced liver fibrosis in male Sprague Dawley rats	15, 30 and 60 mg/kg per day for 7 days	Decrease in serum level and mRNA expression of TNF-α, IL-6 and IL-1β Increase SOD enzyme	
Bao et al. (2018)	STZ (35 mg/kg) induce type 2 diabetes in male Sprague-Dawley rats	10 mg/kg per day for 8 weeks	Downregulate IL-6, TNF-α and IL-1β production	CGA suppress Nrf2-NF-κB pathway
Chen et al. (2018a)	Spinal cord injury in female Wistar rats	10-100 mg/ml once three days for 3 weeks	Concentration dependent decrease in TNF-α, IL-6, IL-1β, iNOS and COX-2	CGA suppress p38 and NF-κB activation pathway
Chen et al. (2018b)	Duroc × Landrace × Yorkshire weaned pigs	1000 mg/kg per day for 14 days	Decrease in serum level of TNF-α, IL-6 and IL-1β No significant difference in IL-10 production	CGA suppress NF-κB pathway
Clinical trials				
Zampelas, Panagiotakos, Pitsavos, Chrysohoou,	Cross-sectional survey using FFQ	Most of the participants consumed ≥1 cup per day	Men and women drinking ≥200 ml coffee per day have high IL-6 and TNF-α serum level	

and Stefanadis (2004)				
Lopez-Garcia, van Dam, Qi, and Hu (2006)	Cross-sectional survey using FFQ	Most of the participants consumed ≥1 cup per day	Decrease in TNF receptor in serum of healthy women	
Kempf et al. (2010)	Single blind, 3-stage clinical trial (n=47)	4 cups per day of coffee for 1st month and 8 cups per day for 2nd month (150 ml/cup)	Decrease in IL-18 serum level No change in IL-6, IL-1 and MIF levels	No quantification /analysis of CGA composition in coffee
Yamashita et al. (2012)	Cross-sectional survey	0-4 cups per day	Decrease in IL-6 and TNF-α serum level	

SEB: staphylococcal exotoxins B; IL: interleukin; TNF-α: tumour necrosis factor alpha; IFNγ: interferon γ; MCP-1: monocyte chemoattractant protein-1; MIP: macrophage inflammatory protein; LPS: lipopolysaccharide; COX-2: cyclooxygenase-2; PGE_2: prostaglandin E_2; iNOS: inducible nitric oxide synthases; NO: nitric oxide; NF-κB: nuclear transcription factor kappa-B; JNK: c-jun N-terminal kinase; AP-1: activating-protein-1; cAMP: cyclic adenosine monophosphate; DNP: dinitrophenyl; IκB: inhibitor κB; H_2O_2: hydrogen peroxide; HSV: herpes simplex virus; ROS: reactive oxygen species; Nrf2: nuclear factor 2-related factor 2; PMA: phorbol 12-myristate 13-acetate; CCl_4: carbon tetrachloride; DSS: dextran sulphate sodium; SOD: superoxide dismutase; MPO: myeloperoxidase; MTX: methotrexate; MIF: macrophage migration inhibitory factor; STZ: streptozotocin; FFQ: food-frequency questionnaire

2.5.2.2 Metabolism of chlorogenic acid

Absorption of CGA starts in the stomach and ends in the intestine. Experiments with rats showed that a small amount of CGA is absorbed in the stomach in its intact form (Lafay et al., 2006). The remaining part of CGA is hydrolysed to CA and FA in the intestine. The esterases enzyme in the mucosa of the small intestine and microbial produced esterases in the large intestine initiate the hydrolysis process (Konishi & Kobayashi, 2004). FA and CA are absorbed into the cells by diffusion. After absorption metabolites are conjugates with glucurnide and sulphate, as in the case of curcumin (Nardini, Cirillo, Natella, & Scaccini, 2002).

2.5.2.3 Safety of chlorogenic acid

An average cup of coffee made using soluble instant coffee powder (2 g per cup) contains between 50-150 mg of 5-CQA (Gil & Wianowska, 2017), a dose which has been reported

safe for human consumption (Lopez-Garcia et al., 2006; Yamashita et al., 2012; Zampelas et al., 2004). In a double-blind, placebo-controlled, randomized clinical trial conducted 14 subjects suffering from mild hypertension were given drinks containing 140 mg/day of CGA for 12 weeks and no adverse health effects were observed (Watanabe et al., 2006). In a recent study, green coffee extract (700-1050 mg containing 56.6 % of total CGA) was administered daily for 22 weeks to 16 overweight adults and no sign of toxicity was reported (Vinson, Burnham, & Nagendran, 2012).

2.5.3 Lupeol

Lupeol is also a less studied bioactive compound that has gained researchers interest in the last two decades. Lupeol is the key triterpene bioactive compound of the phytosterol family (Saleem, 2009). Triterpenes are naturally present in the human diet. Individuals from Western countries are reported to consume about 250 mg of triterpene per day whereas those from the Mediterranean countries consume 400 mg per kg of body weight per day per person (Moreau, Whitaker, & Hicks, 2002). Lupeol is present in fruits such as olives, mango, figs, red grapes, guava, melons, mulberries, dates, strawberries and plums, and vegetables such as cabbage, pepper, carrot, tomato, cucumber, bitter-root, aloe plant, black tea, pea and soybean (Duke, 2001; Patil, 2018; Saleem, 2009; Hifzur Rahman Siddique & Mohammad Saleem, 2011; Tsai, Lin, & Wu, 2016; A. Wal, Srivastava, Wal, Rai, & Sharma, 2015). Further, lupeol is also reported to be present in some medicinal plants like *Tamarindus indica, Leptadenia hastate, Celastrus paniculatus, Sebastiania adenophora, Zanthoxylum riedelianum, Crataeva nurvala, Himatanthus sucuuba, Bombax ceiba, Aegle marmelos* and Shea butter plant (Saleem, 2009; Wal et al., 2015; Wal, Wal, Sharma, & Rai, 2011).

Lupeol ($C_{30}H_{50}O$) is also known as fagarasterol, clerodol, monogynol B and farganasterol, (Tsai et al., 2016; Wal et al., 2011). The chemical structure of lupeol is shown in Figure 2.7a. It is an element involved in stabilizing plant cell membrane (Liby, Yore, & Sporn, 2007). The process of biosynthesis of this simple compound is very complex in the cytosol of plant cells. The key steps involved in the mevalonate (MVA) biosynthesis pathway of lupeol are shown in Figure 2.7b. In brief, acetyl CoA is converted into 5-carbon molecule isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). IPP and DMAPP are then catalyzed to farnesyl pyrophosphate (FPP) in the presence of farnesyl pyrophosphate synthase (FPS). FPP is then transformed into squalene and 2,3-oxidosqualene which is then cyclized into lupenyl cation in a chain-chain-chain conformation by oxidosqualene cyclase

(OSC). Finally, lupeol is formed by de-protonation of 29-methyl group in presence of lupeol synthase (LUS) (Phillips, Rasbery, Bartel, & Matsuda, 2006; Siddique & Saleem, 2011; Thimmappa, Geisler, Louveau, O'Maille, & Osbourn, 2014; Tsai et al., 2016).

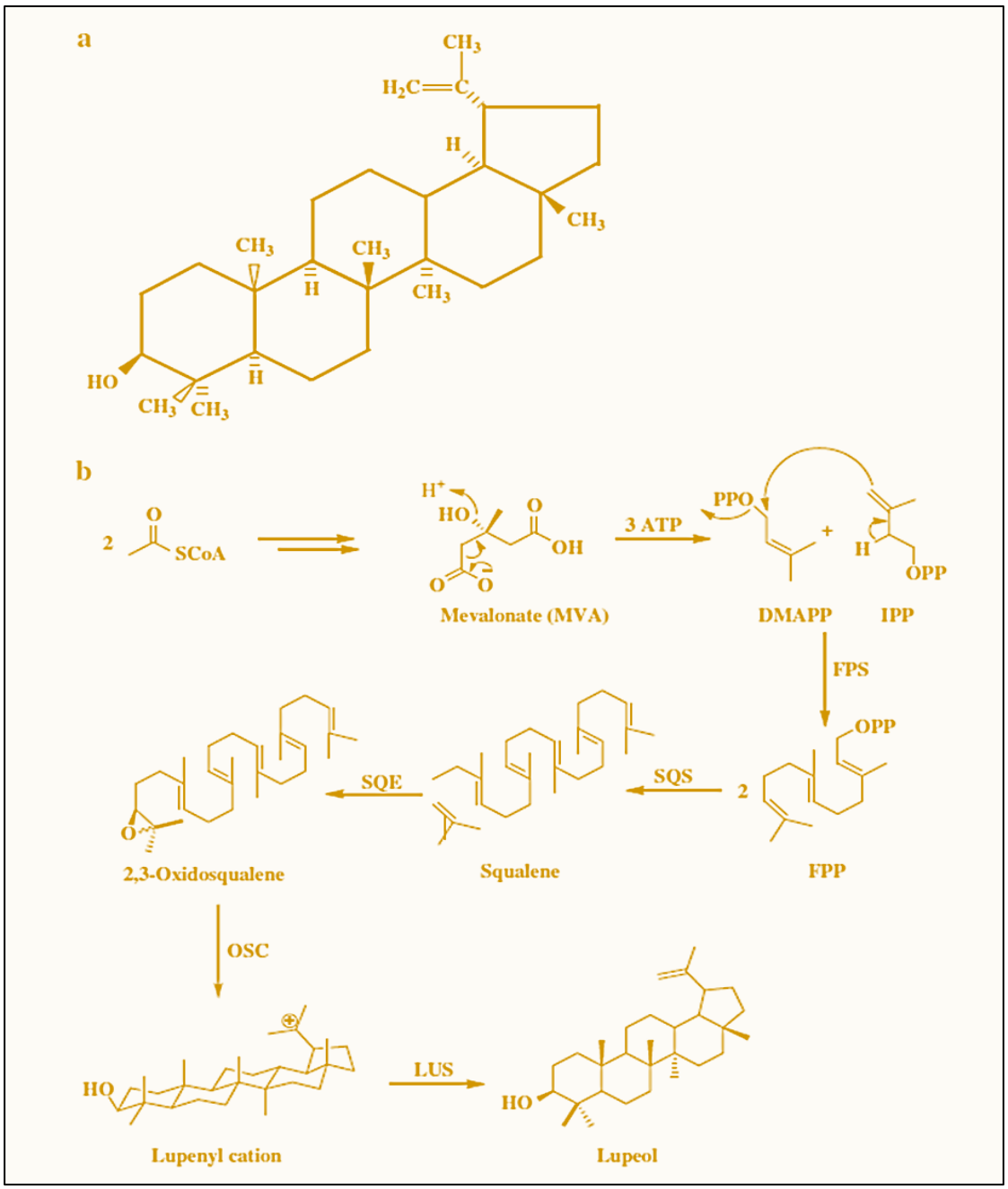


Figure 2.7: (a) Chemical structure of lupeol, and (b) key steps of mevalonate (MVA) biosynthesis pathway of lupeol in plant cells.

ATP: adenosine tri-phosphate; DMAPP: dimethylallyl pyrophosphate; IPP: isopentenyl pyrophosphate; FPS: farnesyl pyrophosphate synthase; FPP: farnesyl pyrophosphate; SQS: squalene synthase; SQE: squalene epoxidase; OSC: oxidosqualene cyclase; LUS: lupeol syhthase. Source: (Siddique & Saleem, 2011) *(Reproduced with permission)*

2.5.3.1 Lupeol and inflammation

Similar to curcumin and CGA, lupeol also targets multiple molecules and exhibits anti-inflammatory and anti-oxidant properties. It is evident from the studies (Table 2.6) carried out by administrating lupeol that it can downregulate the production of pro-inflammatory cytokines like IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, and TNF-α or upregulate production of anti-inflammatory cytokines like IL-10. It also acts as an anti-oxidant agent thereby scavenging free radical species (ROS and RNS). Furthermore, lupeol can inhibit the expression and production of COX-2, iNOS and SOD enzymes and downregulate the activation of NF-κB, Nrf2, Akt, p38, MAPK and JNK signalling pathway.

Evidence for the anti-inflammatory properties of lupeol

In vitro and animal trials demonstrating the anti-inflammatory effects of lupeol are summarised in Table 2.6. In the early studies lupeol containing plant extract was used and in some of the studies lupeol concentration was not quantified (Arciniegas, Apan, Pérez-Castorena, & de Vivar, 2004; Ashalatha et al., 2010; A. Fernández, Álvarez, García, & Sáenz, 2001; Geetha & Varalakshmi, 1999; Lambertini et al., 2005; Lima, Perazzo, Carvalho, & Bastos, 2007; Nguemfo et al., 2009; Sudhahar, Kumar, Varalakshmi, & Sujatha, 2008). Therefore the observed effect may not only be attributed to lupeol due to the presence of other compounds in the extract. To best of our knowledge, there is no published clinical trial supporting the anti-inflammatory effects of lupeol administered in human.

Evidence for in vitro studies

Effects of lupeol on inflammation mediators are studied in both animal and human cell lines. Administration of lupeol with LPS infected human intestinal epithelial cells (IECs) COLO 205 and murine macrophages RAW 264.7 cells downregulated IL-6, IL-8, IL-12 and TNF-α secretion by blocking NF-κB signaling pathway *via* suppressing IκBα phosphorylation (Lee et al., 2016). A similar decrease in pro-inflammatory cytokines was observed on treating CD14+ (monocytes) cells with lupeol (Zhu et al., 2016). The authors further reported that lupeol can shift the differentiation of CD14+ monocytes from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages. On the contrary, lupeol was not effective in reducing IL-6 expression in human umbilical vein endothelial cells (HUVECs) and KKU-M213 cholangiocarcinoma (CCA) cells (Kangsamaksin et al., 2017). Lupeol also

Table 2.6: Pre-clinical and clinical studies showing the anti-inflammatory effects of lupeol.

Researcher(s) ***(reference)***	**Experiment Design**	**Dose and Duration**	**Key Observations**	**Mechanism Purposed/ Comment** ***(if any)***
		In vitro studies		
Kumari and Kakkar (2012b)	Acetaminophen (675 μM) induced hepatotoxicity in rat hepatocytes	10 μM for 1.5 h	Decrease in ROS and increase in activation of SOD enzyme Upregulate Bcl-2 and downregulate Bax	
Chairez-Ramirez et al. (2015)	TNF-α (10 ng/mL) treated Caco-2 (human epithelial cells) cells	5, 10, and 20 mM for 24 h	Decrease in NF-κB in cytosol and nucleus of the cells	Lupeol inhibits NF-κB signaling pathway
Lee et al. (2016)	LPS (10 μg/ml) treated human intestinal epithelial cells (IECs) COLO 205 and the RAW 264.7 (murine macrophages) cell lines	10, 50 and 100 μM/ml for 24 h	Decrease in IL-6, IL-8, IL-12 and TNF-α	Lupeol blocks the NF-κB signaling pathway by blocking IκBα phosphorylation
Srivastava et al. (2016)	Mancozeb (5 μg/ml) induced genotoxicity and apoptosis in cultured human lymphocytes	25 and 50 μg/ml for 24 h	Increase in SOD and catalase enzyme and decreased ROS generation Upregulate Bcl-2 and downregulate Bax and COX-2	Lupeol attenuated NF-κB activation
Zhu et al. (2016)	LPS (1 ng/ml) and IFN-γ (20 ng/ml) treated $CD14^+$ (monocytes) cells	0.01-10 μM for 24 h	Decrease in IL-12, IL-6, IL-1β and TNF-α Increase in IL-10	Shift in pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages
Kangsamaksin et al. (2017)	Human umbilical vein endothelial cells (HUVECs) and KKU-M213 cholangiocarcinoma (CCA) cell lines	1 and 5 μM for 48 h	Reduce mRNA level of TNF-α No effect on IL-6 level	
Guo et al. (2018)	High glucose (100 mM) induced apoptosis in rabbit nucleus pulposus cells	30-70 μg/mL for 24 h	Decrease in level of ROS, Bax and cytochrome C Upregulate Bcl-2 expression	

Pereira Beserra et al. (2018)	Human primary epidermal keratinocytes and dermal fibroblasts cell lines	0.1-20 μg/mL for 24 h	Increase in MMP-2 level Activation of Akt and p38 signaling proteins	Lupeol inhibits NF-κB signaling pathway
		Animal trials		
Saleem, Afaq, Adhami, and Mukhtar (2004)	TPA (3.2 nmol/ 0.4 ml acetone/animal) promoted skin tumor in female CD-1 mice	1 or 2 mg/ 0.2 ml acetone for 6-48 h	Decrease in COX-2 and NOS enzyme Downregulate activation of PI3K, Akt signaling and degradation and phosphorylation of IκBα	Lupeol inhibits NF-κB signaling pathway
Vasconcelos et al. (2008)	Chicken egg ovalbumin (10 μg) induced inflammation in male BALB/c mice	60 mg/kg per day for 5 days	Downregulate IL-4, IL-5 and IL-13	
Ahmad, Pandey, Kour, and Bani (2010)	Saline containing 1 % carrageenan (0.1 mL) administrated in male Swiss albino mice	25-200 mg/kg for 24 h	Downregulate IL-2, TNF-α and IFNγ No significant effect on IL-4 and IL-5	
Lucetti, Lucetti, Bandeira, Veras, Silva, Leal, Lopes, Alves, Silva, and Brito (2010)	Carrageenan (50 μL of 1 %) induced paw edema in male Swiss mice	10-50 mg/kg for 5 h	Decrease in iNOS and TNF-α expression	
Kumari and Kakkar (2012a)	Acetaminophen (1 g/kg) induced hepatotoxicity in Wistar rats	150 mg/kg per day for 30 days	Decrease in ROS and activation of SOD enzyme Upregulate Bcl-2 and downregulate Bax	
Kim et al. (2015)	Cerulean (50 μg/kg) induced acute C57BL/6 mice	10-50 mg/kg for 7 h	Inhibit IL-1β, IL-6 and TNF-α expression and secretion Decrease in MPO activity	
Prabhu, Balakrishnan, and Sundaresan (2016)	BBN (150 mg) induced bladder carcinogenesis in male albino Wistar rats	50 mg/kg per day for 28 weeks	Inhibit COX-2 enzyme	Lupeol suppress Nrf2-NF-κB signaling pathway
Badshah et al. (2016)	LPS (250 μg/kg) induced neuro-	50 mg/kg per day for	Downregulate TNF-α, iNOS and IL-1β	Lupeol inhibits p38-MAPK and JNK

	inflammation in the cortex and hippocampus of adult C657LB male mice	7 days		signaling pathways
Singh, Arora, and Shukla (2017)	Benzo[a]pyrene (5 mg/animal) treated male Swiss albino mice	500 mM/ 0.2 ml acetone twice or thrice a week for 32 weeks	Decrease in ROS	
Kasinathan et al. (2018)	DSS (2 % w/v) induced colitis in male Swiss albino mice	1 mg/25 g of animal for 14 days	Downregulate TNF-α, IL-1 and IL-2	Lupeol inhibits NF-κB signaling pathway

ROS: reactive oxygen species; SOD: superoxide dismutase; TNF-α: tumour necrosis factor alpha; NF-κB: nuclear transcription factor kappa-B; LPS: lipopolysaccharide; IL: interleukin; IκB: inhibitor κB; COX-2: cyclooxygenase-2; IFNγ: interferon γ; mRNA: messenger ribonucleic acid; MMP: matrix metalloproteinase; TPA: 12-Otetradecanoyl-phorbol-13-acetate; iNOS: inducible nitric oxide synthases; PI3K: phosphatidyl inositol 3-kinase; MPO: myeloperoxidase; BBN: N-Butyl-N-(4-hydroxybutyl) nitrosamine; Nrf2: nuclear factor 2-related factor; MAPK: mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; DSS: dextran sodium sulphate

reduced ROS and COX-2 and increased the activity of SOD enzyme in mancozeb infected human lymphocytes by attenuating NF- κB activation (Srivastava, Mishra, Ali, & Shukla, 2016).

Evidence for animal studies

Lupeol was first reported by Geetha and Varalakshmi (1999) to be effective in reducing arthritis inflammation in a mouse model by generating inflammatory factors and modulating the immune system. Fernández, de las Heras, Garcia, Sáenz, and Villar (2001) applied 0.5 and 1 mg of lupeol on each ear in 12-o-tetradecanoyl-phorbol acetate (TPA) induced inflammation in an ear mouse model and reported a decrease in MPO activity thereby reducing cell infiltration at the inflammation site. Treatment of dextran sodium sulphate (DSS) infected male Swiss albino mice with 1 mg of lupeol per 25 g of body weight for 14 days downregulated TNF-α, IL-1 and IL-2 *via* supressing of NF-κB activation pathway (Kasinathan et al., 2018). However, lupeol (25-200 mg/kg) was not effective in reducing IL-4 and IL-5 levels in carrageenan stimulated male Swiss albino mice (Ahmad et al., 2010). Lupeol can also inhibit several enzymes like COX-2 and iNOS. Administration of 50 mg/kg/day of lupeol for 7 days downregulated iNOS production in LPS induced neuro-

inflammation in the cortex and hippocampus of adult C657LB male mice by inhibiting p38-MAPK and JNK signalling pathways (Badshah et al., 2016). Lupeol (50 mg/kg/day for 28 weeks) could also inhibit Nrf2-NF-κB activation pathway thereby downregulating COX-2 enzyme in N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN, 150 mg) induced bladder carcinogenesis in Wistar rats (Prabhu et al., 2016).

2.5.3.2 Safety of lupeol

The safety of lupeol has been studied in many animal models including rats, mice and dogs. Lupeol administered at doses ranging from 25-2000 mg/kg body weight for a period of 1 day to 180 days did not result in any signs of mortality or systematic toxicity (Al Rehaily, El Tahir, Mossa, & Rafatullah, 2001; Bani et al., 2006; Hata et al., 2010; Preetha, Kanniappan, Selvakumar, Nagaraj, & Varalakshmi, 2006; Saleem et al., 2004; Sunitha, Nagaraj, & Varalakshmi, 2001; Vidya, Lenin, & Varalakshmi, 2002; You, Nam, Kim, Bae, & Ahn, 2003; Yokoe et al., 2015).

2.6 Delivery of bioactive compounds *via* food matrices

Traditionally, bioactive compounds were available in the human diet from their native food matrices. However, were only limited to their source of production. Hippocrates, the father of Western medicine stated that *"Let food be thy medicine and medicine be thy food"*. In today's world, with the discovery of enormous therapeutic benefits associated with consumption of the bioactive compounds has increased the acceptance of Hippocrates's belief which has resulted in the global availability of these compounds (Chen, McClements, & Decker, 2013). In 2012, turmeric and/or curcumin supplements were amongst the top 10 bestselling supplements in the USA, accounting for $100 million sales which further increased to about $200 million by 2015 (Cassandra, 2016; Kumar, 2016). However, bioactive compounds are now more available as dietary supplements (i.e. in the form of capsules and powders) and are less consumed in their native food matrix. One reason for this shift in the way of consumption may be the changing food habits of people.

Bioactive compounds such as curcumin, CGA and lupeol are poorly soluble in water and hence have low bioavailability following consumption. Therefore, the dose absorbed may not be sufficient in providing the desired health benefits. In recent studies, the effect of different types of food matrices on the bioavailability of bioactive compounds is being analysed.

Carotenoids are lipophilic compounds, when delivered through milk and fruit juice mixed drink were more bioaccessible in an *in vitro* model on comparison with fruit juice mixed in water or soymilk (Rodríguez-Roque et al., 2016). The influence of the food vehicle on bioaccessibility of vitamin C, polyphenols and carotenoids has been also reported by Rodríguez-Roque et al. (2015), Rodríguez-Roque, Rojas-Graü, Elez-Martínez, and Martín-Belloso (2014a), and Rodríguez-Roque, Rojas-Graü, Elez-Martínez, and Martín-Belloso (2014b). In another study by Zou, Liu, Liu, Xiao, and McClements (2015), curcumin powder delivered by mixing in an oil-in-water emulsion was reported to increase in bioaccessibility in a simulated gastrointestinal tract model.

Milk and milk products can be an effective vehicle for delivery of hydrophobic bioactive compounds as the lipid constituents of the milk may stabilise bioactive compounds and enhance absorption and bioavailability of functional ingredients (Jakobek, 2015). Duarte and Farah (2011) studied the effect of milk on the bioavailability of CGA from coffee by testing the urine sample of 5 subjects for 24 h. The authors reported that subjects consuming coffee in milk had less CGA in urine than subjects consuming coffee in water.

Yogurt is a fermented milk product, highly popular across the globe and a good carrier for beneficial probiotics and prebiotics, could be used for the delivery of bioactive compounds (Allgeyer, Miller, & Lee, 2010; Ejtahed et al., 2011; Weerathilake, Rasika, Ruwanmali, & Munasinghe, 2014). During the fermentation process, the pH of milk is reduced to 4.5-4.6 by the action of starter microorganisms on milk sugar (lactose) to produce lactic acid (Aguirre-Ezkauriatza et al., 2008; De Brabandere & De Baerdemaeker, 1999; Fazilah et al., 2018). The acidic environment in yogurt enhances the stability of compounds (such as curcumin, CGA and lupeol) making them more bioavailable for absorption in the human body. A study by Fu et al. (2016) showed that curcuminoids (0.3 % w/w) delivered through buttermilk yogurt were 15 times more bioaccessible than curcuminoids in aqueous dispersion. Furthermore, curcuminoids also increased the viscosity of yogurt during storage and reduced syneresis hence positively influencing the sensory attributes. Thus, yogurt is a promising potential vehicle for delivery of bioactive compounds as it is commercially feasible, acceptable to consumers and improves the functionality of ingredients.

CHAPTER 3

Research Methodology

3.1 Introduction

The study aimed at developing a coconut yogurt as a delivery vehicle for two characterised anti-inflammatory bioactive compounds. This chapter describes the experimental design used to achieve the desired aim. The research methodology was divided into two parts. In part 1, the effect of bioactive compounds on inflammatory biomarkers was studied using an *in vitro* model. In part 2, coconut yogurt fortified with bioactive compounds was developed and its physiochemical, microbiological and sensory stability were analysed over 15 days during storage at 4±1ºC. All the chemicals used in this study were of reagent grade or high and were used without any further purification. The ingredients used for the preparation of yogurt were food grade.

3.2 Part 1: *In vitro* studies

Human THP-1 cells were used to investigate the effect of bioactive compounds on reducing the production of TNF-α. THP-1 cells were differentiated using PMA for 72 h followed by incubation with the bioactive compounds. Cells were pre-treated with 1-25 μM of curcumin, lupeol and CGA for 1 h and inflammation was induced by adding LPS to the medium containing the bioactive compound. Cells were also treated with several combinations of curcumin + lupeol and, curcumin + CGA. The level of TNF-α was measured using ELISA and cell viability was measured using MTT assay. Lastly, the effect of the most promising combination of bioactive compounds on the mRNA expression of various pro and anti-inflammatory biomarkers was studied using qRT-PCR.

3.2.1 Cell line

THP-1 cells (ATCC®; American Tissue Culture Collection, TIB-202, isolated from the peripheral blood of a 1-year-old human male infant with acute monocytic leukaemia) with passage number 4 were provided by Dr Fran Wolber, Massey University, Palmerston North, New Zealand (NZ).

3.2.2 Medium and solutions

3.2.2.1 Cell work

Complete RPMI cell culture medium

Complete RPMI 1640 cell culture medium was prepared by aseptically supplementing Gibco™ RPMI 1640 (GlutaMAX™ supplement, HEPES, catalogue number 72400120, ThermoFisher Scientific™, NZ) with 10 % Fetal Bovine Serum (FBS, catalogue number MG-FBS0820, MediRay, NZ) and 1 % antibiotic solution of Penicillin and Streptomycin (catalogue number 15070063, ThermoFisher Scientific™, NZ). The prepared medium was stored at 4ºC (Small, Lansdown, Al-Baghdadi, Quach, & Ferrante, 2018).

Phorbol 12-myristate 13-acetate (PMA) stock solution

A stock solution of 1 mM was prepared by dissolving 1 mg of PMA (catalogue number P1585, Sigma-Aldrich®, NZ) in 1.62 ml of dimethyl sulfoxide (DMSO, catalogue number D4540, Sigma-Aldrich®, NZ) and stored at -20ºC until required for use. The working solution of 400 nM was prepared by diluting 20 µl of stock solution in 50 ml of complete RPMI medium. The working solution was syringe-filtered (0.2 µm, catalogue number 4906, Acrodisc®) before use.

Lipopolysaccharide (LPS) stock solution

The LPS stock solution (0.1 mg/ml) was prepared by diluting 1 part of LPS (1 mg/ml, from Escherichia coli O111:B4, catalogue number L5293, Sigma-Aldrich®, NZ) in 9 parts of complete RPMI medium and stored at 4ºC until required for use.

3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) stock solution

MTT stock solution was prepared by dissolving 5 mg of the dye (catalogue number M5655, Sigma-Aldrich®, NZ) in 1 ml of phenol free Gibco™ RPMI 1640. The solution was syringe filtered (0.2 µm) and stored at 4ºC until used. The working solution was prepared by ten-fold dilution of MTT stock solution in phenol free Gibco™ RPMI 1640.

Acidified isopropanol

To prepare 200 ml of acidified isopropanol, 785 µl of 32 % HCl (catalogue number 1789, Unilab, NZ) was mixed in 199 ml of absolute isopropanol (≥95.0%, catalogue number 1274, Unilab, NZ) and stored at room temperature.

3.2.2.2 Preparation of bioactive compounds

Curcumin stock solution

A 20 mM stock solution was prepared by dissolving 10 mg of curcumin (catalogue number 08511, Sigma-Aldrich®, NZ) in 1.36 ml of DMSO and stored at -80ºC until required for use.

Chlorogenic acid (CGA) stock solution

A 50 mM stock solution was prepared by dissolving 25 mg of CGA (catalogue number 500590, Sigma-Aldrich®, NZ) in 1.41 ml of DMSO and stored at -80ºC until required for use.

Lupeol stock solution

To prepare 10 mM stock solution, 10 mg of lupeol (catalogue number 18692, Sigma-Aldrich®, NZ) was dissolved in 1.172 ml of DMSO and 1.172 ml of ethanol (96 %). The stock solution was stored as 100 µl aliquote at -80ºC until required for use.

3.2.2.3 Enzyme-linked immunosorbent assay (ELISA)

All the buffers, reagents and standard for ELISA were prepared according to the manufacturer's instructions (TNF-α ELISA MAX™ Deluxe Set Kit BioLegend®, USA).

Phosphate buffer saline (PBS)

PBS was prepared by dissolving 8 g sodium chloride (NaCl), 0.2 g potassium chloride (KCl), 2.68 g disodium hydrogen phosphate (Na_2HPO_4) and 0.24 g potassium dihydrogen phosphate (KH_2PO_4) in 800 ml milli-Q water and the pH was adjusted to 7.4 using 0.1 M HCl. After adjusting the pH to 7.4, the final volume was made up to 1 L.

Wash Buffer

Wash buffer or Tween PBS (0.1 % v/v) was prepared by diluting 1 ml of Tween 20 (catalogue number AJA2510, ThermoFisher Scientific™, NZ) in 1 L of PBS buffer.

Stop Buffer

Stop buffer (2 N H_2SO_4) was prepared by mixing 5.5 ml of 98 % H_2SO_4 (Unilab, NZ) in 94.5 ml of milli-Q water.

Coating buffer (1x)

1x coating buffer was prepared by diluting 2 ml of 5x stock in 8 ml of milli-Q water.

Capture antibody solution

1x capture antibody solution was prepared by mixing 50 µl of 200x stock solution in 9.950 ml of 1x coating buffer.

Assay diluent A

1x assay diluent A was prepared by mixing 12 ml of 5x assay buffer in 48 ml of milli-Q water.

Detection antibody solution

1x detection antibody solution was prepared by mixing 50 µl of 200x stock solution in 9.950 ml of 1x assay diluent A.

Avidin-horseradish peroxidase (Avidin-HRP) solution

Avidin-HRP solution was prepared by diluting 10 µl of 1000x stock solution in 9.990 ml of 1x assay diluent A.

Reconstitution of standards for ELISA

TNF-α standard (50 ng/ml) was prepared by reconstituting 10 ng of lyophilized Human TNF-α in 0.2 ml of 1x assay diluent A. 10 µl of this stock was diluted in 990 µl of 1x assay diluent A to obtain the highest concentration of 500 pg/ml. Using 500 pg/ml standard, six two-fold dilutions were prepared in 1x assay diluent A to obtain a series of standards ranging from 500-7.8 pg/ml. 1x assay diluent A served as the blank.

3.2.2.4 Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

All the buffers and reagents used in qRT-PCR were supplied in the kits provided by the manufacturer. The necessary dilutions were prepared in Diethyl-pyrocarbonate (DEPC) treated water.

DEPC treated water

DEPC water (0.1 %, v/v) was prepared by dissolving 1 ml of DEPC (catalogue number D5758, Sigma-Aldrich®, NZ) in 1 L of milli-Q water. The solution was left at room temperature for 2 h followed by 15 min autoclaving to remove the traces of DEPC.

Primer mix

Primers were reconstituted in mili-Q water, as per the manufacturer's instructions, to make a stock of 200 µM. Primer mix was prepared by further diluting the stock, such that a concentration of 10 µM for forward and reverse primer was achieved.

3.2.3 Methods for *in vitro* studies

3.2.3.1 Culturing of THP-1 cells

On receipt of the Falcon tube containing THP-1 cells, it was centrifuged (Megafuge 1.0R, ThermoFisher Scientific™, NZ) at 136 relative centrifugal force (rcf) for 3 min at 25ºC. The supernatant was discarded and the cells were re-suspended in 20 ml of fresh complete RPMI medium. The cells were then transferred into a T75 flask and incubated at 37ºC under 5 % carbon dioxide (CO_2) in a humidified incubator (Mitre 4000 series, Contherm™, NZ). The growth of cells was regularly monitored by viewing under an inverted phase-contrast microscope (CKX31, Olympus™, Japan). The cells were sub-cultured every 2-3 days with the dilution factor depending on the concentration of the cells (Wang et al., 2013).

3.2.3.2 Differentiation of THP-1 Cells

Before initiating the differentiation, cell viability was determined using the trypan blue exclusion method. Equal volumes of cell containing medium and trypan blue (0.4 %, catalogue number 93595, Sigma-Aldrich®, NZ) were mixed in an Eppendorf tube and left at room temperature for approximately 2-3 minutes. Trypan blue can penetrate in the dead cells and are thus stained blue while viable cells remain unstained (Louis & Siegel, 2011; Strober, 1997). About 10-15 µl of the mixture was loaded on the haemocytometer and analysed under an inverted microscope. A detailed protocol for counting the cells is given in Appendix 2. The concentration of cells in the medium was calculated using equation (1).

$$Viable\ cells/ml = Average\ number\ of\ cells \times Dilution\ factor \times 10^4 \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

THP-1 monocyte cells at a density of 0.25×10^6 cells/ml were seeded in a 6-well plate (2 ml/well) and differentiation was induced by adding 2 ml of PMA solution such that the final concentration of 5-200 nM PMA was achieved in each well. Plates were left undisturbed for 3 days at 37ºC under 5 % CO_2 in a humidified incubator (Daigneault, Preston, Marriott, Whyte, & Dockrell, 2010; Parthiban, Jaiganesh, Aruni, Meignanalakshmi, & Ramadass, 2007; Small et al., 2018). After incubation, THP-1 cells stick to the cell surface and the

medium containing PMA was discarded, followed by washing the cells with fresh complete RPMI medium. Prior to further treatment, cells were allowed to rest in 2 ml of complete RPMI medium for about 1 h.

3.2.3.3 Treatment of THP-1 cells with bioactive compounds and LPS

The differentiated THP-1 cells were treated with curcumin, lupeol and CGA (individually and combined) at a concentration ranging from 1-25 µM. The stock solutions of bioactive compounds were diluted in complete RPMI medium to achieve the different concentrations. The cells were also treated with varying concentrations of solvents used for dissolving the bioactive compounds i.e. DMSO (0.001-0.2 %) and DMSO + ethanol (0.01-0.2 % each). The concentration range of solvents corresponds to their amount in diluted solutions of bioactive compounds. The cells that were rested in the incubator following differentiation were removed after 1 h and the medium was discarded. 2 ml of complete RPMI medium containing an appropriate amount of the bioactive compounds (individually and combined) or solvent was added to each well and incubated for 1 h at 37ºC under 5 % CO_2. After incubation, cells were treated with LPS (5-200 ng/ml). LPS was added to the wells containing cells with bioactive compounds in complete RPMI medium and the plate was further incubated for 0.5-6 h at 37ºC under 5 % CO_2. At the end of the incubation period, the supernatant was collected and stored at -80°C until required for TNF-α quantification by ELISA and the cells were immediately utilised to assess the cytotoxicity *via* MTT assay or to perform qRT-PCR.

3.2.3.4 MTT assay

The cells after treatment with bioactive compounds and LPS were washed with 2 ml of phenol red free RPMI medium. After washing, 2 ml of MTT working solution (0.5 mg/ml) was added to the cells in each well of the 6-well plate and incubated at 37ºC under 5 % CO_2 for 2 h. The cells treated with LPS alone (i.e. without bioactive compounds) served as the negative control and the well without cells as the blank. Following incubation, the MTT dye was removed and the violet crystals formed by the reduction of MTT to formazan in the cells (Popovich & Kitts, 2002; van Meerloo, Kaspers, & Cloos, 2011) were solubilized by adding 1 ml acidified isopropanol. The plate was wrapped in aluminium foil and placed on an orbital shaker (Bellco Glass Inc., New Jersey, USA) for 20 min to completely solubilize the formazan. The content from each well was collected in a 1.5 ml Eppendorf tube and centrifuged (Centrifuge 5425 Eppendorf™, MediRay, NZ) at 17500 rcf for 2 min. 200 µl

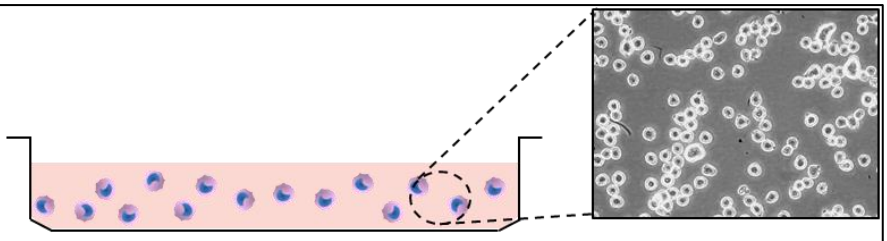
supernatant was transferred into the wells of a 96-well plate and the absorbance was measured at 595 nm using a micro-plate reader (FLUOstar® Optima, BMG Labtech™, Germany). Cell viability was calculated using equation (2).

$$\%\ cell\ viability = \frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{Absorbance\ of\ control - Absorbance\ of\ blank}\ \text{x}\ 100 \quad \text{.....(2)}$$

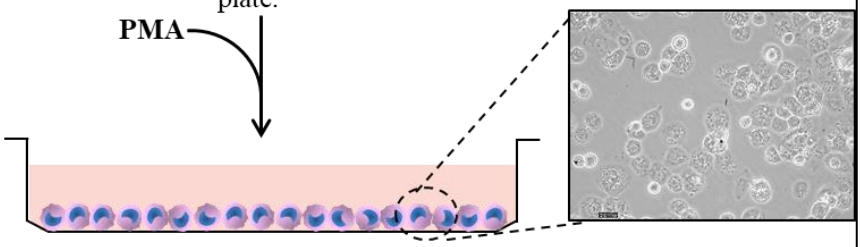
A summary of the protocol for the treatment of THP-1 cells and cytotoxicity analysis are shown in Figure 3.1.

3.2.3.5 Quantification of TNF-α using ELISA

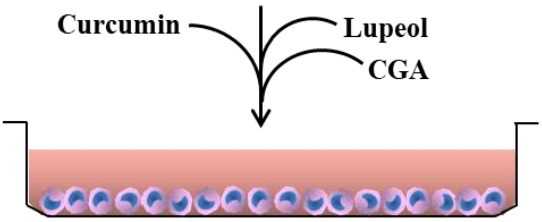
The amount of TNF-α produced by the THP-1 cells in response to LPS was quantified using a Human TNF-α ELISA MAX™ Deluxe Set Kit (BioLegend®, USA). All the reagents were stored at 4ºC and were allowed to equilibrate to room temperature before use. The assay was performed according to the manufacturer's instructions. In brief, TNF-α specific capture antibody was coated on the surface of a 96-well plate. Appropriate dilutions of standards and samples were added to the wells and TNF-α binds to its specific capture antibody. After washing, anti-human-TNF-α detection antibody was added to produce an antibody-antigen-antibody sandwich. Avidin-HRP was added to the sandwich followed by the addition of TMB substrate solution that produced a blue colour depending on the concentration of TNF-α. To stop the enzymatic conversion of TMB substrate solution, stop solution was added producing a yellow colour. The absorbance was recorded at 450 nm using a micro-plate reader. The protocol used is summarised in Figure 3.2. Wells containing assay diluent only were treated as the blank and their absorbance was subtracted from the absorbance of samples and standards to minimise the background. A standard curve was generated using known concentrations of human TNF-α standards and was used for quantifying the amount of TNF-α in samples.



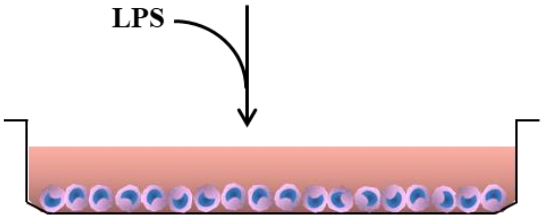
2 ml of complete RPMI medium containing 0.25X10^6 THP-1 cells/ml was transferred into 6-well plate.



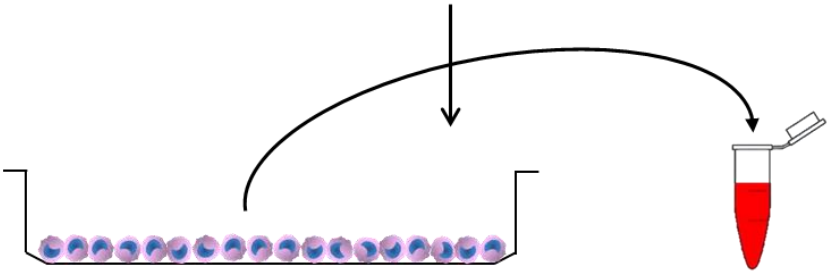
Cells were differentiated using PMA (5-200 nM) and incubated at 37°C for 3 days.



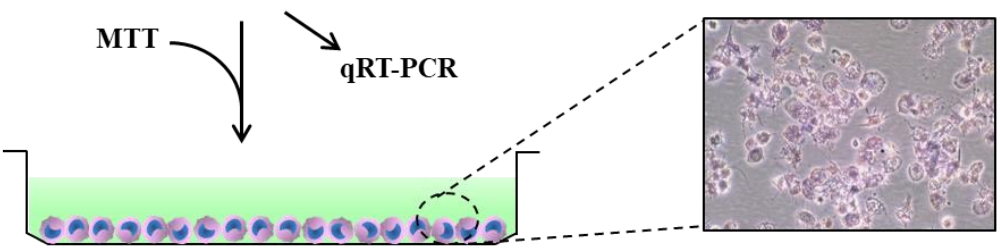
Cells were washed and incubated with 1-25 µM concentrations of bioactive compounds for 1 h at 37°C under 5 % CO_2.



LPS (5-200 ng/ml) was added and further incubated for 0.5-6 h at 37°C.



The supernatant was collected and stored at -80°C for TNF-α ELISA.



1 ml of 0.5 mg/ml MTT was added to each well and incubated for 2 h at 37°C.

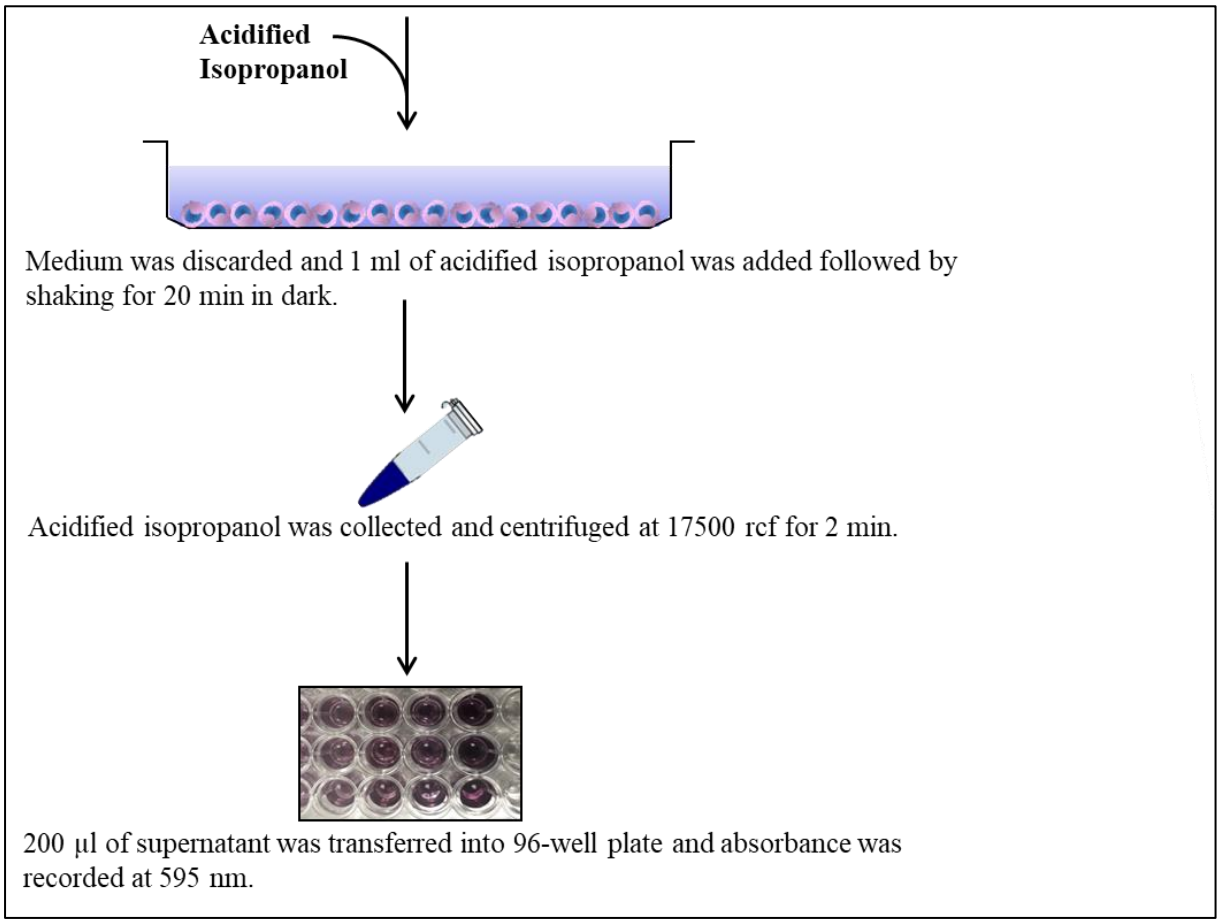
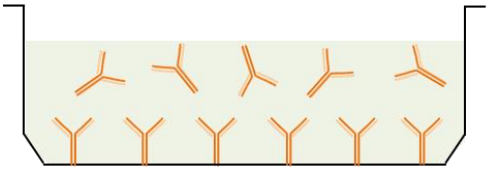


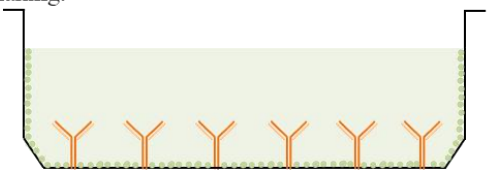
Figure 3.1: Treatment of THP-1 cells with bioactives and LPS, and cytotoxicity analysis using MTT assay.

THP-1 cells were differentiated with phorbol 12-myristate 13-acetate (PMA), washed and treated with bioactive compounds and lipopolysaccharide (LPS). The supernatant containing tumour necrosis factor-α (TNF-α) was collected and stored at -80°C and the cells were used for 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay or quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

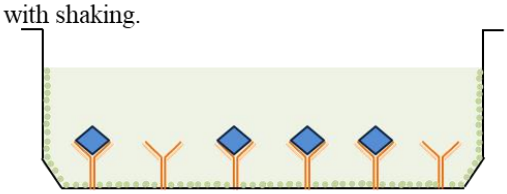
1. 96-well plate was coated with 100 µl of diluted capture antibody and incubated at 2-8°C overnight.



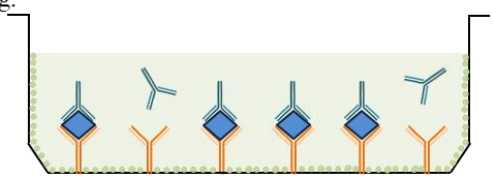
2. The plate was washed for 4 times (4x5 sec soak) with 300 µl washing buffer. The non-specific binding sites were blocked by adding 200 µl 1 X Assay Diluent A and incubated for 1 h at room temperature (RT) with shaking.



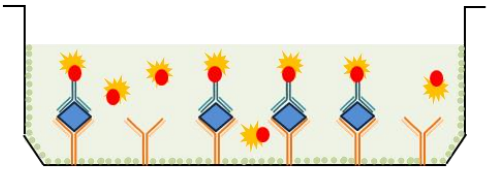
3. The plate was washed 4 times and 100 µl diluted standard/sample was added. Plate was then incubated for 2 h at RT with shaking.



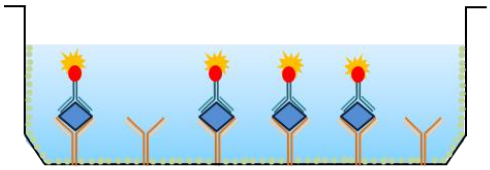
4. The plate was washed 4 times and 100 µl diluted detection antibody was added and incubated for 1 h at RT with shaking.



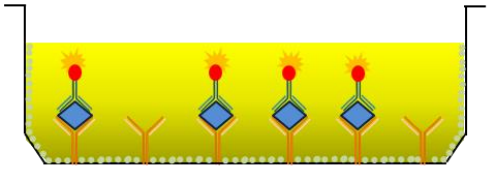
5. The plate was washed 4 times and 100 µl diluted Avidin-HRP was added and incubated for 30 min at RT with shaking.



6. The plate was washed 5 times (5x45 sec soak) and 100 µl freshly mixed TMB substrate solution was added and incubated in dark for 15 min at RT.



7. 100 µl of stop solution was added to each well.



8. Absorbance was measured at 450 nm within 15 min.

Figure 3.2: Summary of protocol used for the detection of TNF-α using sandwiched ELISA.

3.2.3.6 Relative gene expression using two-step qRT-PCR

(a) Extraction and purification of total RNA

The cells after treatment with bioactive compounds and LPS were washed twice with sterile PBS (2 ml) and then utilised for RNA extraction using ISOLATE II RNA Mini Kit (catalogue number BIO-52073, Bioline, NZ). The assay was performed according to the manufacturer's instructions. In brief, the cells were quickly lysed in buffer supplemented with 20 mM dithiothreitol (DTT) followed by filtration of the lysate. After filtration, ethanol was added to the sample. The RNA sample was then bound to a silica membrane by processing it through a silica-containing spin column. The column was loaded with DNase I to digest any genomic DNA contamination. Any other contamination such as salts, cell components and metabolites were removed simply by washing the silica membrane containing RNA with two different buffers. The purified RNA was then eluted in RNase-free water and was stored at -80ºC until required for further analysis.

(b) Complementary DNA (cDNA) synthesis from RNA

The RNA was used to synthesise the cDNA strand using a High-Capacity cDNA Reverse Transcription Kit (catalogue number 4368813, ThermoFisher Scientific™, NZ). 2x reverse transcription (RT) master mix was prepared by mixing (per reaction): 2 µL 10X RT buffer + 0.8 µl 25X deoxyribonucleotide triphosphate (dNTP) + 2 µl 10x RT random primers + 1 µl reverse transcriptase + 4.2 µl nuclease free water. In a PCR tube, 10 µl of 2x RT master mixes was pipetted, to which an equal volume of RNA sample was added to give a final RNA concentration of 0.5 µg. The PCR tubes were then loaded into the thermal cycle under the following conditions: 25ºC for 10 min, 37ºC for 120 min, 85ºC for 5 min and 4ºC to infinity. The cDNA was stored at -20ºC until required for PCR analysis.

(c) qRT-PCR

The experimental design for qRT-PCR was created using StepOne™ software v2.3, where information about the target and sample gene, replicate number, reference sample, endogenous control, reaction size and the position of each sample in the well were defined (Appendix 3). After designing the experiment, the qRT-PCR reaction was setup by mixing 1 µl cDNA template, 1µl of 10µM forward and reverse primer mix, 5 µl 2x SYBR® green PCR master mix (PowerUp™, catalogue number A25742, ThermoFisher Scientific™, NZ) and 3 µl water in a 48-well PCR-plate. The master mix consisted of the buffer, dNTPs, SYBR-green fluorescent dye which binds to PCR product and ROX reference dye to normalise the

fluorescent signal. The primers used to target specific gene are given in Table 3.1. All the primers were supplied by Integrated DNA technologist (IDT, NZ). The PCR-plate was loaded into the StepOne Real-Time PCR System (ThermoFisher Scientific™, Singapore) for 40 cycles under the following conditions: denaturing temperature of 95ºC for 5 sec and combined annealing and extension temperature of 60ºC for 30 sec. Each sample was also subjected to melt curve analysis at temperature between 60-95ºC. The relative mRNA expression was expressed as fold change relative to the control and was calculated as ΔΔCt:

$$\Delta\Delta Ct = 2^{-(\Delta Ct_{\beta\text{-}Actin} - \Delta Ct_{target\ gene})} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, Ct is the cycle number at which the fluorescence signal of the reaction crosses the threshold. cDNA from LPS alone treated cells was the control and β-Actin gene was considered as the endogenous control.

Table 3.1: Primer sequences used for qRT-PCR.

Gene	Accession Number	Primer Sequence (5'→3')	Size Amplified (BP)
IL-6	NM_001318095.1	F: TGGCAGAAAACAACCTGAACC R: TTTCACCAGGCAAGTCTCCTCAT	89
IL-10	NM_000572.2	F: GTGATGCCCCAAGCTGAGA R: CACGGCCTTGCTCTTGTTTT	138
TNF-α	NM_000594.2	F: CTGCTGCACTTTGGAGTGAT R: AGATGATCTGACTGCCTGGG	93
iNOS	NM_000625.3	F: CATCCTCTTTGCGACAGAGAC R: GCAGCTCAGCCTGTACTTATC	118
COX-2	NM_000963.3	F: TCCCTTGGGTGTCAAAGGTAAAA R: AACTGATGCGTGAAGTGCTG	144
TLR-4	NM_003266.3	F: GGTCAGACGGTGATAGCGAG R: TTTAGGGCCAAGTCTCCACG	180
IκB-α	NM_020529.2	F: AAGTGATCCGCCAGGTGAAG R: CGTGTGGCCATTGTAGTTGG	281
IκB-β kinase	NM_001556.3	F: TCCGATGGCACAATCAGGAAA R: GCAGACCACAGCAGTTCTCA	264
NF-κB	NM_003998.2	F: TGAGTCCTGCTCCTTCCA R: GCTTCGGTGTAGCCCATT	103
β-Actin	NM_001101.4	F: CACTCTTCCAGCCTTCCTTC R: GTACAGGTCTTTGCGGATGT	104

3.3 Part 2: Preparation of coconut cream yogurt with added bioactive compounds

This part of the study aimed to develop coconut cream yogurt supplemented with coffee and turmeric to deliver CGA and curcumin, respectively, to the consumers. The development of functional coconut yogurt was divided into two stages. In stage 1, the optimum concentrations of CGA and curcumin to be added in coconut yogurt were determined. Different doses of coffee and turmeric were added to the coconut cream to give a ratio of 1:1 (CGA : curcumin) and their effects on sensory attributes and fermentation process were studied. In stage 2, the stability of coconut yogurt enriched with CGA and curcumin was studied for 15 days of storage at 4±1ºC. The experiment design for part 2 of the study is summarised in Figure 3.3.

3.3.1 Ingredients in yogurt

Commercial food grade ingredients were used to prepare coconut cream yogurt (Table 3.2).

Table 3.2: List of ingredients used in the production of coconut cream yogurt.

Items	Ingredients	Processing Technique	Producer	Code Used (if any)
Kara™ coconut cream	Fresh natural coconut cream (99.9%), stabilizers (xanthan gum (E415), guar gum (E412), carrageenan (E407))	Ultra-high temperature pasteurization	Kara Marketing SdnBhd.	
Turmeric (Curcumin C^3 complex® AU)	Curcumin, demethoxycurcumin, bisdemethoxycurcumin	Solvent extraction	Sabinsa Corporation	
Nescafe® Gold Green Blend	Coffee beans	Green and roasted beans	Nestle®	C1
Avalanche® Espresso style	Coffee beans	Freeze dried	Avalanche coffee	C2
Robert Harris Colombian Blend	Coffee beans	Freeze dried	Robert Harris	C3
Squeeze Me Honey	Pure honey	---	Arataki Honey	

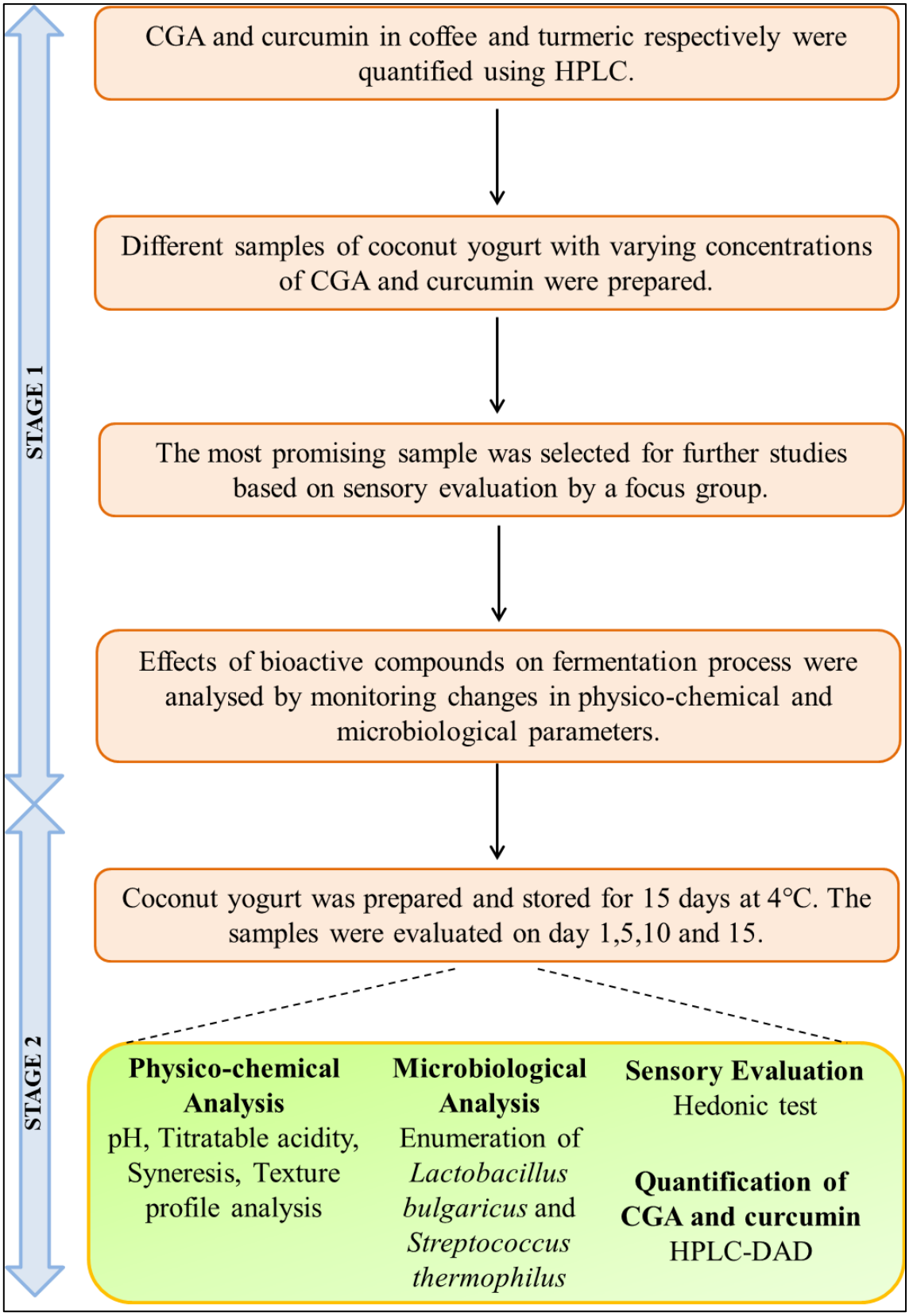


Figure 3.3: Experimental design used for the development of coconut cream yogurt enriched with chlorogenic acid (CGA) and curcumin.

3.3.2 Stage 1: Quantification and optimisation of bioactive compounds added to coconut cream yogurt

Different samples of coconut yogurt containing 100, 150, 200 and 300 mg (per 150 g of coconut cream) of CGA and curcumin were prepared. Before preparation of the yogurt, the approximate amount of CGA and curcumin in coffee and turmeric, respectively, were quantified using HPLC. A focus group was used to screen the most accepted yogurt sample based on the sensory profile. The sample found to be most acceptable by the focus group was used for further studies. The effect of bioactive compounds on the fermentation process was studied by measuring pH, colour, titratable acidity and *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* cell counts at 2 h interval during fermentation.

3.3.2.1 Quantification of bioactive compound in coffee and turmeric

Three coffee samples and one turmeric sample were analysed using HPLC to quantify the amounts of CGA and curcumin, respectively.

(a) Extraction of curcumin and CGA

Curcumin and CGA were extracted from the test samples using acetone (≥99.9 %, Fisher Scientific, UK) and methanol (≥99.9 %, Fisher Scientific, UK) respectively (Chen, Jimmy Yu, Li, Luo, & Liu, 2007; Saleh et al., 2016; Singh, 2018). One g of sample was dispersed in 5 ml of solvent and ultra-sonicated for 10 min at room temperature (20ºC) in an ultrasonic water bath (Sonorex Super RK 501, Bandelin electronic GmbH & Co. KG, Berlin). Ultra-sonication was done to facilitate the dispersion and extraction of the bioactive compounds (Saleh et al., 2016). The resulting mix was centrifuged at 11200 rcf for 10 min. The supernatant was collected and the sediment was re-dispersed in 5 ml of solvent to extract any remaining compound. The two supernatants were combined, filtered and diluted prior to HPLC analysis. The amount of bioactive compound was quantified using HPLC-DAD, as discussed in the following section.

(b) HPLC analysis for CGA

Quantitative determination of CGA in the test sample was carried out using the protocol described by Özbilgin et al., 2015; Saleh et al., 2016 and Xue et al., 2016 with minor modifications. The reverse phase HPLC system (Shimadzu Prominence Series, Japan) equipped with pump (LC-20 AD), degasser (DGU-20 A_5), sample injector (SIL-20ACHT), column oven (CTO-20AC) and a photodiode array detector (SPD-M20A) was used for the

analysis. The sample (20 µl) was injected into the stationary phase consisting of C-18 Phenomenex Luna column (250 x 4.6 mm, particle size 3 µm) at 22ºC. The mobile phase used to separate CGA consisted of:

A: 0.1 % Trifluoroacetic acid in water

B: 0.1 % Trifluoroacetic acid in 40 % Acetonitrile

The sample was separated at a flow rate of 0.75 ml/min using a discontinuous gradient from 100 % A at time 0 min to 35 % B after 15 min, and finally to 50 % B after 26 min. The CGA in the sample was detected using a diode array detector (DAD) at 327 nm. Chromatographic data were recorded and integrated using LC solution software (Shimadzu Prominence UFLC, Japan). Analytical standards for CGA diluted in methanol at different concentrations were also analysed, and a standard curve was generated by plotting peak areas versus concentrations of the compound. The amount of CGA in the test sample was then quantified by comparing the retention time and peak area with the standard.

(c) HPLC analysis for curcumin

Curcumin in the test sample was quantified using similar HPLC equipment as for CGA. The sample (10 µl) was injected onto a C-18 Grace Smart column (250 x 4.6 mm, particle size 5 µm) *via* an auto-sampler (Singh, 2018). The mobile phase used to analyse curcumin consisted of:

A: 2 % Acetic acid + 4 % Methanol + 25 % Acetonitrile + 69 % water

B: 2 % Acetic acid + 10 % Methanol + 88 % Acetonitrile

The gradient (0 to 35 % of B) was run for 17 min at a flow rate of 1 ml/min and curcumin was detected using DAD at 425 nm. Curcumin standard was diluted in acetone to obtain different concentrations and analysed as previously described to generate a standard curve based on peak area. The amount of curcumin in the test sample was quantified using the standard curve.

3.3.2.2 Preparation of yogurt

Coconut yogurt was prepared as described in Figure 3.4. Kara™ coconut cream was mixed with different amounts of CGA and curcumin (100-300 mg/150 g of coconut cream) at low speed using a Kitchen Aid mixer bowl. Honey (5 g/150 g of coconut cream) was also added to mask the bitter taste of the coffee (Sohi, Sultana, & Khar, 2004; Sun-Waterhouse &

Wadhwa, 2013). The mixture was then heated to 42±1ºC in a water bath (Grant, Global Science, NZ), followed by inoculation with 2 % (w/v) of YO MIX® 726 LYO 50 DCU starter culture into the mixture (Muniandy, Shori, & Baba, 2016). The culture was mixed and the mixture was then sub-divided into glass jars of 500 g and 150 g. Glass jars were then placed in a water bath at 42±1ºC until pH of 4.5±0.1 was achieved. The fermented samples were stored at 4ºC without disturbing the gel.

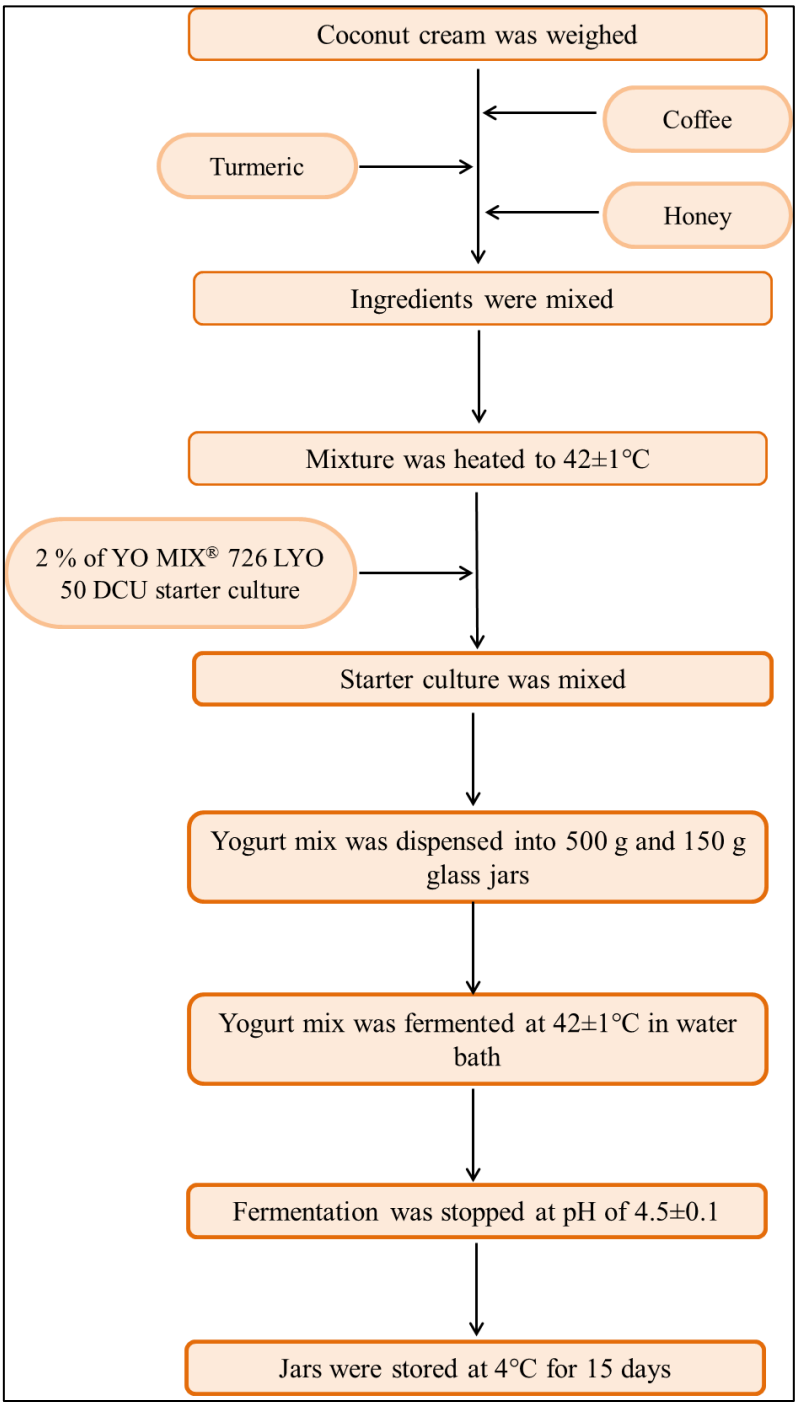


Figure 3.4: Production of coconut yogurt containing curcumin and chlorogenic acid *(CGA).*

3.3.2.3 Sensory evaluation

A descriptive focus group sensory evaluation (Hall & Wendin, 2008; Laureati, Pagliarini, Calcinoni, & Bidoglio, 2006) was used to screen the various yogurt samples prepared with different concentrations of CGA and curcumin (Section 3.3.2.2). The focus group consisted of 5 members who consumed yogurt on a regular basis. The group evaluated one sample at a time by describing its appearance, mouthfeel, flavour, aftertaste and texture. The most promising sample was selected for further analysis.

3.3.2.4 Fermentation of yogurt in the presence of CGA and curcumin

Effect of CGA and curcumin on fermentation was studied by monitoring the changes in pH, titratable acidity, colour and microbial counts at every 2 h interval during fermentation. The sample without bioactive compounds was used as controls.

(a) pH and titratable acidity

A bench-top pH meter (Sartorius® PB-11, Germany), pre-calibrated using pH buffers 4, 7 and 9 was used to measure the pH of yogurt samples. The electrode was dipped into 30 g of the sample until a stable reading was obtained.

Titratable acidity was determined using the AOAC 947.05 method (AOAC, 2005). Ten g of sample was weighed in a flask and mixed with 30 ml of distilled water. The sample was titrated against standardised 0.1 M NaOH until the end-point of pH 8.1 was achieved on pH meter (Sartorius® PB-11, Germany). Titratable acidity (%) was calculated using equation (4):

$$\%\, Titratable\ acidity = \frac{Average\ volume\ of\ NaOH\ (ml) \times 0.1 \times 90 \times 100}{Sample\ weight\ (g) \times 1000} \quad \text{.....(4)}$$

Where, 0.1 = molarity of NaOH and

90 = molecular weight of lactic acid

(b) Colour

The colour of yogurt was measured with a digital chroma meter (CR-300, Konica Minolta, Japan) in the CIE system (L*, a* and b*) (Sah, Vasiljevic, McKechnie, & Donkor, 2016) following the manufacturer's instructions. About 2 g of the sample was measured into a plastic petri dish and placed on the compartment of the instrument in dark conditions.

(c) Microbiological analysis

Microbiological analysis was performed to enumerate *L. bulgaricus* and *S. thermophilus* bacteria in yogurt samples. The two starter cultures were plated using the pour-plate method. Five g of the sample was weighed and mixed with 45 ml of 0.1 % peptone water (Merck, Germany) in a sterile stomacher bag (Global Science, NZ) giving a 10^{-1} dilution. The content in the stomacher bag was mixed for about 1 min using paddle stomacher blender (Masticator 400 ml, ILU, Spain). Ten-fold serial dilutions up to 10^{-9} were prepared by mixing 1 ml of 10^{-1} dilution sample with 9 ml of peptone water (Espírito Santo, Perego, Converti, & Oliveira, 2012).

Enumeration of L. bulgaricus* and *S. thermophilus

Enumeration of *L. bulgaricus* was done using MRS agar (Bracquart, 1981). MRS powder (62 g, Oxoid™, UK) was dissolved in 1 L of distilled water and autoclaved (Astell Scientific™, NZ) at 121ºC for 15 min. Sterile molten agar was cooled to 45-48ºC and 15-18 ml of molten agar was gently mixed with 1 ml of diluted sample in sterile petri dishes. Upon solidification, about 5 ml of more MRS agar was added to maintain an anaerobic condition. Solidified agar plates were then incubated at 43±1ºC for 72 h. Agar plates with 30-300 grown bacterial colonies were counted and counts were expressed as colony forming unit per gram of yogurt sample (cfu/g).

S. thermophilus was enumerated on M17 agar (Bracquart, 1981). M17 agar (48.25 g, Himedia, NZ) was mixed in 950 ml of distilled water. Simultaneously, 10 % lactose solution was prepared by dissolving 10 g of lactose (ThermoFisher Scientific™, NZ) in 100 ml of distilled water. Both solutions were autoclaved individually at 121ºC for 15 min. Post-autoclave, the media was cooled to 45-48ºC and 50 ml of 10 % lactose solution was added to 950 ml of M17 agar as a nutrient supplement. Samples were plated, incubated at 37±1ºC for 48 h and developed colonies were counted as previously described.

3.3.3 Stage 2: Stability of coconut yogurt with added bioactive compounds during storage at 4°C for 15 days

Yogurt sample that was most accepted by the focus group (section 3.3.2.3) based on sensory attributes was selected to study the stability during refrigerated storage. Coconut yogurt containing CGA and curcumin was prepared (section 3.3.2.2) and stored at 4±1ºC for 15 days. The changes in physio-chemical properties, microbial counts and sensory attributes

were analysed on every 5th day. Also, the amounts of CGA and curcumin in yogurt were quantified using HPLC at day 1, 5, 10 and 15 of storage. Coconut yogurt without added bioactive compounds was considered the control.

3.3.3.1 Analysis of yogurt during storage

(a) pH, titratable acidity, colour and microbiological count

pH, titratable acidity, colour and viable cell counts of the yogurt samples were determined as described in section 3.3.2.4.

(b) Syneresis

Syneresis of yogurt samples was measured as described by Amatayakul, Sherkat and Shah, (2006). Briefly, the container containing yogurt was weighed and placed at an angle of about 45º for 10-15 s. The supernatant was carefully collected and weighed. Syneresis (%) was calculated using equation (5):

$$\%\ Syneresis = \frac{Weight\ of\ supernatant\ (g)}{Initial\ weight\ of\ sample\ (g)} \text{x}100 \quad \text{.....(5)}$$

(c) Texture profile analysis

The firmness of yogurt gel was analysed by a single penetration test using a TA-XT plus Texture Analyser (Stable Micro Systems, Godalming, UK) equipped with 5 kg load cell on 150 g packed sample (Espírito Santo et al., 2012; Mudgil, Barak, & Khatkar, 2017). A cylindrical probe of 25-mm diameter, moving at a constant test speed of 1 mm/s through 15 mm within the sample was used. The force-distance curve was obtained and the firmness of the gel was determined as the highest force point on the curve.

(d) Quantification of bioactive compounds using HPLC

CGA and curcumin were extracted from coconut yogurt on day 1, 5, 10 and 15 of storage and quantified using HPLC as described in section 3.3.2.1.

(e) Consumer sensory evaluation

A consumer sensory acceptance test was performed over 15 days of storage at 4±1ºC for the most promising sample. Samples were evaluated on day 1, 5, 10 and 15, by 25 participants. The participants mainly consisted of academic and administrative staff, and students at Massey University (Albany, NZ) and local inhabitants in the Auckland region. The

participants were selected based on their familiarity with the fermented products and consumed it once a week. The sensory sessions were conducted in individual sensory booths at Product Development Laboratory, Massey University, NZ. Before tasting the sample, the participants were introduced to the sensory process and were informed about the ingredients in accordance with the human ethics requirements (Application no.: 4000020553). For each session, participants were required to evaluate two samples for appearance, mouthfeel, flavour and texture on a 9-point hedonic scale ranging from dislike extremely to liked extremely (Appendix 4). Each participant was provided with 15 g of samples (at 4±1ºC) in 25 ml plastic cups coded with a random three-digit number. Participants were asked to clean their palate with bottled still water before evaluating each sample.

3.4 Data analysis

All the experiments were replicated at least three times. The results were expressed as mean ± standard deviation. One-way ANOVA (Analysis of variance) with Duncan's post hoc test at 5 % significance level was used to determine significant differences between the means using the statistical software (IBM SPSS version 25). Graphs were created using ORIGIN software (OriginPro, 64 Bit, 2018).

CHAPTER 4

Investigation of Anti-Inflammatory Activity of Bioactive Compounds using an *In Vitro* Model

4.1 Introduction

In humans, invasion by a foreign body immediately results in the activation of monocytes which are responsible for the production of inflammatory defence responses, such as secretion of pro-inflammatory cytokines like TNF-α (Kumar et al., 2012c). However, a prolonged and/or repetitive inflammatory response can lead to the onset of chronic-inflammation related diseases such as obesity, arthritis, Alzheimer, cardiovascular diseases and several forms of cancer (Hewlings & Kalman, 2017; Meirow & Baniyash, 2017). Therefore, research into the role of bioactive compounds in reducing chronic-inflammatory responses and their ability to combat the associated diseases is important.

Bioactive compounds are naturally available in small quantities which may not be sufficient to fully elicit the desired anti-inflammatory effects (Long, Yang, Xu, Hao, & Li, 2015). In addition, combining different bioactive compounds may result in synergistic effects, thus achieving anti-inflammatory effects at lower doses. Curcumin is a bioactive compound reported to suppress inflammation, but its low bioavailability in humans requires a high dose to elicit significant effects. In this study, the combined effect of curcumin with lupeol and CGA on inflammation was investigated. As described in Chapter 2, individually, all three of these bioactive compounds are reported to produce anti-inflammatory effects but their combined effects have not yet been determined.

This chapter investigates the anti-inflammatory effects of the three selected bioactive compounds, both individually and in combination, in an *in vitro* model using the human leukaemia monocyte THP-1 cell line. Using THP-1 cells is advantageous as they can replicate within 35-50 h, do not produce any toxic products, do not contain any infectious viruses, have minimum genetic variation with increasing passage number which yields more reproducible results and after differentiation they mimic primary human macrophages (Chanput, Peters, & Wichers, 2015; Tedesco et al., 2018). Briefly, THP-1 monocytes were

differentiated into macrophages using PMA, followed by treatment with the bioactive compounds and then the inflammatory response was stimulated using LPS. The supernatant was collected to allow quantification of TNF-α secretion by ELISA, while the cells were utilised for measuring cell viability using MTT assay or for studying mRNA expression using qRT-PCR as previously described in Chapter 3.

4.2 Results and discussion

4.2.1 PMA induced differentiation of THP-1 cells

Phorbol 12-myristate 13- acetate (PMA), also known as 12-O-Tetradecanoylphorbol-13-acetate (TPA) is a potential tumour promoter which is commonly used in biomedical research to activate expression of protein kinase C (PKC) (Pham et al., 2017; Schwende, Fitzke, Ambs, & Dieter, 1996). PMA induced activation of PKC results in differentiation of THP-1 monocyte cells into macrophages and subsequently an increase in the expression of several surface markers which causes cell adhesion (Starr, Bauler, Malik-Kale, & Steele-Mortimer, 2018). The differentiated macrophages lose their ability to divide and become more sensitive to bacterial invasion, which results in a strong immune response (Takashiba et al., 1999). In several *in vitro* studies, PMA concentrations ranging from 6-500 nM have been used to differentiate the THP-1 cells (Lund, To, O'Brien, & Donnelly, 2016). A high concentration of PMA aberrantly expresses several genes in the cells during differentiation and highly expressed genes may overpower the effect of stimuli on macrophages (Park et al., 2007). In contrast, at a low PMA concentration, all the cells may not differentiate completely into macrophages. Hence, it is important to select the lowest possible concentration of PMA that still maintains a high differentiation rate.

After incubation with 5-200 nM PMA the THP-1 cells spread and adhered to the surface of the plate, which are hallmarks of macrophages (Park et al., 2007), while the undifferentiated monocytes remained floating in suspension. As evident from Figure 4.1A the cells without PMA were small, spherical and of uniform morphology, whereas the PMA treated cells had a larger and irregular shape. These results are consistent with those from previously reported studies (Daigneault, Preston, Marriott, Whyte, & Dockrell, 2010; Gatto et al., 2017; Lund et al., 2016; Pham et al., 2017; Takashiba et al., 1999). However, it must be noted that the differentiation of monocytes into macrophages was dependent on PMA concentration.

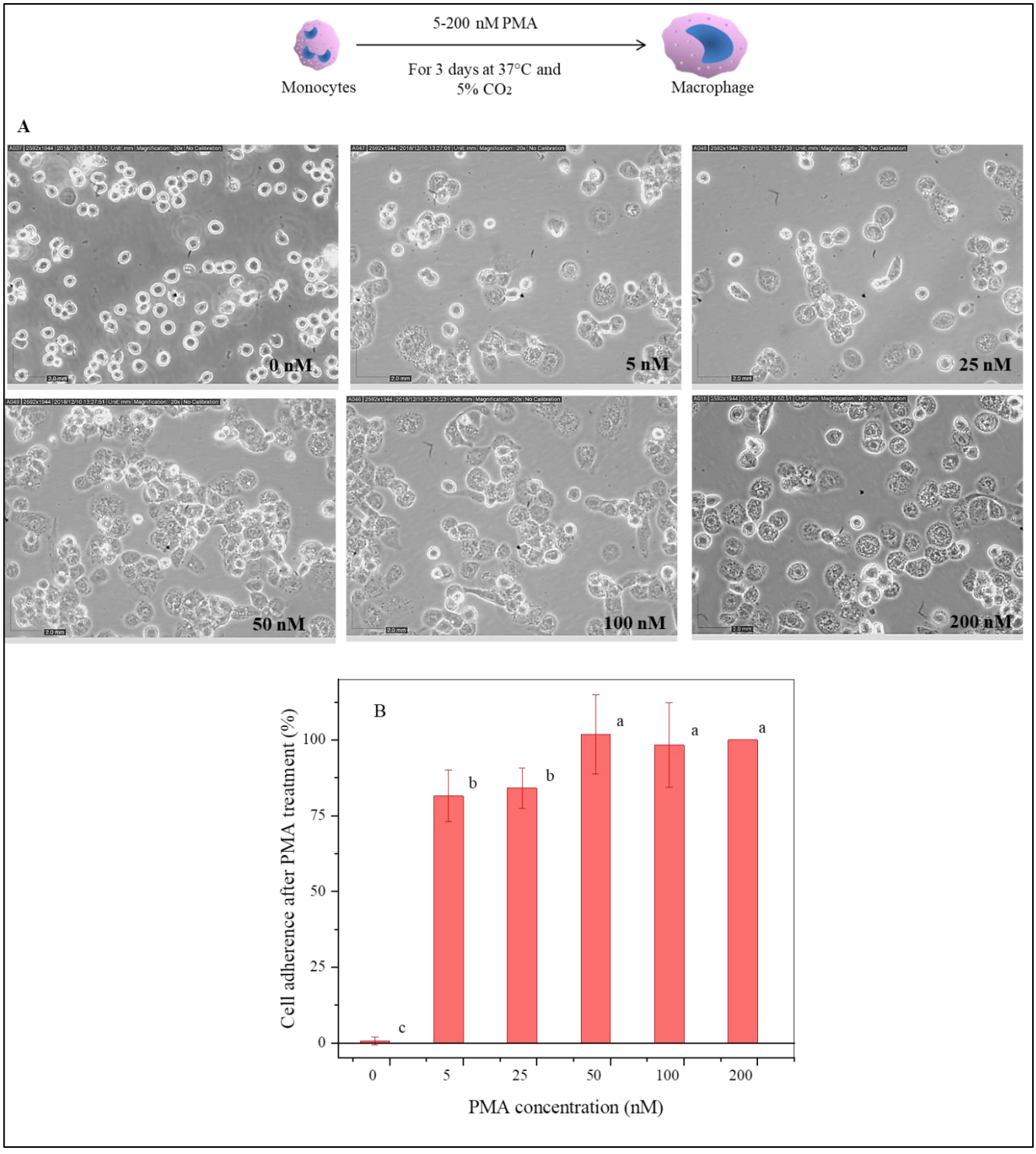


Figure 4.1: Effect of PMA concentration on differentiation of THP-1 cells.

THP-1 monocytes were differentiated into macrophages using Phorbol 12-myristate 13-acetate (PMA) ranging from 5-200 nM treated for 3 days at 37°C and 5 % CO_2. After incubation period phase contrast microscopic images (40X) (A) and percentage cell adhesion (B) with respect to 200 nM dose were analysed. Adhesion was quantified using MTT assay. **Note:** Data represents the mean of two biological replicates with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

The two lowest PMA doses of 5 nM and 25 nM were not effective in differentiating all the cells as the macrophage population was low in comparison to that observed at higher PMA doses (Figure 4.1A). In comparison to the 200 nM PMA dose, about 81.18±8.47 % and 83.84±6.63 % cells adhered to the surface of the plate at PMA concentration of 5 nM and 25 nM respectively, reflecting the lower differentiation rate of the cells (Figure 4.1B). Incubation of the THP-1 cells with PMA at 50-200 nM resulted in high levels of differentiation, with no significant difference ($p \leq 0.05$) in percentage cell adhesion between these concentrations. A similar dose-dependent result was reported by Park et al. (2007) when differentiating THP-1 cells using 2.5-100 ng/ml PMA. Since differentiation of THP-1 cells at 50 nM PMA was similar to that achieved at higher doses, 50 nM was selected as the lowest possible dose of PMA with high differentiation rate. Thus, all subsequent experiments were performed with 50 nM PMA.

4.2.2 LPS induced inflammation in THP-1 macrophages

Lipopolysaccharide (LPS) is an endotoxin commonly present on the outer cell membrane of gram-negative bacteria (Raetz & Whitfield, 2002). LPS is a highly immunogenic compound that can stimulate an inflammatory response in humans. The lipid portion of LPS interacts with cluster differentiation (CD) marker CD14 surface protein which results in the activation of Toll-like receptor-4 (TLR-4) receptor (Meng & Lowell, 1997; Ngkelo, Meja, Yeadon, Adcock, & Kirkham, 2012; Tapping & Tobias, 1997). Activation of TLR-4 receptors further activates several kinase enzymes resulting in the initiation of NF-κB, AP-1 and MAPK signalling pathways (Chanrot et al., 2017; Ngkelo et al., 2012). Treatment of macrophages with LPS results in the production of several pro-inflammatory cytokines such as TNF-α, IL-6 and IL-8 and anti-inflammatory cytokines such as IL-10 and superoxide species (Li et al., 2003; Lund et al., 2016; Meng et al., 2013). The amount of cytokines produced by THP-1 cells will be dependent on the concentration of the LPS and the exposure time. Thus, it was important to identify the optimum conditions where a significant amount of TNF-α would be secreted by the cells following stimulation with LPS. TNF-α is a trans-membrane protein secreted by macrophages and is extensively used as a key biomarker to study anti-inflammatory effects of compounds (Gurol et al., 2016; Kotlyarov et al., 1999; Mahmoud, El-Nagar, & El-Bassossy, 2012; Roome et al., 2019; Taylor, Porter, & Gonzalez, 2014).

4.2.2.1 Optimisation of dilution factor for TNF-α measurement from LPS stimulated cells

Post LPS (200 ng/ml) treatment of differentiated THP-1 cells for 4 h, the supernatant containing TNF-α was collected and TNF-α was quantified by ELISA (Section 3.2.3.5). As per the manufacturer's instructions, the supernatant containing TNF-α needed to be diluted to obtain results within the detection limits of the kit. The supernatant was diluted to 1/10, 1/50, 1/100 and 1/200 times in 1x assay diluent A to quantify TNF-α production.

The amount of TNF-α produced by the differentiated THP-1 cells in response to LPS was quantified by interpolation from the standard curve (Figure 4.2A). The absorbance of the sample without any dilution and 10 fold dilution was 3.18±0.27 and 2.39±0.12, respectively, which was well above the absorbance of the highest standards of 500 pg/ml (1.77±0.09). Thus, these two samples were too concentrated and were therefore not considered suitable for further studies. The absorbance for the other three dilutions was within the detection range of the standard curve and once corrected for the dilution factor, the total TNF-α quantified was not significantly different ($p \leq 0.05$) for these dilutions (Figure 4.2B). However, the absorption value (0.26±0.01) for the 1/200 dilution was in the lower portion of the standard curve which may not be suitable for further experiments involving a reduction in TNF-α level. Hence a dilution ranging between 50 to 100 times was determined to be optimum for further studies.

4.2.2.2 Optimisation of LPS dose-response for stimulation of THP-1 macrophages

Stimulation of differentiated THP-1 cells with different concentrations (0-200 ng/ml) of LPS for 4 h resulted in a dose-dependent increase in TNF-α production (Figure 4.3A). An LPS concentration of 5 ng/ml was not sufficient to produce a detectable amount of TNF-α but at 25 ng/ml and above a quantifiable amount of TNF-α was produced. Cells treated with 200 ng/ml LPS produced the maximum amount of TNF-α (10.69±1.16 ng/ml). However, this amount was not significantly different ($p \leq 0.05$) to that produced by 100 ng/ml (9.99±3.26 ng/ml) LPS treatment. Moreover, as the concentration of the LPS increased, the cell viability decreased (Figure 4.3B), with more than 50 % cell death observed at 200 ng/ml LPS, indicating the highly sensitive nature of THP-1 cells towards LPS.

These results are consistent with Huiyu Shi et al. (2016) who observed a dose-dependent increase in the production of several pro-inflammatory cytokines (TNF-α, IL-6 and IL-1), iNOS enzyme and NO species in LPS stimulated bovine epithelial cells. The increase in LPS dose was shown to enhance the production of various ROS and RNS resulting in oxidative

stress leading to cell death (Kim, Johnson, Shin, & Sharma, 2004; Li, Ma, Peng, Chen, & Zhang, 2011; Nishio et al., 2013). Furthermore, NO can also promote the apoptotic pathway in macrophages by activating Bax, a pro-apoptotic gene (Xaus et al., 2000). Therefore, taking into account the TNF-α production and cell viability results, 100 ng/ml LPS was considered the most suitable concentration to use for further studies.

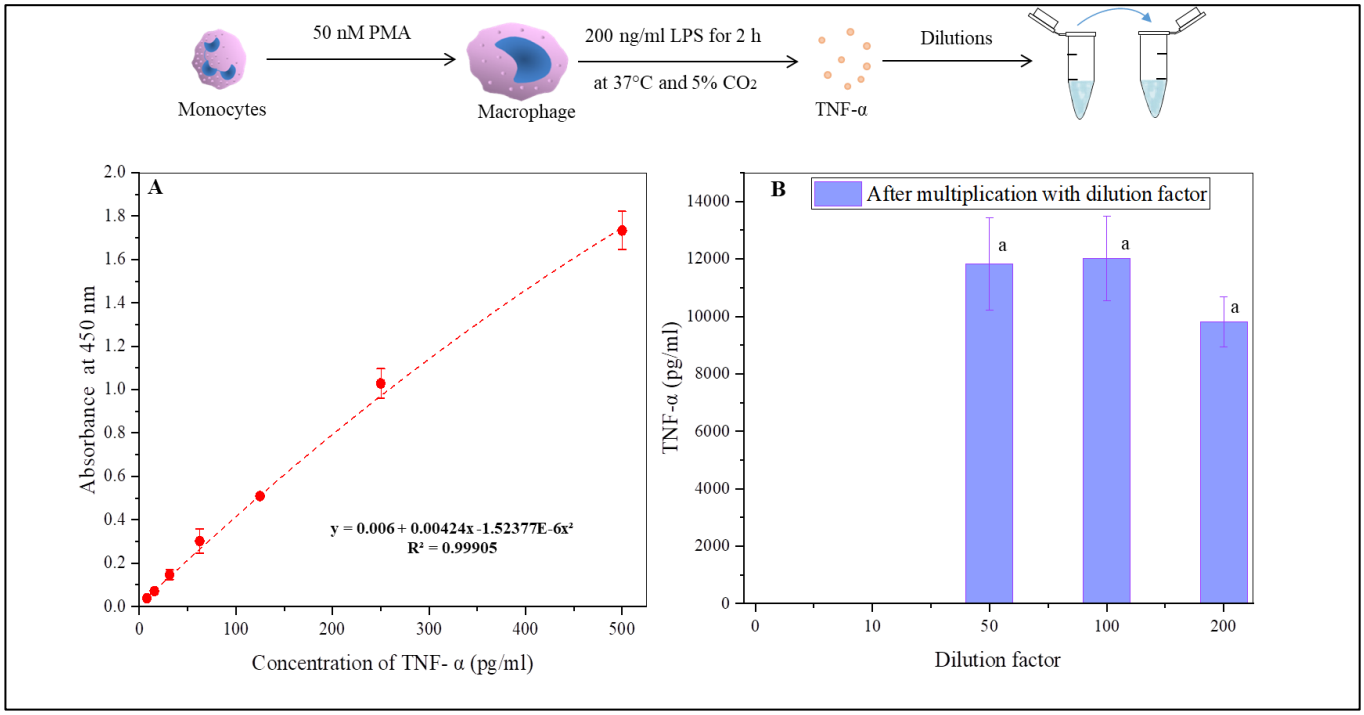


Figure 4.2: Optimum dilution factor for supernatant containing TNF-α.

THP-1 macrophages were exposed to 200 ng/ml lipopolysaccharide (LPS) for 4 h and supernatant was diluted and TNF-α was quantified using ELISA. (A) Standard curve for TNF-α ELISA, (B) adjusted TNF-α level after multiplication with respective dilution factors. **Note:** Data represents the mean of two biological replicates and two replicates in each assay with error bars corresponding to standard deviation. *: TNF-α was above the detection limits of the standard curve. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

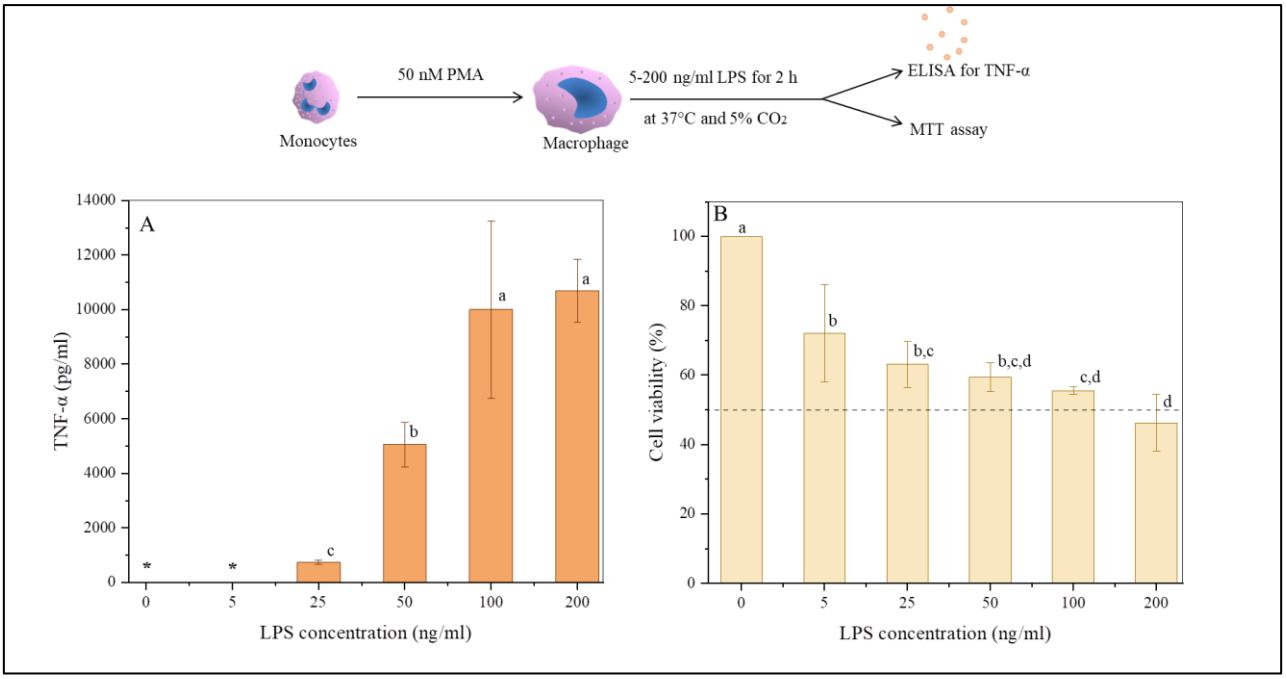


Figure 4.3: Effect of lipopolysaccharide (LPS) concentration on (A) TNF-α production and (B) cell viability.

THP-1 macrophages were stimulated with LPS (5-200 ng/ml) for 2 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of two biological replicates and two replicates in each assay with error bars corresponding to standard deviation. *: TNF-α was below the detection limits. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

4.2.2.3 Optimisation of LPS incubation time

The stimulation of THP-1 macrophages with 100 ng/ml LPS for 0.5, 1, 2, 4 and 6 h revealed a time-dependent increase in TNF-α level (Figure 4.4A). A similar time-dependent increase in TNF-α has been reported by others (Chanput, 2012; Ma et al., 2017; Huiyu Shi et al., 2016). The amount of TNF-α produced by the negative control (t=0 h) and at t=0.5 h was insufficient for detection but at t=1 h and above, a detectable amount of TNF-α was produced. The maximum amount of TNF-α was secreted at t=6 h (3.21±0.17 ng/ml) but was not significantly different ($p \leq 0.05$) from that obtained at t=4 h (3.19±0.31 ng/ml). However, in comparison with t=0, about 60 % cell death was observed at t=6 h (Figure 4.4B) which was higher than at t=4 h. Hence, a 4 h incubation period was considered to be suitable for further studies.

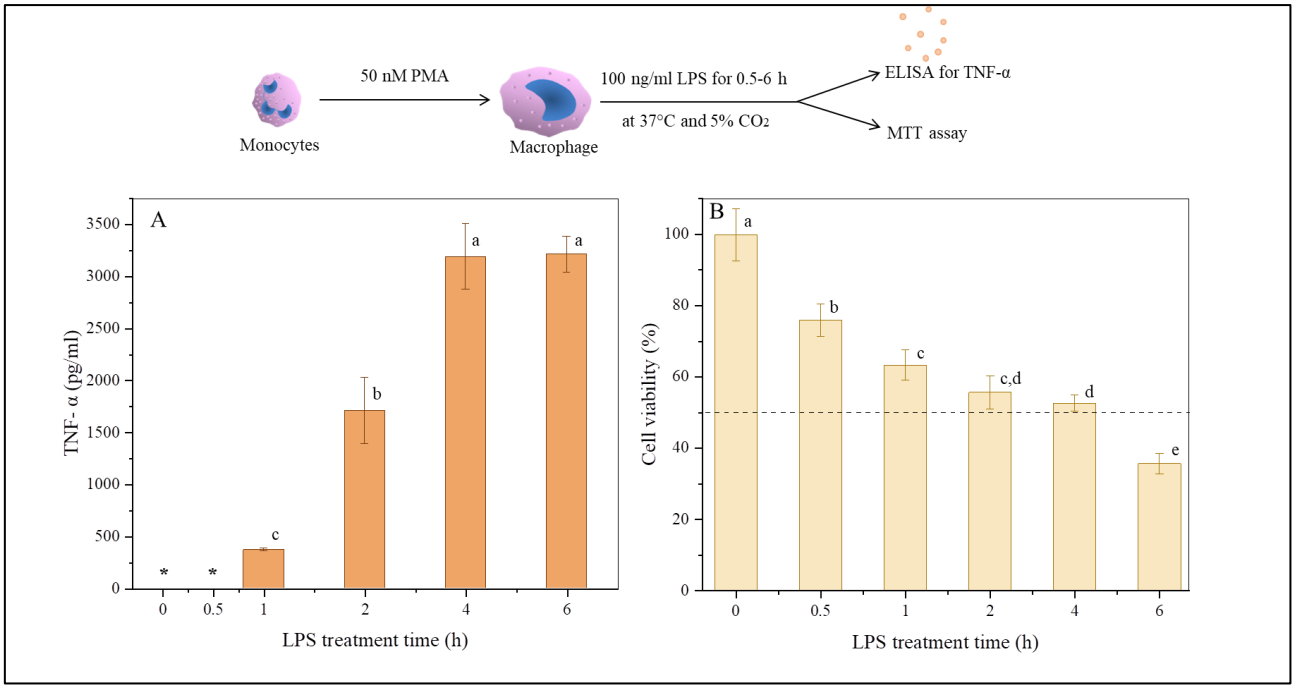


Figure 4.4: Effect of lipopolysaccharide (LPS) incubation time on (A) TNF-α production and (B) cell viability.

THP-1 macrophages were stimulated with 100 ng/ml LPS for 0.5-6 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of two biological replicates and two replicates in each assay with error bars corresponding to standard deviation. *: TNF-α was below the detection limits. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

4.2.3 Anti-inflammatory potential of bioactive compounds in LPS stimulated THP-1 cells

4.2.3.1 Effect of different doses of curcumin, lupeol and CGA

(a) Treatment with curcumin

Curcumin was effective in significantly ($p \leq 0.05$) reducing TNF-α production in a dose dependent manner in LPS stimulated THP-1 macrophages (Figure 4.5A). 1 µM curcumin reduced TNF-α level by about 3 % when compared with the negative control. At a dose of 5 µM curcumin, TNF-α production decreased from 7.97±0.21 ng/ml (only LPS treated cells) to 6.37±0.18 ng/ml, which was further reduced to 1.70±0.08 ng/ml at 25 µM curcumin. These results are consistent with previous observations (Cho, Lee, & Kim, 2007; Lin et al., 2014; Meng et al., 2013; Woo et al., 2007; Yang et al., 2012). Chan (1995) reported a 57 % decrease in TNF-α level on treating LPS stimulated human Mono Mac 6 cells with 5 µM

curcumin for 4 h. Curcumin (5-20 µM) can act on NF-κB and JNK signalling pathway, and downregulate the production and transcription of TNF-α and IL-6 in a dose-dependent manner in 3T3-L1 cells (Shao-Ling et al., 2009). A similar reduction in TNF-α level was reported in LPS induced RAW 264.7 cells pre-treated with 5-15 µM curcumin for 2 h by blocking PI3K/AKT signalling pathways (Ma et al., 2017).

With increasing curcumin concentration a dose-dependent decrease in cell viability was observed (Figure 4.5B). Cell viability was 70-75 % at the two highest doses of 10 and 25 µM curcumin. Exposure of THP-1 macrophages to 50 µM curcumin has been shown to significantly upregulate various caspases pathways and downregulate the PARP-1 pathway, thereby causing apoptosis (Yang et al., 2012). Curcumin (15 µM) was also reported to result in a 15 % reduction in viability of RAW 264.7 macrophages (Ma et al., 2017). In contrast, no such toxic effects of curcumin were reported by Naik, Mujumdar, and Ghaskadbi (2004), Park et al. (2008) and Wang, Boddapati, Emadi, and Sierks (2010).

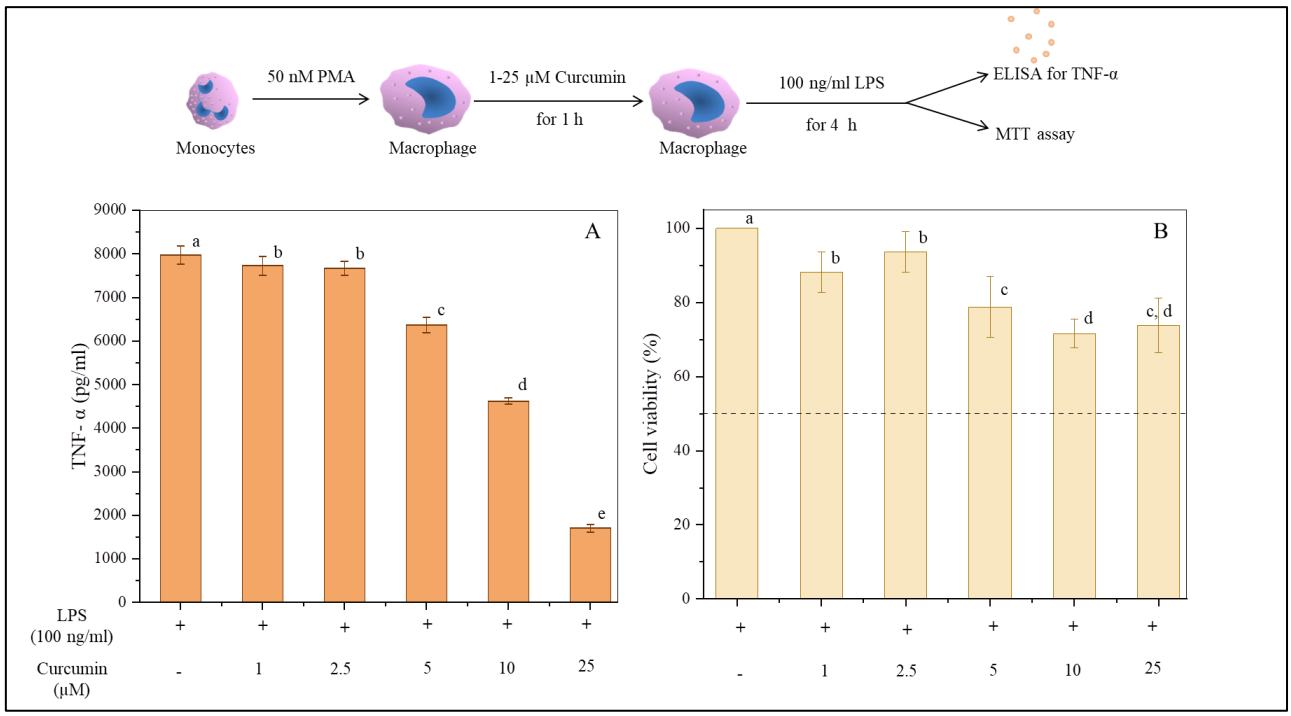


Figure 4.5: Effect of curcumin dose on (A) TNF-α production and (B) cell viability. THP-1 macrophages were pre-treated with 1-25 µM curcumin for 1 h prior to stimulation with 100 ng/ml LPS for 4 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of three biological replicates and three replicates in each assay with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

(b) Treatment with lupeol

Similar to curcumin, 1 h pre-treatment of differentiated THP-1 cells with lupeol concentrations ranging from 1-25 µM resulted in a dose-dependent decrease in the secretion of TNF-α (Figure 4.6A). LPS stimulated THP-1 cells without any treatment with lupeol produced 3.92±0.18 ng/ml TNF-α which was reduced by 10-40 % on pre-treatment with lupeol in a dose-dependent manner. A similar decrease in the production of inflammatory cytokines such as TNF-α, IL-6, IL-8, IL-12 and ROS was observed in epithelial cells, RAW 264.7 and $CD14^+$ monocyte cells on treatment with lupeol (Lee et al., 2016; Srivastava et al., 2016; Zhu et al., 2016).

Simultaneously to TNF-α downregulation, the increased concentration of lupeol caused a decrease in cell viability (Figure 4.6B). On comparing Figure 4.6A and B, the decrease in TNF-α level was closely related to the decrease in cell viability. For instance, at 25 µM dose,

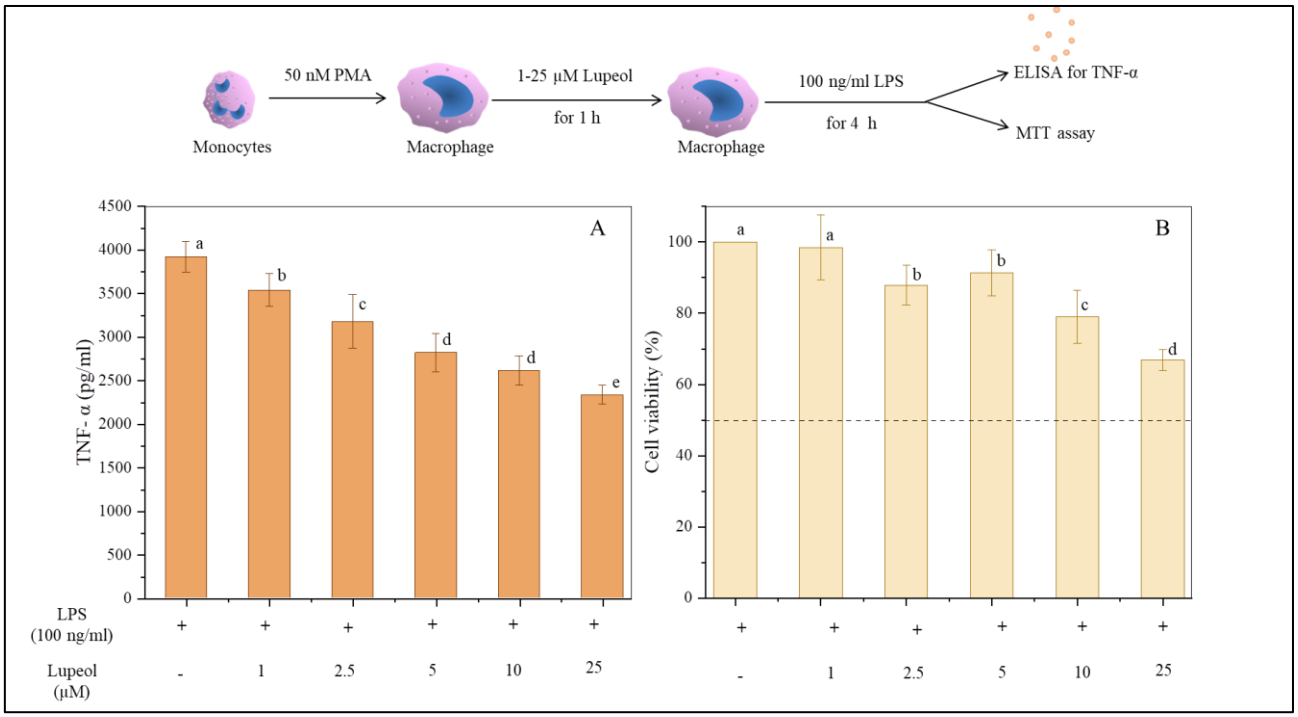


Figure 4.6: Effect of lupeol dose on (A) TNF-α production and (B) cell viability.
THP-1 macrophages were pre-treated with 1-25 µM lupeol for 1 h prior to stimulation with 100 ng/ml LPS for 4 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of three biological replicates and three replicates in each assay with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

about 33 % of the cells died with a decrease of about 40 % in TNF-α level, suggesting that cell death was responsible for cytokine reduction. In previous reports, lupeol was reported to kill 50 % of LNCaP, CWR22Rr1 and human umbilical vein epithelial cells at 21, 18.5 and 10 µM concentrations respectively within 48 h of treatment (Kangsamaksin et al., 2017; Saleem et al., 2005). Lupeol can act on multiple apoptotic pathways by upregulating PARP, Bax and caspase and downregulating Bcl-2 and Bcl-xL expressions and thereby cause cell death (Lee et al., 2007; Pitchai, Roy, & Ignatius, 2014; Prasad, Sabarwal, Yadav, & Singh, 2018; Prasad, Kalra, & Shukla, 2007).

(***c) Treatment with CGA***

Figure 4.7A shows the effect of CGA on TNF-α produced by differentiated THP-1 cells. As the concentration increased from 1 µM to 2.5 µM the TNF-α levels significantly ($p \leq 0.05$) increased from 2.49±0.27 ng/ml to 2.91±0.31 ng/ml. Above the 2.5 µM dose, the amount of TNF-α produced started to decrease and yield 2.62±0.39 ng/ml at 25 µM dose, although this was still above the amount secreted in the control (2.37±0.37 ng/ml) sample. The increase in the level of TNF-α secretion can be explained by an increase in cell viability of CGA treated THP-1 cells when compared with LPS only treated cells (Figure 4.7 B). At the concentration of 1, 2.5 and 5 µM cell viability increased to 119.00±13.06 %, 123.59±12.09 % and 122.51±11.38 % respectively and was significantly different from the control ($p \leq 0.05$). Further increase in the concentration to 25 µM resulted in elevated cell viability to 140.15± 14.55 %.

Similar protective effect of CGA on cell viability was reported by Gong, Su, Zhan, and Zhao (2018), Pavlica and Gebhardt (2005) and Zhang et al. (2018). Lee et al. (2011) observed the protective effect in beta-amyloid stimulated PC12 cells pre-treated with CGA for 1 h. These authors proposed that CGA can increase the production of Bcl-2 and decrease the production of Bax, anti-apoptotic and pro-apoptotic proteins respectively. Further, they also proposed that CGA can reduce intercellular calcium levels and decrease caspase-3 thereby reducing beta-amyloid-induced apoptosis. Another group of researchers (Taram, Winter, & Linseman, 2016) showed the neuroprotective effect of 10 µM CGA against sodium nitroprusside (50 µM) in cerebellar granule neurons and achieved more than 55 % higher cell viability when compared with the negative control sample (without CGA). The latter study also highlighted that CGA could not protect the cerebellar granule neurons when stimulated with glutamate, indicating that the protective effect of CGA is dependent on the type of stimulator used.

Hence, CGA promotes the viability of THP-1 cells possibly by intervening in the pathway of LPS induced cell death. To best of our knowledge, no publication has highlighted the protective effect of CGA against human immune-cells, therefore, our work is the first report to identify the protective effect of CGA against THP-1 cells and thus, needs further investigations to identify the possible mechanisms for THP-1 cells protection.

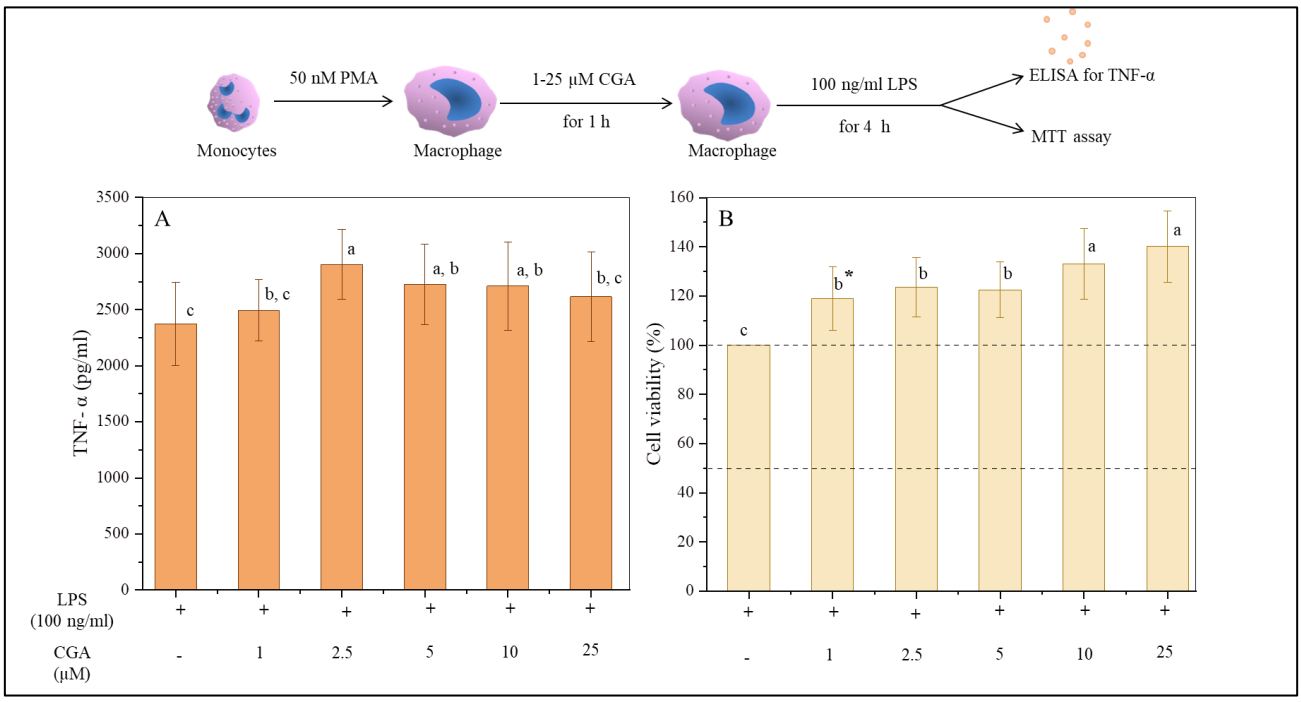


Figure 4.7: Effect of chlorogenic acid (CGA) dose on (A) TNF-α production and (B) cell viability.

THP-1 macrophages were pre-treated with 1-25 µM chlorogenic acid (CGA) for 1 h prior to stimulation with 100 ng/ml LPS for 4 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of six biological replicates and three replicates in each assay with error bars corresponding to standard deviation. *: Data represents the mean of five biological replicates. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

4.2.3.2 Effect of carrier vehicle on LPS stimulated THP-1 macrophages

The solvent used to dissolve the bioactive compounds may interfere with their activity and thus the results obtained could be misleading. In this study, two different types of vehicles were used: dimethyl sulfoxide (DMSO) was used to dissolve curcumin and CGA while a combination of DMSO and ethanol (1:1) was used to solubilise lupeol. To investigate the

influence of the vehicle on THP-1 monocytes, several concentrations of DMSO and DMSO + ethanol were studied. As can be seen in Figure 4.8, with an increase in the concentration of either of the vehicles a small decrease in TNF-α level and cell viability was observed. The small decrease in TNF-α production may be caused by cell death or possibly by the small anti-inflammatory effect of DMSO (Elisia et al., 2016). However, this decrease was small thus the results discussed in Section 4.2.3.1 were considered free from any influence from the respective vehicle used.

4.2.3.3 Effect of combined treatment of curcumin + lupeol and curcumin + CGA on LPS stimulated THP-1 macrophages

To study the effect of combinations of curcumin with lupeol and CGA, the concentration of curcumin was fixed at 5 µM. Further, based on the results obtained in section 4.2.3.1 for the effect of lupeol and CGA on suppressing TNF-α level and cell viability (Figure 4.6 and Figure 4.7) three different concentrations (2.5, 5 and 10 µM) were selected to blend with curcumin. THP-1 macrophages were treated with three concentrations of each: curcumin + lupeol and curcumin + CGA (at ratios of 1:0.5, 1:1 and 1:2) for 1 h. THP-1 cells treated with various concentrations of curcumin or lupeol or CGA alone and cells without any bioactive compound treatment were the controls.

As shown in Figure 4.9A, the combined effect of curcumin and lupeol significantly ($p \leq 0.05$) reduced the TNF-α level when compared with curcumin and lupeol alone treated controls. 5 µM curcumin and 2.5 µM lupeol alone were able to reduce TNF-α production by 15 % and 12 % TNF-α level respectively, while their combined treatment resulted in about 30 % reduction. As the concentration of lupeol was increased from 2.5 to 10 µM, in the combination treatments, the amount of TNF-α produced decreased from 4.3±1.57 ng/ml to 3.24±1.18 ng/ml. At a 1:2 ratio of curcumin to lupeol, about 50 % reduction was observed in TNF-α level which was significantly different ($p \leq 0.05$) from 10 µM lupeol alone (4.74±1.99 ng/ml) and 5 µM curcumin alone (5.33±2.21 ng/ml) controls. These results suggested a small synergistic effect of curcumin + lupeol treatment on TNF-α reduction. However on considering the effect of combined treatments on cell viability (Figure 4.9B) the synergistic effect on TNF-α reduction was found to be misleading because the decrease in TNF-α level was influenced by cell death. For example, 5 µM curcumin and 5 µM lupeol alone resulted in about 21±6.17 % and 15±9.93 % cell death respectively, while their combination resulted in about 37±10.61 % cell death. Thus, the combination of curcumin with lupeol suggested an

additive effect on reducing cell viability, hence was not considered suitable for any further analysis.

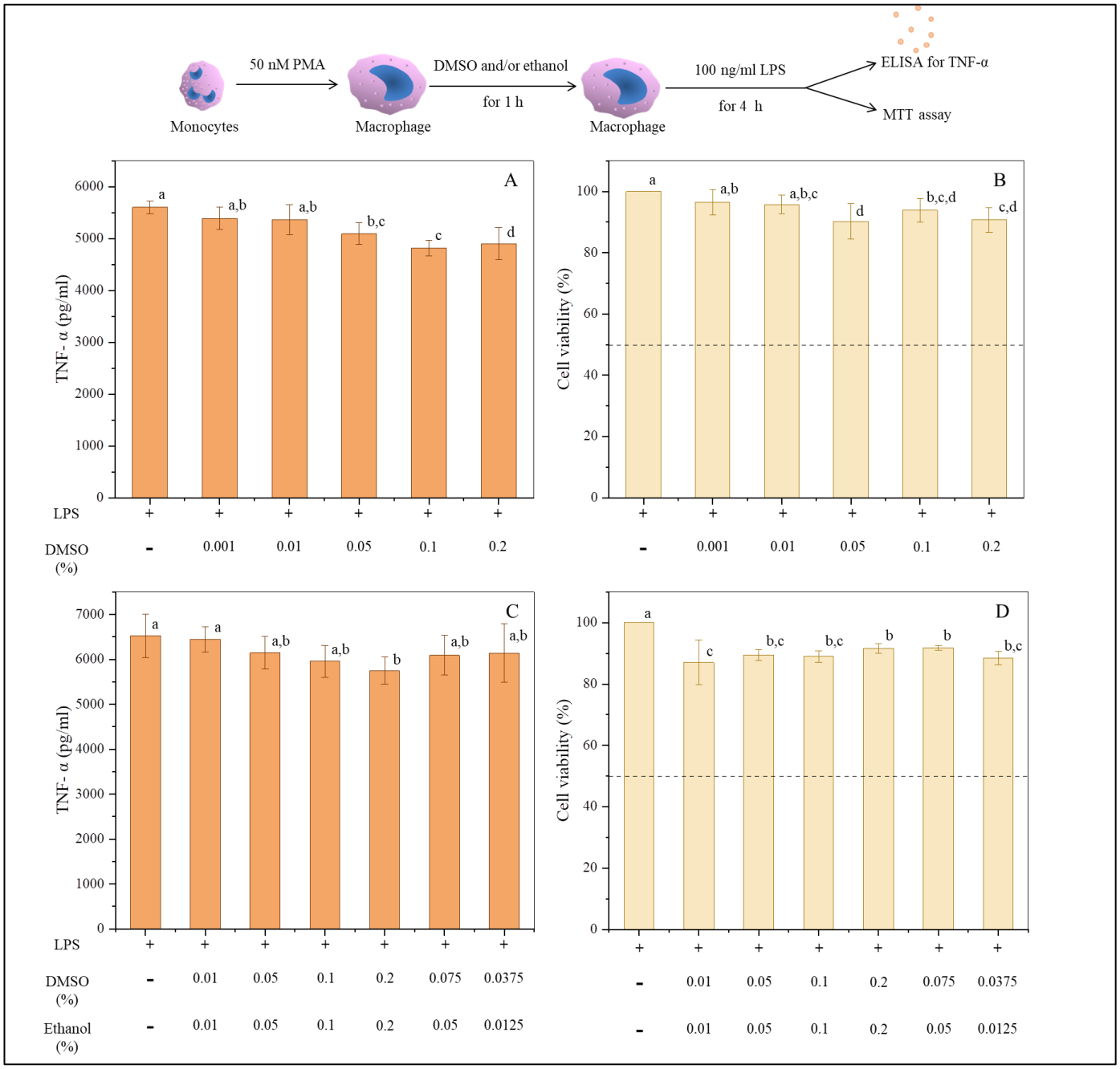


Figure 4.8: Effect of carrier vehicles on (A,C) TNF-α production and (B,D) cell viability. THP-1 macrophages were pre-treated with different concentrations of Dimethyl sulfoxide (DMSO) (A and B) and DMSO + ethanol (C and D) for 1 h prior to stimulation with 100 ng/ml LPS for 4 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of two biological replicates and three replicates in each assay with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

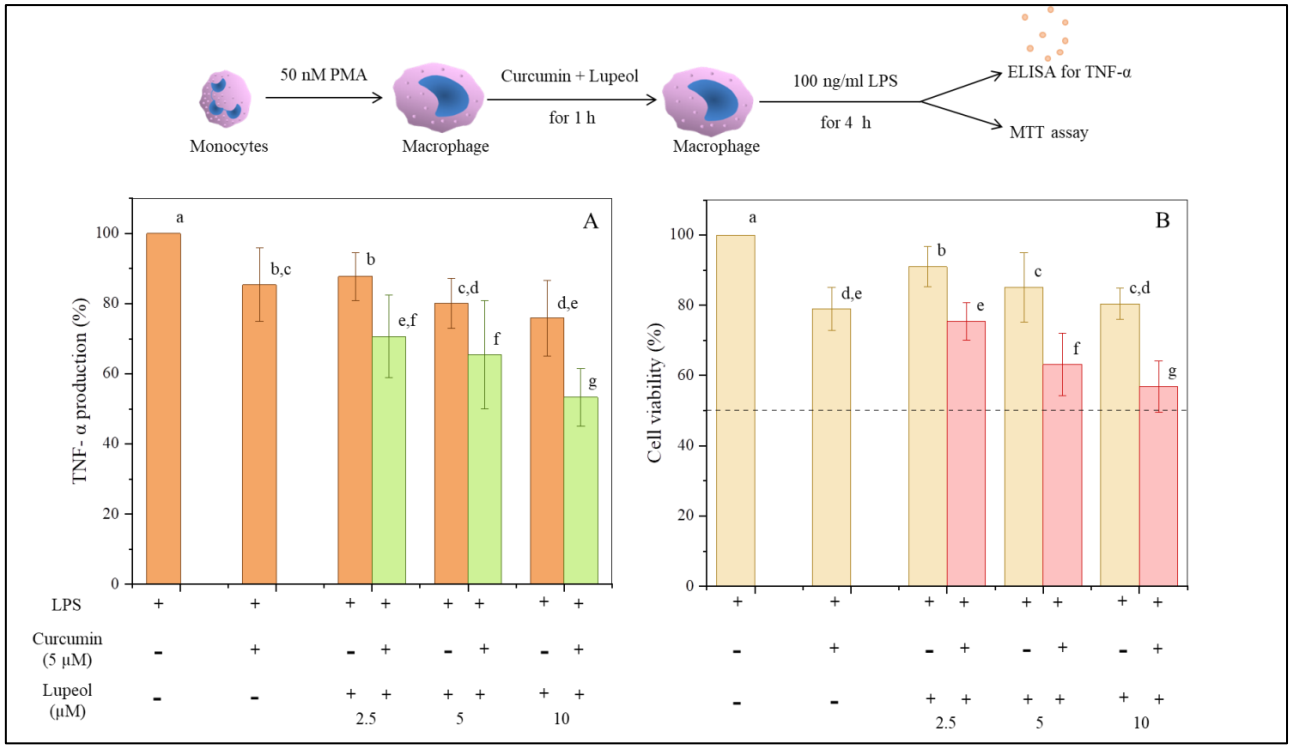


Figure 4.9: Effect of curcumin + lupeol on (A) TNF-α production and (B) cell viability. THP-1 macrophages were pre-treated with curcumin + lupeol for 1 h prior to stimulation with 100 ng/ml LPS for 4 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of six biological replicates and three replicates in each assay with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

The results obtained for the effects of combined treatment of curcumin and CGA on TNF-α production and cell viability are shown in Figure 4.10. Co-administration of curcumin and CGA at ratios of 1:0.5 and 1:2 resulted in about 9 % and 6 % reduction in TNF-α level respectively. This decline in TNF-α level was less than that caused by 5 µM curcumin alone (≈ 16 %). However, at 1:1 ratio, TNF-α level dropped to 2.95±1.17 ng/ml from 3.98±1.80 ng/ml accounting for about 25 % reduction. The decrease in TNF-α production at 5 µM curcumin + 5 µM CGA was significantly lower ($p \leq 0.05$) than the controls. Moreover, for all the combination treatments the cell viability was significantly ($p \leq 0.05$) higher than to that of 5 µM curcumin treated control. Hence, combining equal quantities of curcumin and CGA was found to be effective in reducing both TNF-α production and cell death.

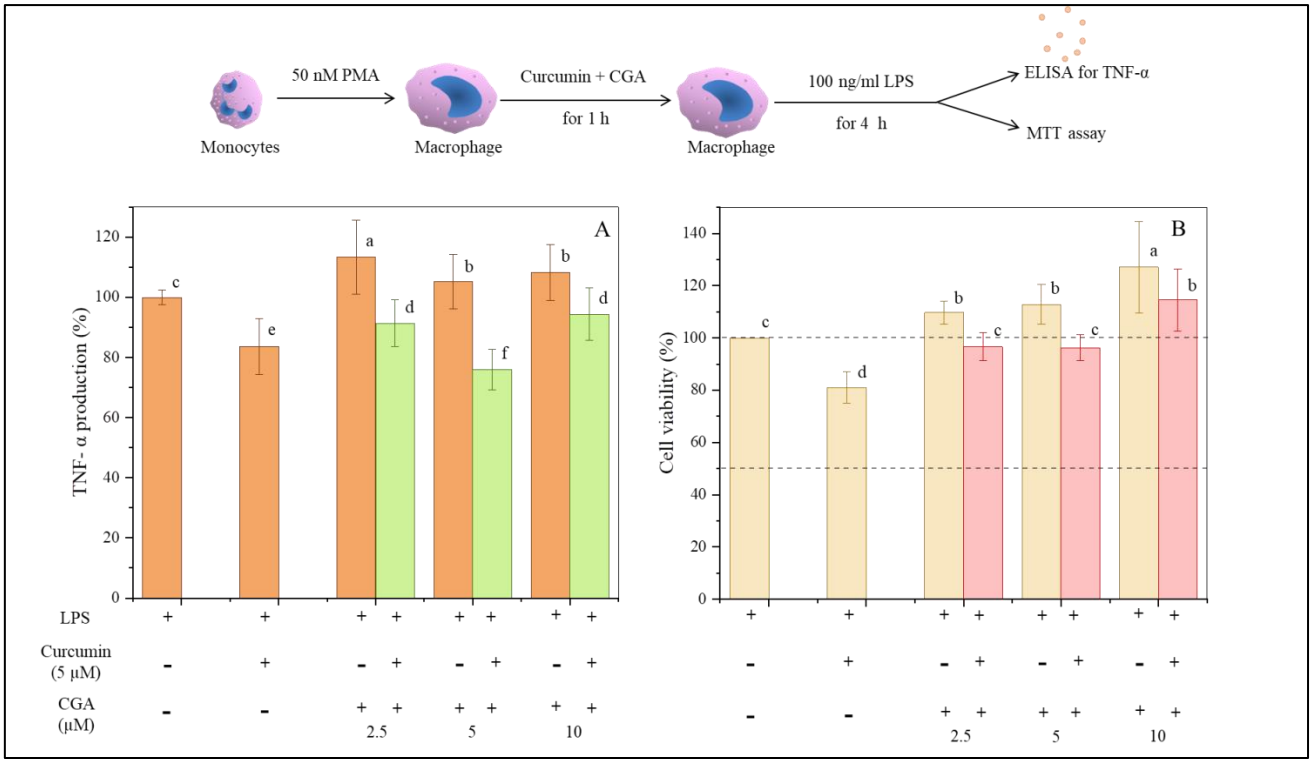


Figure 4.10: Effect of curcumin + chlorogenic acid (CGA) on (A) TNF-α production and (B) cell viability.

THP-1 macrophages were pre-treated with curcumin + CGA for 1 h prior to stimulation with 100 ng/ml LPS for 4 h at 37°C and 5 % CO_2. The TNF-α level was quantified using ELISA and cell viability was analysed using MTT assay. **Note:** Data represents the mean of nine biological replicates and three replicates in each assay with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 5.

4.2.4 Effect of curcumin, CGA and their combination on the NF-κB signalling pathway

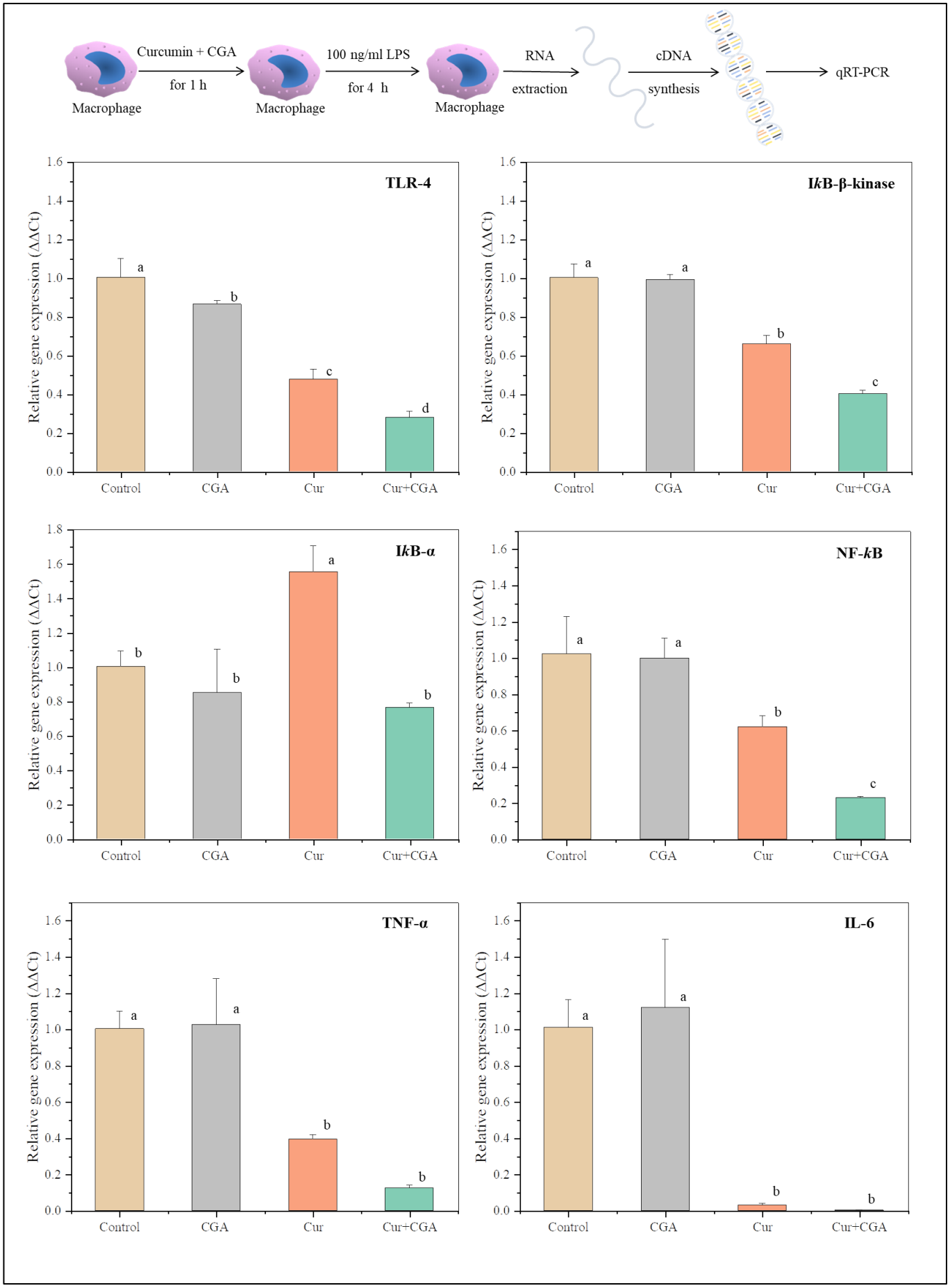
Along with TNF-α, several other pro-inflammatory cytokines such as IL-6, anti-inflammatory cytokines such as IL-10, and enzymes such as COX-2 and iNOS are also produced as an inflammatory response to infection and stress (Kumar et al., 2012c). The production and expression of the aforementioned inflammatory biomarkers can be controlled by activation of JNK pathway, p38 MAPK pathway, P13k/Akt pathway and NF-κB signalling pathway (Qin et al., 2016). Therefore, the effect of curcumin, CGA and their combination (at 1:1 ratio) on mRNA expression of various inflammatory biomarkers and NF-κB signalling pathway was studied using two step qRT-PCR (Section 3.2.3.6)

The relative mRNA expression for TLR-4, IκB-β-kinase, IκB-α, NF-κB, TNF-α, IL-6, IL-10, COX-2 and iNOS are shown in Figure 4.11. Our results suggest that, other than TLR-4, CGA was not effective in reducing the mRNA expression of inflammatory genes. In contrast,

curcumin could suppress the expression of all biomarkers, except for iNOS. The addition of CGA to curcumin potentiates the anti-inflammatory activity of curcumin. On exposing the cells to LPS, the TLR-4 receptor is activated which then activates IκB kinase (IKK), resulting in phosphorylation and degradation of IκB-α, an inhibitory subunit of NF-κB in the cytoplasm (Shen et al., 2012). Treatment with CGA and curcumin alone could suppress the expression of TLR-4 receptor by ≈13 % and ≈52 % respectively and their combination suppresses it by ≈72 %. Curcumin alone downregulates the expression of IκB-β-kinase and NF-κB, and on combining curcumin with CGA an additional significant ($p \leq 0.05$) decrease of ≈39 % and ≈63 %, respectively, in gene expression was observed. Similar decreases in NF-κB expression and production on treatment with curcumin have been reported previously (Bachmeier et al., 2007; Meng et al., 2013; Murakami et al., 2008; Pan et al., 2000). The combination of curcumin and CGA was not effective in reducing IκB-α expression.

Upon activation, NF-κB is translocated into the nucleus where it can influence the transcription of more than 500 inflammation-related genes (Buhrmann et al., 2011). The treatment with the combination of bioactive compounds was also able to downregulate the expression of NF-κB regulated genes. The expression of pro-inflammatory cytokines such as TNF-α and IL-6, was decreased by the combination treatment but was not significantly different ($p \leq 0.05$) from curcumin only treatment. It was expected that curcumin and CGA would promote the expression of anti-inflammatory cytokine such as IL-10, but in fact, the opposite result was obtained. The production of IL-10 is controlled by NF-κB, thus a decrease in NF-κB expression might have knocked out IL-10 expression. A similar reduction was observed in the mRNA level of COX-2, a key enzyme involved in arachidonic acid metabolism. In contrast, the expression of iNOS, an enzyme that initiates the production of RNS to kill bacteria in phagocytes, was upregulated by curcumin alone and curcumin + CGA treatment. Curcumin alone increased the expression of iNOS by ≈80 % while an increase of ≈285 % was observed in the presence of both curcumin and CGA. The response of curcumin, CGA and their combination (1:1) on NF-κB signalling pathway is summarised in Figure 4.12.

In addition to measuring mRMA expression levels, quantification of protein translated from their respective mRMA may enhance our understanding regarding the mechanisms of action of the bioactive compounds used in this study. However, investigating the effect of bioactive compounds on protein expression was beyond the scope of this project.


Macrophage
Curcumin + CGA
for 1 h
Macrophage
100 ng/ml LPS
for 4 h
Macrophage
RNA
extraction
cDNA
synthesis
qRT-PCR
TLR-4
IκB-β-kinase
IκB-α
NF-κB
TNF-α
IL-6
Relative gene expression (ΔΔCt)
Control
CGA
Cur
Cur+CGA

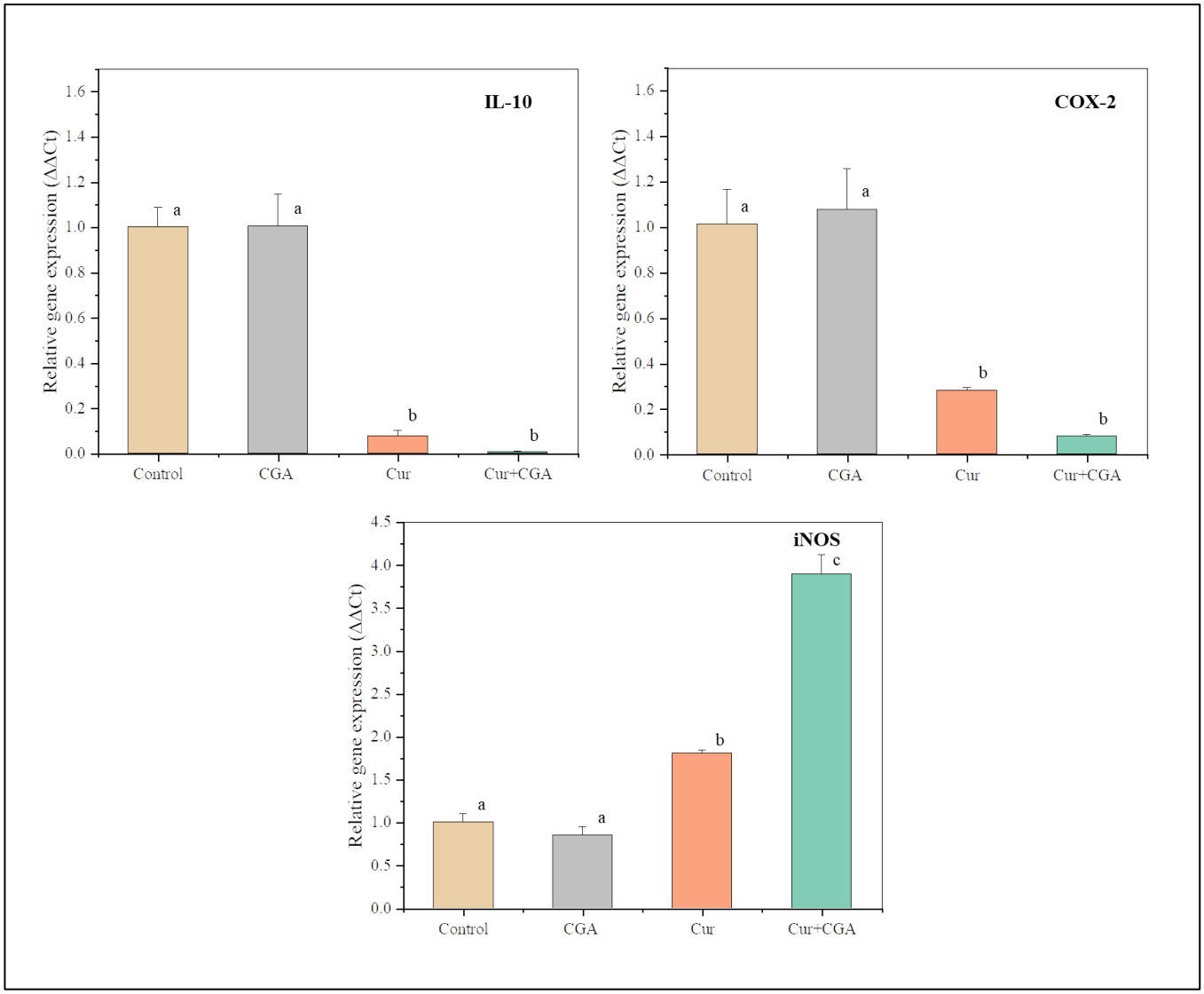


Figure 4.11: Effect of curcumin, chlorogenic acid (CGA) and their combination on mRNA expression of several inflammatory biomarkers and NF-κB signalling pathway.

THP-1 macrophages were pre-treated with curcumin (Cur), chlorogenic acid (CGA) and 1:1 ratio of Cur + CGA for 1 h prior to stimulation with 100 ng/ml LPS for h at 37°C and 5 % CO_2. The mRNA expression was quantified using qRT-PCR. **Note:** Data represents the mean of three biological replicates with error bars corresponding to standard deviation. Samples that do not share the same letters are significantly different ($p \leq 0.05$). Raw and statistically analysed data are shown in Appendix 6.

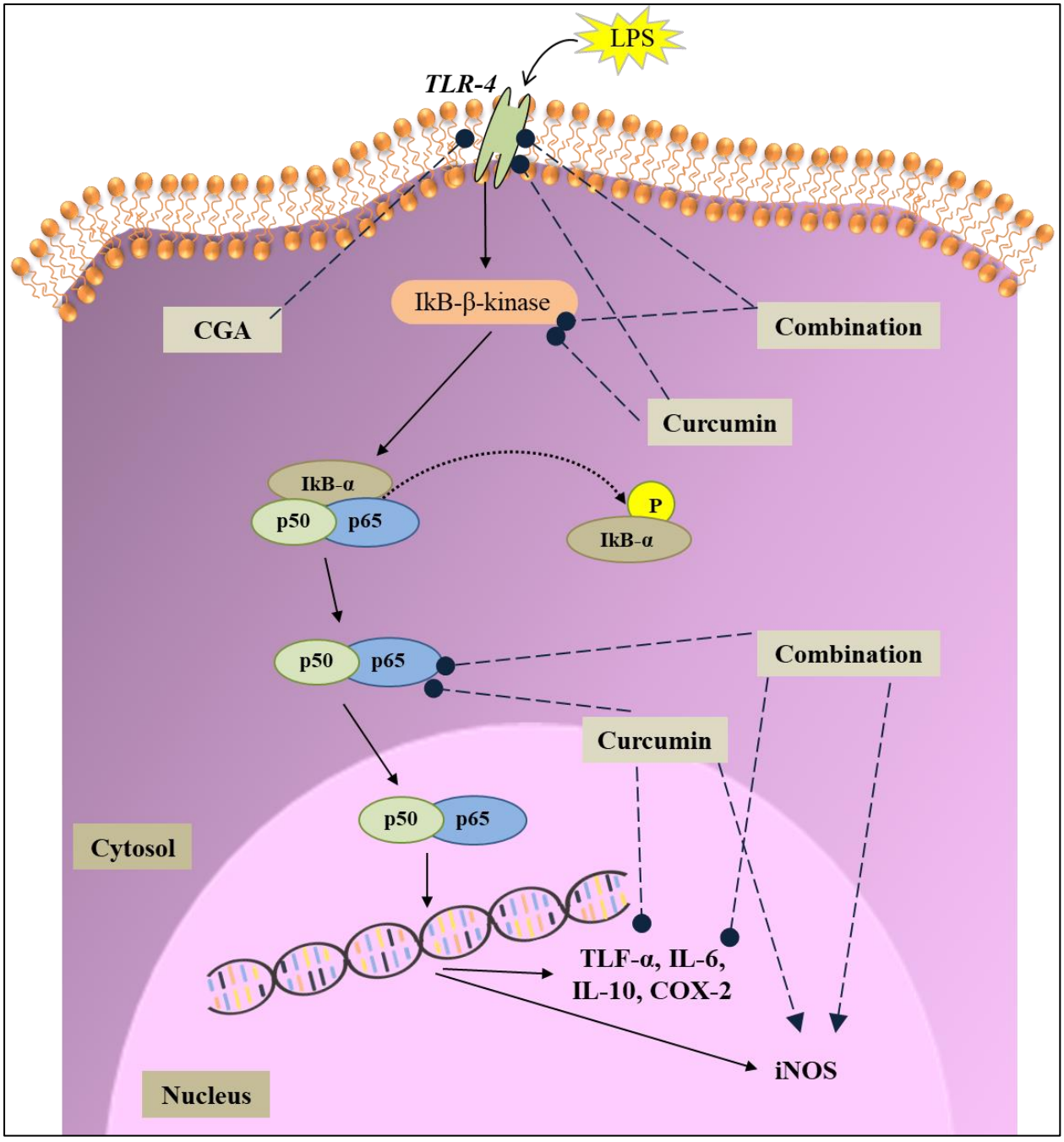


Figure 4.12: Response of curcumin, chlorogenic acid (CGA) and their combination on mRNA expression of NF-κB signalling pathway.

LPS: lipopolysaccharide; TLR-4: toll-like receptor-4; IκB-α: inhibitor κB-α; TNF-α: tumour necrosis factor-α; IL: interleukin; COX-2: cyclooxygenase-2; iNOS: inducible nitric oxide synthase.

----► means upregulations and ----● means downregulations of mRNA level

4.3 Summary

In this study, the anti-inflammatory potential of curcumin, lupeol and CGA (both individually and in combination) was studied using an *in vitro* model of inflammation using THP-1 macrophages. The results suggest that curcumin and lupeol alone were able to downregulate TNF-α production, but also caused a slight decrease in cell viability of LPS stimulated THP-1 macrophages. On combining these two bioactive compounds a large reduction in TNF-α levels was observed, however, this reduction was due to cell death rather than an anti-inflammatory property. In contrast, CGA protected the THP-1 cells from LPS toxicity and co-administration of CGA and curcumin in a 1:1 ratio significantly suppressed TNF-α production without any significant decrease in cell viability. Further, qRT-PCR results indicate that CGA can potentiate the effect of curcumin. The combination was effective in reducing mRNA expression of pro-inflammatory cytokines and COX-2 enzyme possibly by the suppression of NF-κB, IκB-β-kinase and TLR-4 receptor at the mRNA level. Thus, combining CGA and curcumin in 1:1 ratio was considered optimum for introducing an anti-inflammatory effect and this ratio was selected for the fortification of coconut cream yogurt.

CHAPTER 5

Development of Coconut Cream Yogurt Fortified with Curcumin and Chlorogenic Acid

5.1 Introduction

The popularity of functional foods developed using natural ingredients have increased among the consumers looking for healthier products (Granato, Nunes, & Barba, 2017). To cater for this demand, researchers have been focusing on developing food matrices to deliver healthy bioactive compounds destined for human consumption, particularly *via* dairy products (Ndife, Idoko, & Garba, 2014). Yogurt, a fermented dairy product, is consumed across the globe not only for its appealing taste but also for its nutritional value and health-promoting benefits (Saint-Eve, Lévy, Martin, & Souchon, 2006). The presence of peptides and probiotics in yogurt can enhance the immune system, improve digestion, reduce serum cholesterol levels and protect against colon cancer when consumed in adequate amounts (El-Abbadi, Dao, & Meydani, 2014; Helal & Tagliazucchi, 2018; Ndife et al., 2014; Tien et al., 2006; Vijayendra & Gupta, 2012; Weerathilake et al., 2014). The health benefits of yogurt can further be improved by fortifying with bioactive compounds such as resveratrol from grapes, EGCG from green tea, curcumin from turmeric and CGA from coffee.

Traditionally, yogurt is prepared by fermentation of mammalian milk by lactic acid bacteria such as *S. thermophilus* and *L. bulgaricus* (Fadela, Abderrahim, & Ahmed, 2009; Weerathilake et al., 2014). However, because of the limited available options for lactose intolerant individuals, attempts are being made to develop yogurt from non-dairy sources such as soy milk, rice milk, almond milk, legume milk and coconut milk (Jiménez-Martínez, Hernández-Sánchez, & Dávila-Ortiz, 2003; Dorota Zaręba & Małgorzata Ziarno, 2017). Coconut milk or ‘santan’ refers to the white colour protein-oil-water emulsion extracted from the endosperm of *Cocos nucifers L.* fruit using mechanical force (Narataruksa, Pichitvittayakarn, Heggs, & Tia, 2010; Yaakob, Ahmed, Daud, Malek, & Rahman, 2012). Coconut milk contains 55.1-74.9 % moisture, 2-4 % protein, 20-35 % fat, 2.7-4.7 % carbohydrates and 0.6-1 % ash (Shana, Sridhar, Roopa, Varadaraj, & Vijayendra, 2015; Singh, 2018; Tansakul & Chaisawang, 2006). The presence of sucrose, starch, amino acids

such as lysine, methionine and tryptophan, and minerals such as calcium, phosphate, magnesium and potassium make coconut milk a suitable medium for the growth of lactic acid bacteria (Yuliana, Rangga, & Rakhmiati, 2010).

This chapter investigated the development of coconut yogurt fortified with coffee and turmeric to potentially deliver the anti-inflammatory benefits derived from CGA and curcumin respectively. Yogurt seems to be a good medium for the delivery of curcumin and CGA due to its low pH post-fermentation, which increases the stability of bioactive compounds during storage (Chouchouli et al., 2013; Helal & Tagliazucchi, 2018). The presence of fat and protein help maintain the integrity of bioactive compounds during digestion thereby improving their bioavailability (Helal & Tagliazucchi, 2018; Jakobek, 2015; Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014). Briefly, in this study, coconut cream was enriched with sufficient coffee and turmeric to result in a 1:1 ratio of CGA and curcumin followed by fermentation using 2 % starter culture at 42±1ºC. The effect of the bioactive compounds on the fermentation process and stability of the fortified coconut yogurt over a 15 days storage period at 4±1ºC was studied following the procedure previously described in Chapter 3.

5.2 Results and discussion

5.2.1 Stage 1: Optimising the amount of bioactive compounds added to coconut cream yogurt

5.2.1.1 Quantification of bioactive compounds present in coffee and turmeric

To deliver the known concentration of bioactive compounds *via* yogurt, it was first necessary to quantify their concentration in coffee and turmeric sources to be used for delivering the bioactive ingredients. CGA is a temperature-sensitive compound and its concentration in instant coffee powder is greatly influenced by the processing conditions and techniques. Roasting is an important step in developing the desired aroma and flavour in coffee, however, with an increase in roasting intensity, the concentration of CGA decreases (Corso, Vignoli, & Benassi, 2016; Fujioka & Shibamoto, 2008). In addition, drying techniques also degrade the CGA. For this study, 3 different types of freeze-dried coffee powders (Table 3.2) were selected because CGA is preserved at low-temperature. Moreover, the selected coffee blends

(gold bend, espresso style and Colombia blend) were previously reported to have high amounts of CGA (Ludwig et al., 2014).

The amount of CGA in the coffee samples was determined by HPLC following interpolation from a standard curve (Appendix 7). The maximum amount of CGA was found in sample C1 with a concentration of 38.15±1.75 mg/g coffee. The C1 sample was a mix of green (unroasted) and roasted beans, of which the unroasted beans may have contributed to high CGA concentration. A similar concentration of CGA (35 mg/g) was reported by Ludwig et al. (2014) in the same brand of coffee. In contrast, the CGA concentrations in samples C2 and C3 were 8.811±0.395 and 12.298±1.01 mg/g, respectively. These two samples were completely roasted, indicating that CGA might have degraded during roasting. A similar amount of CGA in regular ground-coffee was previously reported to range from 2.10 to 17.1 mg/g and the variation was significantly affected by roasting time and temperature (Fujioka & Shibamoto, 2008). Since the highest amount of CGA was detected in sample C1, this sample was selected for addition to yogurt as the desired amount of CGA could be delivered using the least amount of coffee.

Curcumin C^3 complex® AU has been used in several animal and clinical studies and has been reported to successfully deliver the desired health benefits of curcumin (Ganjali et al., 2014; Kanai et al., 2011; Lev-Ari et al., 2005; Panahi, Badeli, Karami, & Sahebkar, 2015; Panahi, Mahboobeh Sadat Hosseini, et al., 2015; Panahi et al., 2016; Ringman et al., 2012). Thus, in this study Curcumin C^3 complex® AU was used to introduce curcumin in yogurt. The amount of curcumin in Curcumin C^3 complex® AU was quantified using HPLC (Appendix 7) and was found to be 790.36± 1.29 mg/g.

5.2.1.2 Sensory characteristics of coconut cream yogurt with added CGA and curcumin

Different samples of coconut yogurt were prepared by adding 100, 150, 200 and 300 mg of each curcumin and CGA per 150 g coconut cream. As the concentrations of curcumin and CGA were increased from 100 to 300 mg, the sensory parameters of the coconut yogurt were adversely affected. A combination of yellow-orange turmeric (for curcumin) with brown coffee (for CGA) resulted in mustard-brown coloured coconut yogurt. All the sensory panellists (n=5) liked coconut yogurt containing the bioactive compounds as the appearance was more natural than the control (without bioactive compound) which appeared artificial. The samples containing 100 and 150 mg of each bioactive compound had a clean and

homogenous appearance. In contrast, samples containing 200 or 300 mg of curcumin and CGA produced a dark, bubbly and heterogeneous appearance. In the latter samples, very distinct dark brown patches and small insoluble granules of curcumin were observed. Phase separation was also noticed in the samples with 300 mg of each the compounds. Thus, based on appearance, the yogurt samples containing 100 and 150 mg of the bioactives were most liked by the panellists.

Upon tasting, all the samples were described as having a creamy to smooth mouthfeel. The 'coconut taste' of all the samples containing the bioactive compounds was less intense than the control, and was well-liked by all the panellists. The bioactive ingredients added to the yogurt may have masked the coconut taste and thus, was less intense in bioactive containing samples. The sample with 100 mg of curcumin and CGA produced a firm and compact gel with a clean flavour of each ingredient. The 100 mg bioactive sample had a balanced sour flavour and no aftertaste. In contrast, the coffee flavour was dominant in the samples with 150, 200 and 300 mg of each bioactive and had a bitter aftertaste. The intensity of coffee flavour and bitterness increased as the concentration of the bioactive compounds increased. The sample with 150 mg of each bioactive produced a thick gel structure similar to the sample with 100 mg of bioactives but had an undesirable aftertaste and lack clean flavour of each ingredient. Samples containing 200 and 300 mg of each bioactive compound resulted in a thin and watery texture which resembled a fermented beverage rather than a set yogurt. Based on the overall sensory profile, panellists indicated that they would buy the yogurt samples with 100 and 150 mg curcumin and CGA but not the other two samples (200 and 300 mg).

The comments received from sensory panellists were used to design a Pugh decision matrix (Table 5.1) which helped in screening the most promising sample based on sensory attributes (Cervone, 2009). The Pugh decision matrix is a quantitative technique commonly used to handle data and choose between a list of alternatives based on specific criteria. In Table 5.1, 1: parameter liked by panellists; -1: parameter not liked by panellists, and 0: parameter neither liked nor disliked by panellists. The highest score of 5 was calculated for coconut yogurt sample with 100 mg each of curcumin and CGA, hence this concentration was selected for further experiments.

Table 5.1: Pugh decision matrix for screening coconut yogurt fortified with curcumin and chlorogenic acid (CGA).

Amount (mg/150g)		Appearance	Flavour	Texture	Taste	Overall Acceptance	Total score
Curcumin	CGA						
100	100	1	1	1	1	1	5
150	150	1	0	1	-1	1	2
200	200	-1	-1	-1	-1	-1	-5
300	300	-1	-1	-1	-1	-1	-5

5.2.1.3 Effect of bioactive compounds on fermentation of coconut cream

(a) Growth of L. bulgaricus and S. thermophilus

The growth of starter culture bacteria i.e. *L. bulgaricus* and *S. thermophilus* was monitored every 2 h during 8 h fermentation and the results are shown in Figure 5.1. As the fermentation time progressed, the viable cell counts (log cfu/g) of both starter culture bacteria increased, but at different rates, with the growth of *S. thermophilus* always being higher than *L. bulgaricus.* At t=0 h, *L. bulgaricus* count was 6.7-6.8 log cfu/g and increased to 7.36-7.44 log cfu/g at the end of fermentation. While *S. thermophilus* grew significantly ($p \leq 0.05$) from 6.88-6.93 log cfu/g (at t=0 h) to 9.64-9.70 log cfu/g within 6 h of fermentation. The viable count of *S. thermophilus* further increased to 9.81-9.85 log cfu/g at t=8 h but was not significantly ($p \leq 0.05$) higher than growth at t=6 h. There was no significant difference ($p \leq 0.05$) in the growth of bacteria in each of the four samples at each time point, indicating that addition of curcumin and CGA to coconut cream did not inhibit bacterial growth.

It is well-documented that the initial phase of fermentation is dominated by *S. thermophilus* and later by *L. bulgaricus* (Aguirre-Ezkauriatza et al., 2008). The starter culture bacteria have symbiotic growth. During fermentation, the proteolytic activity of *L. bulgaricus* produces amino acids such as valine and histidine which are rapidly consumed by *S. thermophilus* for its growth (Horiuchi & Sasaki, 2012; Mahdian & Tehrani, 2007). The fast growth of *S. thermophilus* during fermentation acidifies the environment which limits its own growth but favours the growth of *L. bulgaricus* (Mahdian & Tehrani, 2007; Rajagopal & Sandine, 1990).

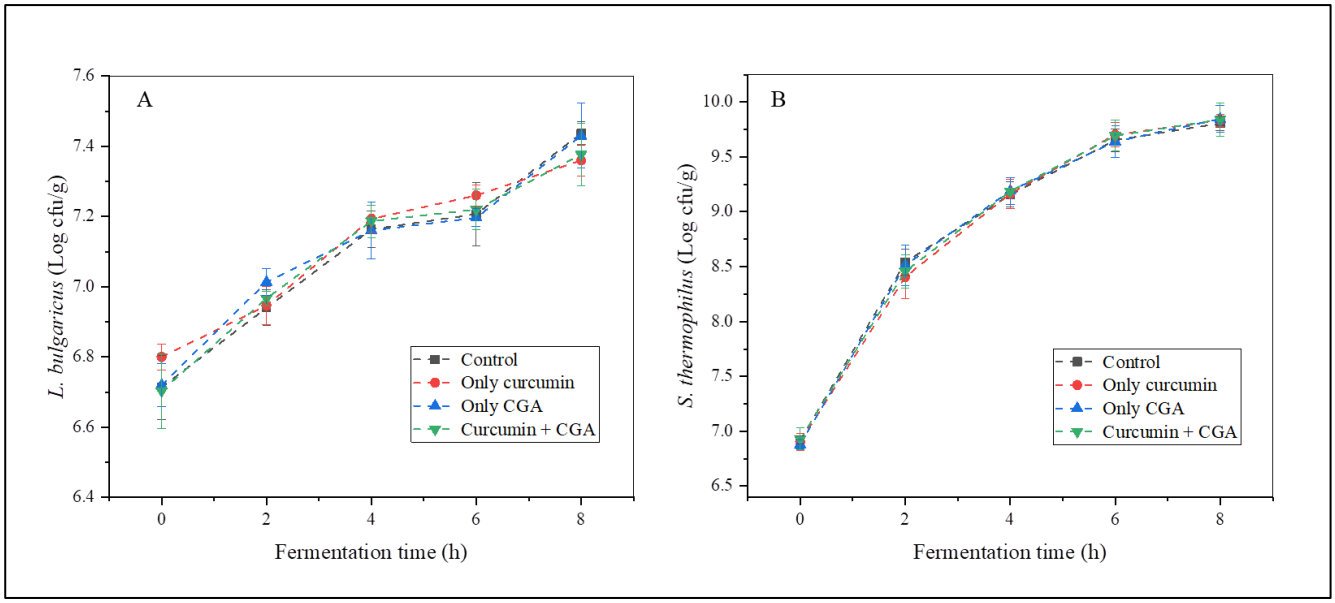


Figure 5.1: Viable cell counts of (A) L. bulgaricus and (B) S. thermophilus in coconut cream yogurt (with or without bioactive compounds) during fermentation at 42±1ºC for 8 h.

Note: Data represents the mean of three biological replicates and three assay replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

(b) Development of acidity

During the fermentation process, *L. bulgaricus* and *S. thermophilus* utilise the sugars in the medium to produce acids thereby creating an acidic environment (Aguirre-Ezkauriatza et al., 2008). Changes in the acidity during fermentation were studied by monitoring pH, and titratable acidity and the results are presented in Figure 5.2. The initial pH of coconut cream was 5.85±0.02, which decreased significantly ($p \leq 0.05$) to 5.71±0.06 and 5.66±0.02 in the samples immediately following the addition of CGA and curcumin + CGA, respectively, at t=0 h. However, the addition of curcumin did not produce any significant ($p \leq 0.05$) decrease in the initial pH of coconut cream. As fermentation progressed, a drop in pH was observed for all the samples (Figure 5.2A). This decrease in pH could be accounted to an increase in the viable cell counts of *L. bulgaricus* and *S. thermophilus*. By the end of 8 h of fermentation, the pH for all the samples was between 4.35-4.44. According to the Food Standard Code, the pH of the yogurt sample should not exceed 4.50 in order to maintain food safety (Donkor, Henriksson, Vasiljevic, & Shah, 2006). In addition, a decrease in pH is important for gel formation because at isoelectric point (pH 4.6 for casein) the electric repulsion decreases, thereby facilitating protein-protein interactions (Lee & Lucey, 2010).

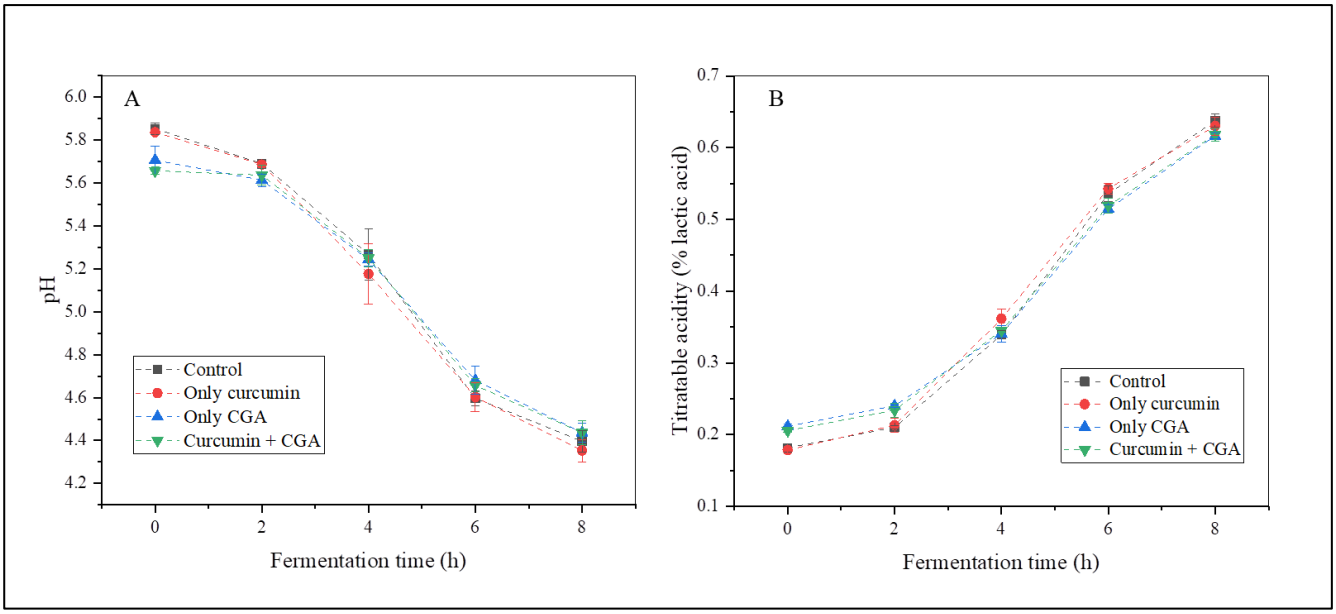


Figure 5.2: Changes in (A) pH and (B) titratable acidity of *coconut cream yogurt (with or without bioactive compounds) during fermentation at 42±1ºC for 8 h.*

Note: Data represents the mean of three biological replicates and three assay replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

The titratable acidity of yogurt refers to the amount of lactic acid and other organic acids produced by the action of starter bacteria on sugar during fermentation (Tamime & Robinson, 2007), and it is inversely proportional to pH (Güler-Akın & Akın, 2007). At t=0 h, titratable acidity for the samples with CGA and CGA+ curcumin was 0.21 % which was significantly ($p \leq 0.05$) higher than the sample with curcumin alone or the control (without bioactive compound). In addition to CGA, other organic acids such as citric and acetic acids present in coffee powder may have contributed to the initially higher titratable acidity (Tan & Korel, 2007). However, during fermentation, the titratable acidity for all the samples increased (Figure 5.2B) to 0.62-0.64 % after 8 h. Similar trends in change in pH and titratable acidity were previously reported (Horiuchi & Sasaki, 2012; Jaziri, Ben Slama, Mhadhbi, Urdaci, & Hamdi, 2009; Rysstad, Knutsen, & Abrahamsen, 1990).

(c) Change in colour

Fortification of coconut cream with curcumin and CGA resulted in visual colour changes (Figure 5.3). Addition of curcumin imparted a yellow-orange colour whereas CGA produced a brown colour to the white coconut cream. The colour of samples with curcumin and curcumin + CGA altered throughout the fermentation, while the colour of the control and

CGA sample was relatively stable. The change in colour was monitored by measuring the L*, a* and b* values (Figure 5.4). L* referrers to the lightness (100) and darkness (0), a* indicates red (positive value) and green (negative value) colour and b* indicates yellow (positive value) and blue (negative value) colour (Tan et al., 2018). L* values were stable in all the samples throughout 8 h of fermentation. The control and curcumin alone samples were very bright (L*>90) but the brightness was reduced (L*<75) in samples containing CGA and curcumin + CGA (Figure 5.4A). a* and b* values for control and CGA samples were stable during fermentation (Figure 5.4B,C). However, a* and b* values for the samples with added

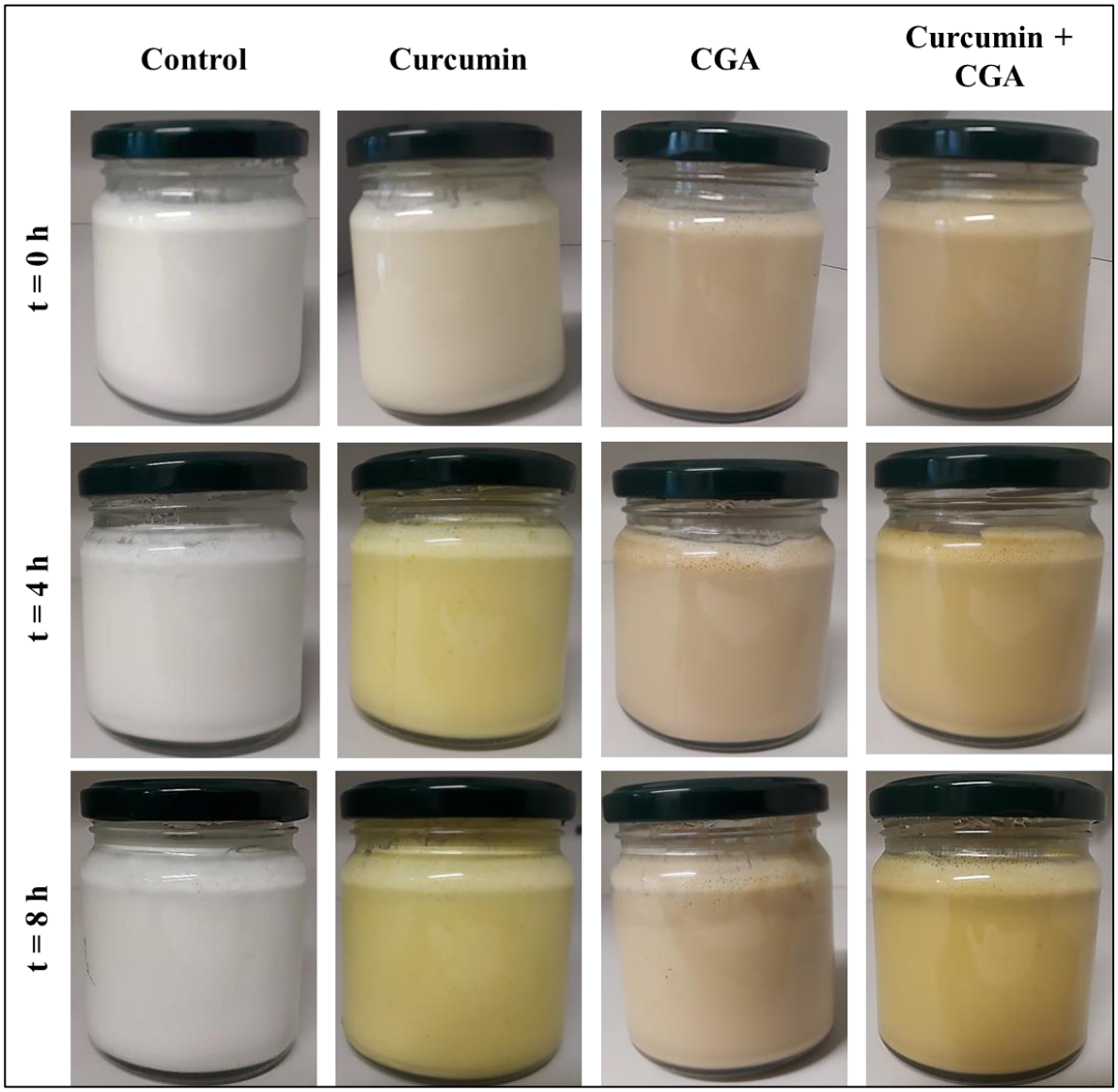


Figure 5.3: Visual changes in the colour of coconut cream yogurt (with or without bioactive compounds) during fermentation at 42±1ºC for 8 h.

curcumin changed from -3.22±0.08 to -8.79±0.15 and 18.56±0.01 to 29.83±0.32, respectively, between 0 to 8 h of fermentation. As observed in section 5.2.1.3(b), as the fermentation time progressed, the pH decreased and the change in pH affects the stability and solubility of curcumin in the medium (Nelson et al., 2017) which may have an influence on a* and b* values. A similar trend in a* and b* values was observed for curcumin + CGA sample which might be due to the presence of curcumin.

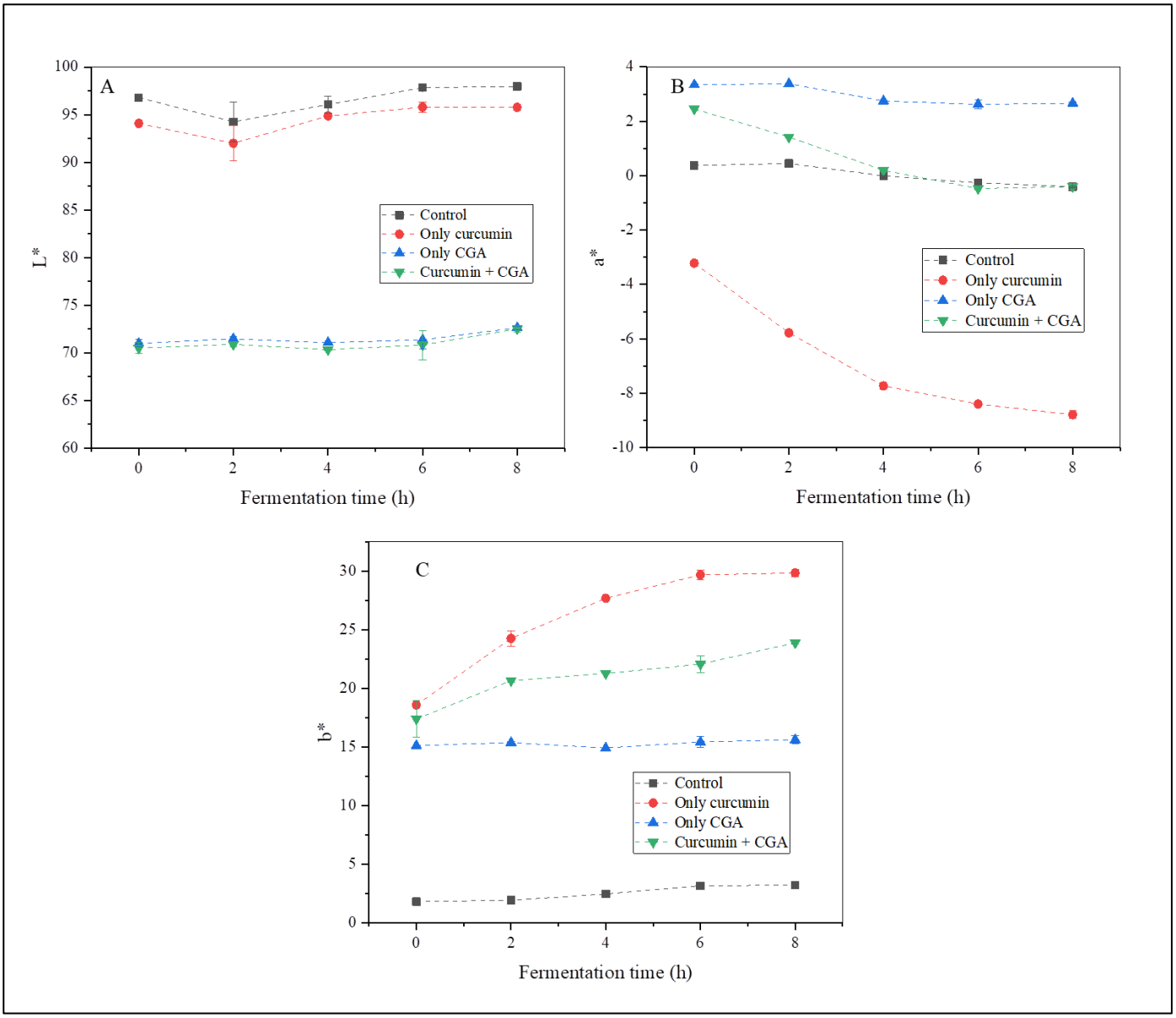


Figure 5.4: Changes in (A) L, (B) a* and (C) b* values of coconut cream yogurt (with or without bioactive compounds) during fermentation at 42±1ºC for 8 h.*

Note: Data represents the mean of three biological replicates and three assay replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

5.2.2 Stage 2: Stability of coconut yogurt with added bioactive compounds during storage at 4±1°C for 15 days

(a) Survival of L. bulgaricus and S. thermophilus

Viable cell counts for *L. bulgaricus* and *S. thermophilus* during storage are shown in Figure 5.5. A decrease in the bacterial count was observed in the control and the bioactive containing samples. Survival of starter culture bacteria in the yogurt sample can be influenced by several factors including pH, presence of dissolved oxygen, duration of fermentation, production of acids, storage time, and temperature, and availability of sugars and nutrients (Azizkhani & Parsaeimehr, 2018; Sharma & Singh Saharan, 2014; Zaręba & Ziarno, 2017). In general, viable cell counts decreases during refrigerated storage (Yaakob et al., 2012). However, it has been suggested that the yogurt samples should contain at least 10^6 cfu/g of cells by the end of shelf-life (FAO/WHO, 2001). In our study, coconut yogurt supplemented with curcumin and CGA had more than 10^8 cfu/g *S. thermophilus* and 10^6 cfu/g *L. bulgaricus* at the end of 15 days refrigerated storage period.

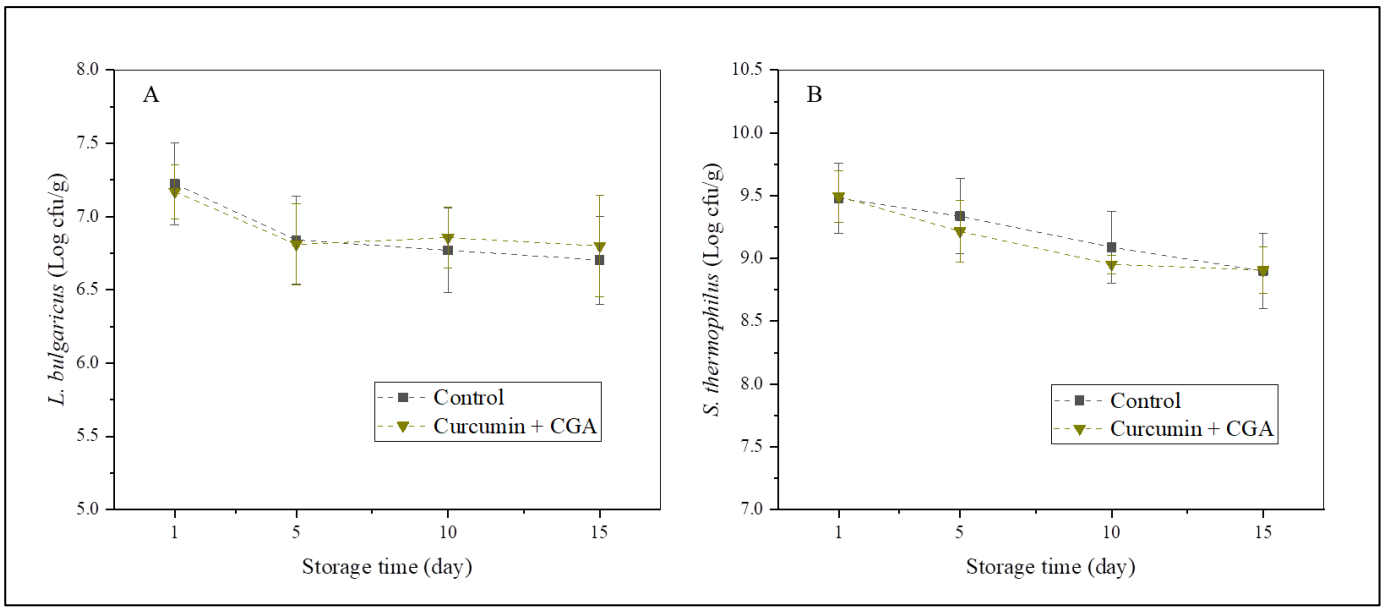


Figure 5.5: Viable cell counts of (A) L. bulgaricus and (B) S. thermophilus in coconut cream yogurt (with or without bioactive compounds) during storage at 4±1ºC for 15 days.

Note: Data represents the mean of three biological replicates and three assay replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

In the control sample, *L. bulgaricus* decreased from 7.223±0.279 to 6.840±0.301 log cfu/g within 5 days of storage. However, a significant ($p \leq 0.05$) decrease to 6.703±0.3 log cfu/g was observed only on day 15. Further, the decline in cell counts for *S. thermophilus* was not significant ($p \leq 0.05$) at day 15. The consistent decrease in bacterial count could be due to a decrease in fermentable sugars and the increase in acidity of the yogurt (Tavakoli, Habibi Najafi, & Mohebbi, 2019). Several studies have previously reported similar decreases in starter culture bacteria during storage at refrigerated conditions (Güler-Akın & Akın, 2007; Ng, Yeung, & Tong, 2011; Turgut & Cakmakci, 2018; Zaręba & Ziarno, 2017). A similar trend of decrease in the bacterial count was observed in coconut yogurt containing curcumin + CGA, hence the addition of these bioactive compounds did not appear to have any effect on the survival of *L. bulgaricus* and *S. thermophilus*.

(b) Acidity

During storage, small changes in pH and titratable acidity were observed (Figure 5.6). The pH of the control and bioactive containing samples significantly ($p \leq 0.05$) decreased, while the titratable acidity significantly ($p \leq 0.05$) increased. The pH and titratable acidity of the control sample changed from 4.387±0.029 to 4.310±0.016 and 0.650±0.006 to 0.683±0.006 %, respectively, between day 1 and day 15. While in the sample with curcumin + CGA the pH decreased from 4.410±0.022 to 4.333±0.025 and titratable acidity increased from 0.626±0.004 to 0.66±0.004 % by the end of the storage period. These changes in acidity were most likely due to the active metabolism of bacteria that utilises sugars to produce acids post-fermentation (Dai, Corke, & Shah, 2016). Since the changes in pH were same for all the samples, it may be concluded that curcumin + CGA did not have any impact on the acidity during storage.

Similar results for acidity have been previously reported in yogurt-related studies (Dabija, Codină, Ropciuc, Gâtlan, & Rusu, 2018; Mosiyani, Pourahmad, & Eshaghi, 2017; O'Sullivan et al., 2016; Petrotos et al., 2012; Turgut & Cakmakci, 2018). Tan and Korel (2007) reported a decrease in pH from 4.56 to 4.21 and increase in titratable acidity from 1.22 % to 1.42 % by the end of 15 days storage of milk yogurt supplemented with coffee. In comparison, the changes observed in our study were small. Coconut naturally contains minerals that may have provided a buffering capacity and anti-microbial compounds that slow down the growth of starter culture bacteria, thereby reducing the rate of acidification post-fermentation (Lutchman et al., 2006; Singh, 2018).

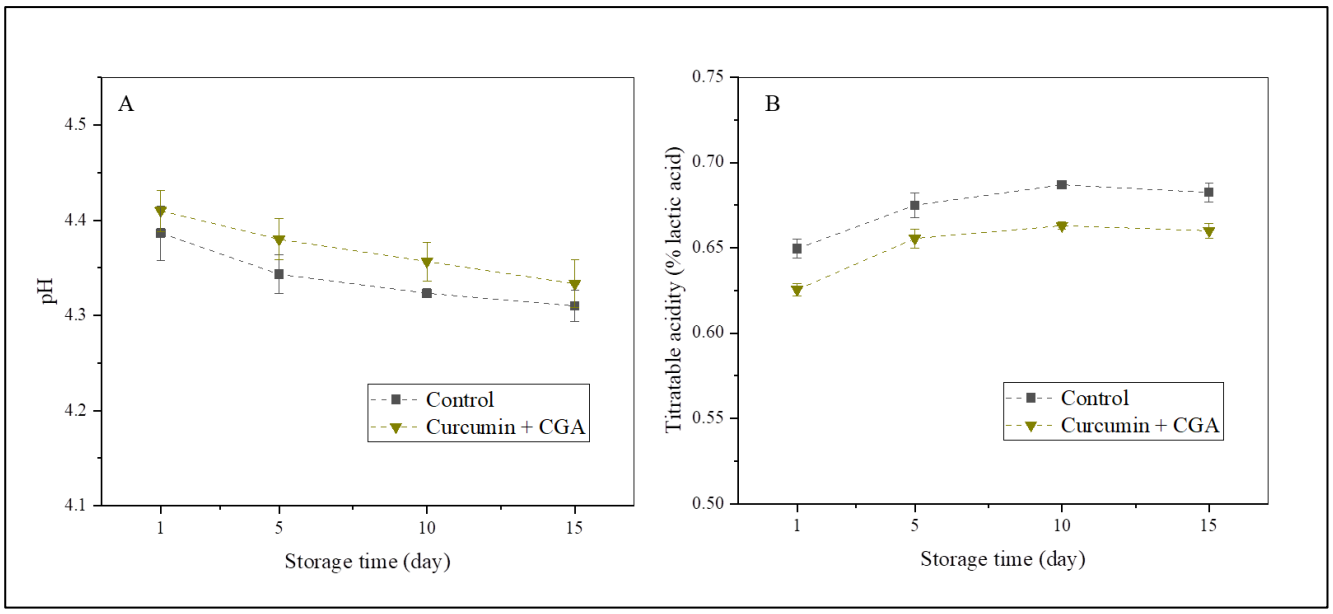


Figure 5.6: Changes in (A) pH and (B) titratable acidity of *coconut cream yogurt (with or without bioactive compounds) during storage at 4±1ºC for 15 days.*

Note: Data represents the mean of three biological replicates and three assay replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

(c) Colour

Colour is an important parameter that may influences the initial judgement of a consumer about purchasing a food product. Changes in colour during storage can indicate visual deterioration of a sample (Coggins, Rowe, Wilson, & Kumari, 2010; Yaakob et al., 2012). The changes in L*, a* and b* values for control and bioactive containing samples are presented in Figure 5.7. During storage, there was no significant change ($p \leq 0.05$) in L* (97.117 to 97.727), a* (-0.350 to -0.430) and b* (3.570 to 4.620) values for the control sample. In contrast, significant changes ($p \leq 0.05$) in a* and b* values were observed on the 10^{th} day of storage in the sample containing curcumin + CGA. The decrease in a* values and increase in b* values indicated that the yogurt sample became more yellow and green during storage. A possible reason for these changes may be the structural changes in light scattering bioactive compounds due to the decrease in pH (Tan et al., 2018). However, the brightness of the yogurt sample with added bioactive compounds was stable ($p \leq 0.05$) during storage.

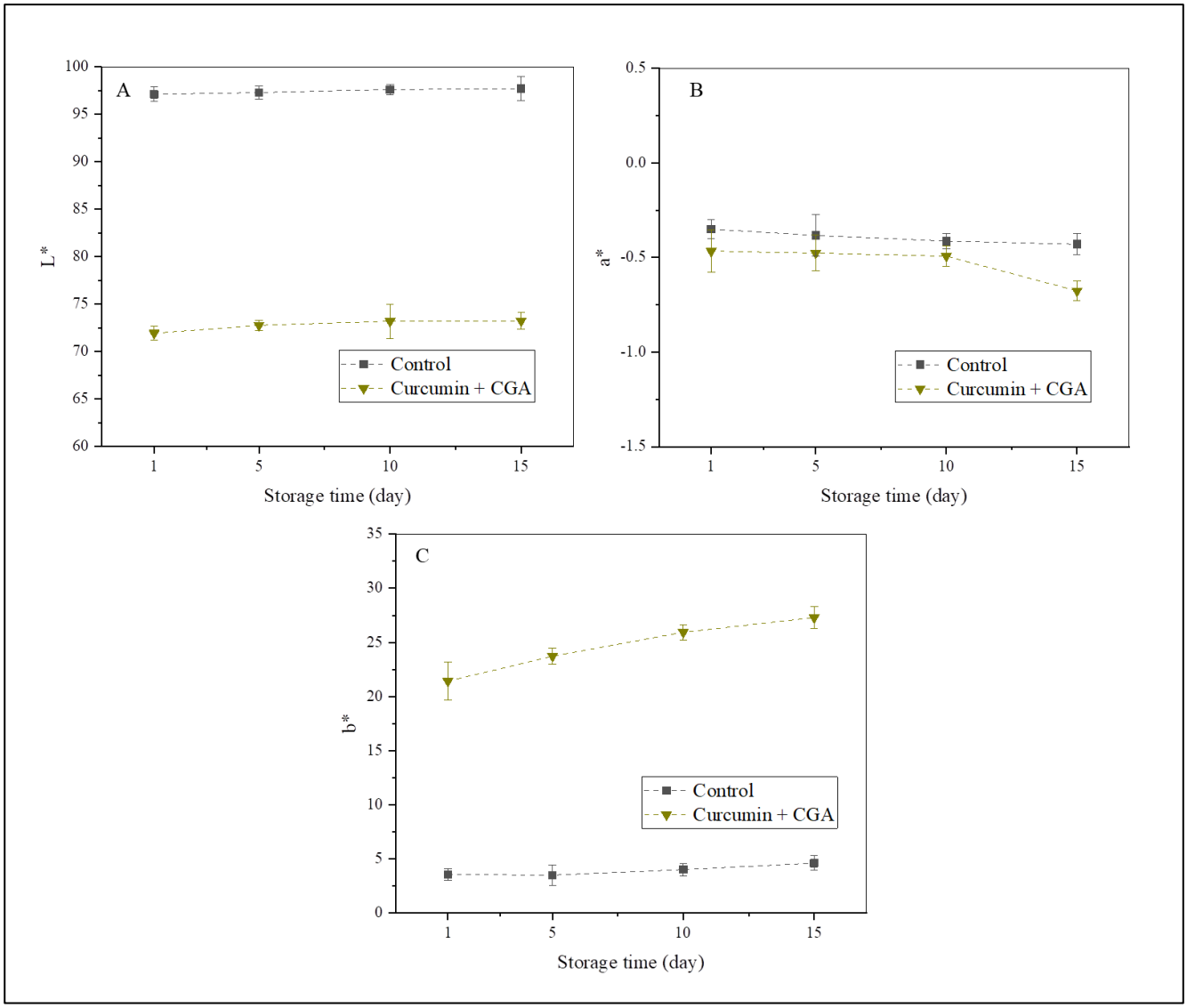


Figure 5.7: Changes in (A) L, (B) a* and (C) b* values of coconut cream yogurt (with or without bioactive compounds) during storage at 4±1ºC for 15 days.*

Note: Data represents the mean of three biological replicates and three assay replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

(d) Syneresis

Syneresis is an important parameter that describes the quality of yogurt during storage (Shakerian et al., 2015). Spontaneous syneresis is the separation of serum without any external force and is related to gel instability and its poor serum retention capacity (Lucey, Munro, & Singh, 1998). Many previous studies have measured syneresis in set yogurt using centrifugation as an external force (Vasiljevic, Kealy, & Mishra, 2007; Zainoldin & Baba, 2009). However, this method is not directly related to spontaneous syneresis for set yogurts

as it measures water-holding capacity and resistance of the gel to external force (Lee & Lucey, 2010). Alternatively, quantifying the spontaneous syneresis of set yogurt by measuring the surface serum that oozed out from the gel is a better method (Lee & Lucey, 2006) and thus was used in this study.

The results obtained for syneresis in the control and the bioactive containing yogurt samples during 15 days of storage are shown in Figure 5.8. No significant change ($p \leq 0.05$) in the serum separation was observed in either of the samples throughout the storage period. However, previous studies have reported that syneresis in set yogurt tends to increase with storage time (Estrada, Boeneke, Bechtel, & Sathivel, 2011; Supavititpatana, Wirjantoro, & Raviyan, 2010; Vasiljevic et al., 2007). The level of syneresis is related to the increase in rearrangements of the gel matrix (Lucey et al., 1998), which can be influenced by several factors such as fermentation temperature, storage time, pH of yogurt and total solids (Vareltzis, Adamopoulos, Stavrakakis, Stefanakis, & Goula, 2016). A small change in pH can disrupt the gel structure by altering the charge on proteins and dissolving the calcium and phosphate ions (Dönmez, Mogol, & Gökmen, 2017; Lucey, 2002). However, in this study despite a significant ($p \leq 0.05$) decrease in pH during storage (section 5.2.2.b) the syneresis level remained stable. One possible reason for this behaviour could be the high total solids (26 % w/w fat and 2% w/w protein) present in the coconut cream. Tavakoli et al. (2019) reported a significant ($p \leq 0.05$) decrease in syneresis of the yogurt samples with an increase in fat content from 0.5-3.5% over 21 days of storage period. A similar decrease in the level of syneresis on increasing total solids has also been reported in several other studies (Amatayakul, Sherkat, & Shah, 2006; Izadi, Nasirpour, Garoosi, & Tamjidi, 2015; Sahan, Yasar, & Hayaloglu, 2008).

The level of syneresis in yogurt samples with added curcumin + CGA was slightly lower than the control sample. This could be possibly due to an increase in total phenolic content. Addition of phenolic compounds has been shown to result in protein-polyphenol interactions which strengthens the gel structure and helps in serum retention (Siebert, Troukhanova, & Lynn, 1996). Dönmez et al. (2017) reported a decrease in the rate of syneresis in milk yogurt on the addition of 1 and 2 % green coffee powder during 21 days of storage.

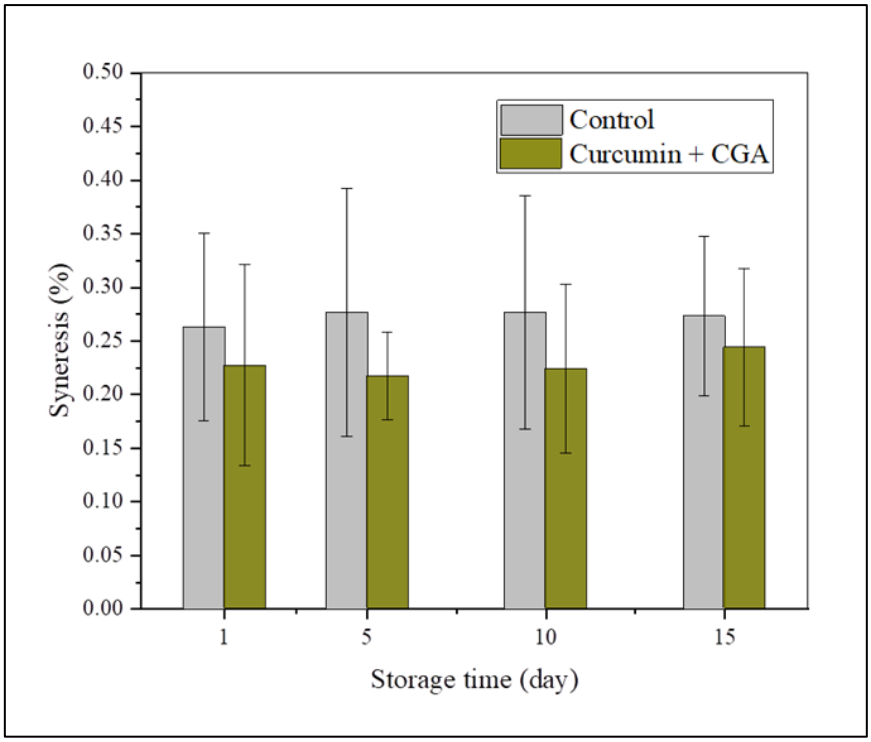


Figure 5.8: Syneresis (%) in coconut cream yogurt (with or without bioactive compounds) during storage at 4±1ºC for 15 days.

Note: Data represents the mean of three biological replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

(e) Firmness

Firmness is another important physical parameter closely related to the gel microstructure (Izadi et al., 2015) that can influence the acceptability of yogurt. The firmness of yogurt samples measured during storage is shown in Figure 5.9. In both samples, a significant ($p \leq 0.05$) increase in gel firmness was observed on the 5th day of storage. In the control sample gel firmness increased from 33.800±0.899 g to 38.757±0.366 g, while in the bioactive containing samples, it increased from 29.183±0.274 g to 36.067±0.149 g towards the end of the storage period. Moreover, the firmness of the control sample was slightly higher than the curcumin + CGA containing sample, indicating a possible negative impact of the bioactive compounds on firmness. Yogurt is a protein gel with entrapped serum, and the gel strength can be affected by the type and amount of fat and protein (Tan et al., 2018). The increase in acidity of yogurt samples (section 5.2.2.b) during storage can influence the net charge on the protein that might cause an increase in gel strength (Singh, 2018). Sahan et al. (2008) reported that gel firmness was not affected during storage in β-glucan added to low-fat yogurt. Several studies have reported a decrease in the strength of set yogurt during

refrigerated storage (Paseephol, Small, & Sherkat, 2008; Salvador & Fiszman, 2004; Tan et al., 2018). However, the reason for an increase in firmness in this study is not fully understood.

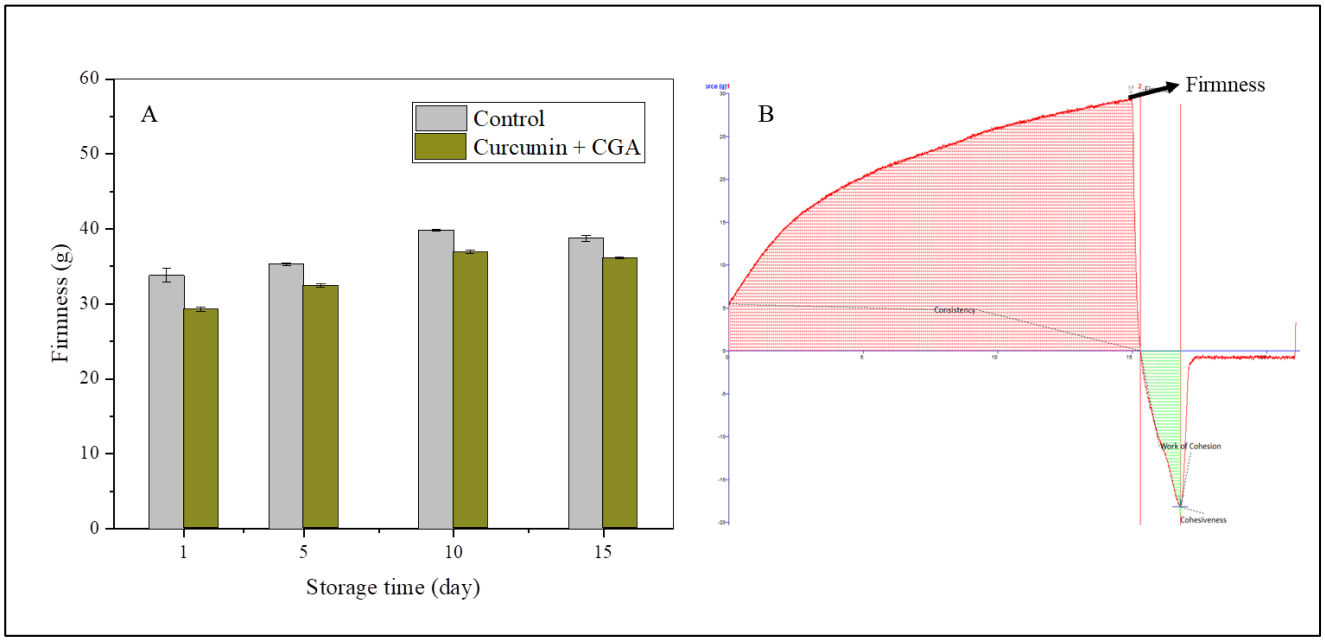


Figure 5.9: Firmness (g) (A) of coconut cream yogurt (with or without bioactive compounds) during storage at 4±1ºC for 15 days. (B) Typical texture profile plot for yogurt with added curcumin and CGA at day 5.

Note: Data represents the mean of three biological replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

(f) Retention of curcumin and CGA in yogurt

The amounts of curcumin and CGA retained in the yogurt during refrigerated storage were analysed using HPLC and the results are shown in Figure 5.10. The retention (%) of curcumin and CGA are expressed with reference to the amount available on day 1. The amount of curcumin and CGA in yogurt sample decreased as storage time increased, with the degradation of curcumin being more rapid than CGA. The retention of curcumin significantly ($p \leq 0.05$) decreased on day 5, while CGA was significantly ($p \leq 0.05$) reduced on day 15. Curcumin decreased to 66.61±1.49 % on day 5, which further reduced to 63.31±3.20 % on day 15. In contrast, the amount of CGA on day 5 and day 15 was 90.23±5.47 % and 84.81±3.17 % respectively. The retention of curcumin and CGA in yogurt sample may be attributed to the stability of bioactive compounds in an acidic environment and their

solubility in the fat matrix (Akulov et al., 2014; Helal & Tagliazucchi, 2018; Kharat, Du, Zhang, & McClements, 2017).

It should be noted that during the extraction of the bioactive compounds from the yogurt samples ultra-sonication was used to improve the extraction process (Saleh et al., 2016). However, it is possible that some fraction of the bioactive compounds may have been strongly bound in the protein matrix and could not be extracted. For example, the addition of milk to cinnamon beverage could reduce the total polyphenol content by 28 % as tannins in the cinnamon form an insoluble complex with milk proteins (Helal, Tagliazucchi, Verzelloni, & Conte, 2014). In addition, the extent of the interactions between proteins and bioactive compounds are high in an acidic environment such as in yogurt (Hagerman & Butler, 1978). Therefore, the bioactive compounds strongly bound in the protein matrix could be a potential source of error while quantifying their amount retained in the yogurt sample.

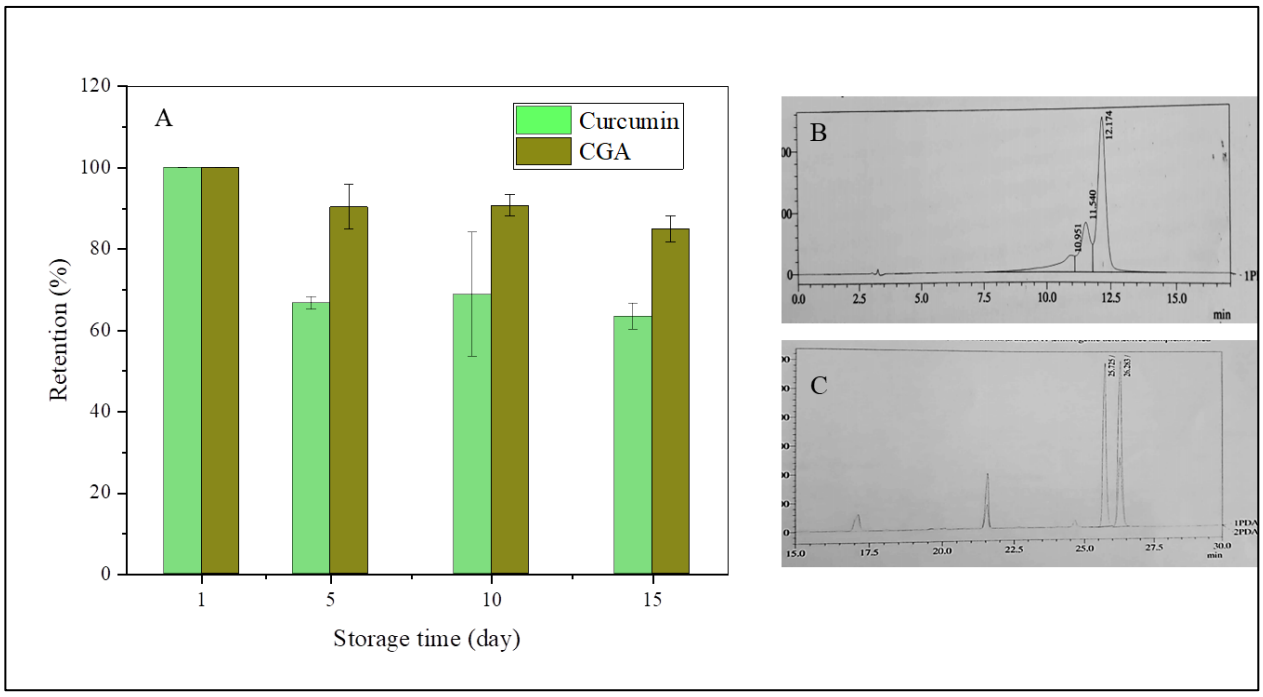


Figure 5.10: (A) Retention (%) of curcumin and CGA in coconut cream yogurt during storage at 4±1ºC for 15 days. HPLC chromatogram for (B) curcumin and (C) CGA.

Note: Data represents the mean of three biological replicates with error bars corresponding to standard deviation. Raw data and statistically analysed data are shown in Appendix 8.

(g) Consumer sensory evaluation

Sensory properties of a product can strongly influence its acceptance by the consumer. Addition of plant-based compounds into dairy products can result in an unpleasant colour and

off-flavours which may have a negative impact on their organoleptic properties (Zoidou et al., 2014). Thus, the effect of curcumin and CGA as functional ingredients on the sensory acceptance of coconut yogurt was studied. In this study, appearance, mouthfeel, texture, flavour and overall acceptability of control and bioactive containing sample were evaluated by panellists (n=100) and results are shown in Figure 5.11.

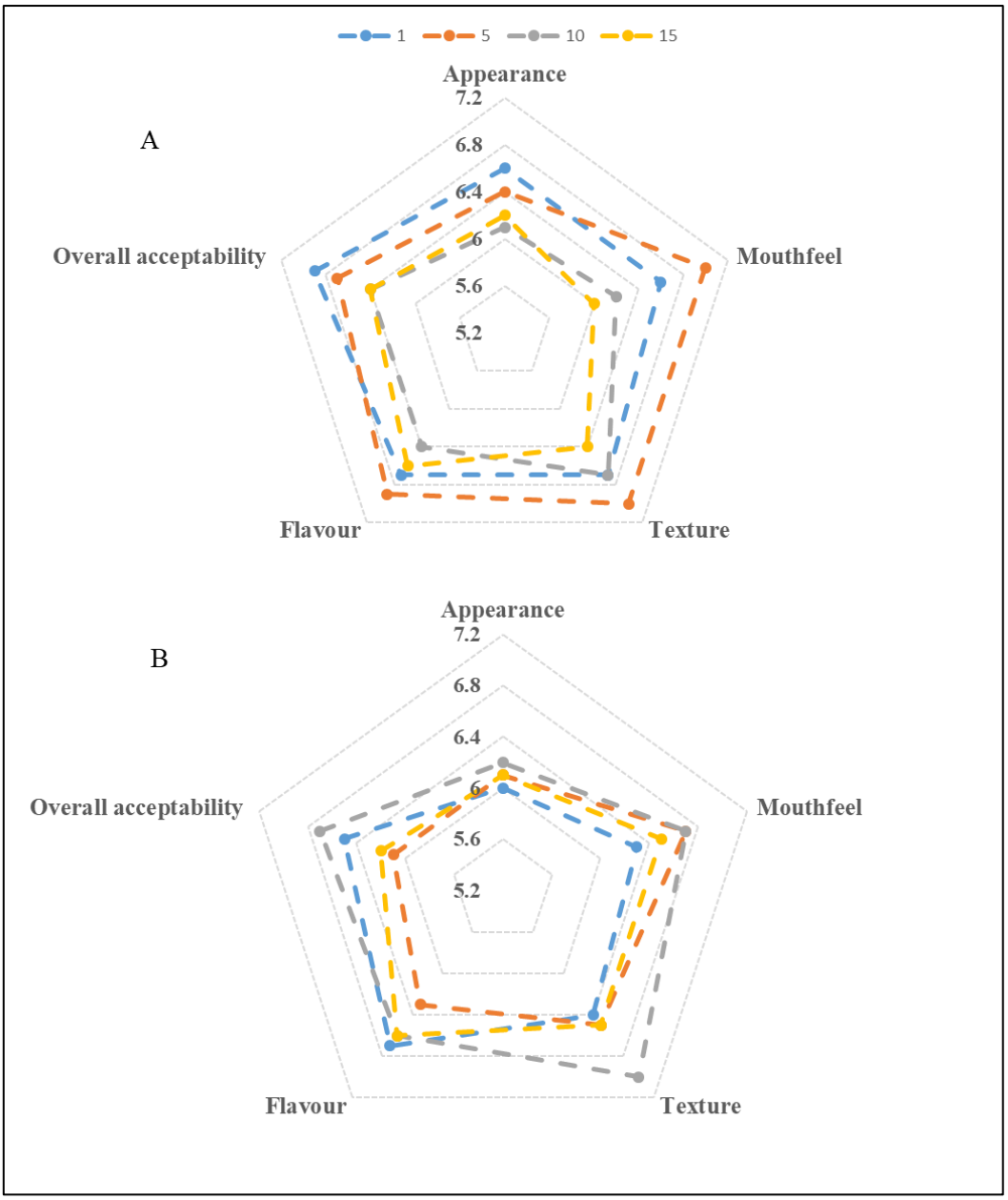


Figure 5.11: Spider web showing the average sensory score for (A) control (B) curcumin and CGA added yogurt during storage at 4±1ºC for 15 days.

Note: Data represents the mean score of sensory panellists (n=100). Raw data and statistically analysed data are shown in Appendix 8.

The average score for overall acceptability of the control and bioactive added yogurt samples was 6.6±1.1 and 6.4±1.1, respectively, on a 9-point hedonic scale. This indicated that the addition of curcumin and coffee did not have any negative impact on the acceptance of coconut yogurt. It must be noted that there were small changes in mean scores for all the attributes during storage in both the samples, but these changes were not statistically significant. Moreover, there was no significant difference in the scores between the two samples for any attribute. The panellists scored slightly high for the appearance of the control sample (6.4±1.5) than the bioactive containing sample (6.1±1.3). The average scores for mouthfeel, texture and flavour for bioactive added sample were 6.6±0.9, 6.6±0.8 and 6.6±1.3, respectively. The mean scores by the panellists for all the parameters throughout the study were between 6 to 7 (Appendix 8).

Previous reports on coconut added to fermented dairy products showed similar high sensory score (Gad, Kholif, & Sayed, 2010; Ndife et al., 2014; Sanful, 2009; Shana et al., 2015). Some panellists described the samples as creamy probably due to the high fat content in the coconut cream. None of the panellists reported any unpleasant off-flavour or undesirable texture in either of the sample. Thus, the sensory profile was unaltered during the storage period.

5.3 Summary

In this study, a coconut yogurt (150 g) fortified with 100 mg of curcumin and CGA was developed and their effects on physico-chemical, microbial and sensory parameters were studied over a 15 days refrigerated storage period. Addition of bioactive compounds did not have any significant ($p \leq 0.05$) effects on fermentation of coconut cream yogurt. However, they did impart a mustard-brown colour which was liked by the sensory panellist. During refrigerated storage significant ($p \leq 0.05$) changes in acidity, viable cell counts of starter culture bacteria and gel firmness were observed in control and test samples, while syneresis remained stable. Retention of curcumin and CGA was high in coconut yogurt, accounting for about 63 % and 85 %, respectively, by the end of storage. Importantly, the coconut yogurt containing bioactives was found to be acceptable in consumer sensory evaluations. Thus, coconut yogurt may be a potential medium to deliver curcumin and CGA to consumers.

CHAPTER 6

Conclusion

This study demonstrated that in an *in vitro* model of LPS stimulated THP-1 macrophages treatment with curcumin and CGA (1:1) could synergistically reduce the production of a pro-inflammatory cytokine TNF-α. This combination was effective in downregulating mRNA expression of pro-inflammatory cytokines and COX-2 enzyme by suppressing the NF-κB pathway. Further, the incorporation of curcumin and CGA in coconut yogurt did not affect the fermentation process. About 63 % curcumin and 85 % CGA were retained in the yogurt samples at the end of refrigerated storage for 15 days. The fermented non-dairy product had acceptable physico-chemical, microbiological and sensory attributes during storage. The coconut yogurt with added curcumin and CGA is a functional product that could confer anti-inflammatory therapeutic benefits to the consumers.

CHAPTER 7

Recommendations

The following recommendations are proposed for future studies:

- In this study, the protective effect of CGA against LPS toxicity in THP-1 monocyte cells was reported for the first time. Further work is required to understand the underlying mechanisms of action for this effect.

- The combination of curcumin and CGA was able to suppress mRNA expression of several biomarkers involved in the NF-κB pathway. In addition, the effect of the combination on other inflammatory pathways such as MAPK and JNK STAT should be investigated at both the protein and mRNA levels. In addition, the anti-inflammatory effects of the proposed combination should be studied using *in vivo* models.

- Previous reports have documented that the delivery of bioactive compounds *via* food matrices may improve the bioaccessibility of the compound in humans. Therefore, the bioavailablity of curcumin and CGA delivered *via* coconut yogurt should be studied using *in vitro* and *in vivo* models to determine the delivery efficacy of the proposed matrix.

- Functional coconut yogurt with added curcumin and CGA was developed to deliver anti-inflammatory health benefits. Thus, it would be desirable to evaluate the anti-inflammatory activity of this product in clinical trials.

References

Aggarwal, B., & Nesaretnam, K. (2012). Vitamin E tocotrienols: Life beyond tocopherols. *Genes and Nutrition, 7*(1), 1-1. doi:10.1007/s12263-011-0234-x

Aggarwal, S., Ichikawa, H., Takada, Y., Sandur, S. K., Shishodia, S., & Aggarwal, B. B. (2006). Curcumin (diferuloylmethane) down-regulates expression of cell proliferation and antiapoptotic and metastatic gene products through suppression of IκBα kinase and Akt activation. *Molecular Pharmacology, 69*(1), 195-206.

Aguirre-Ezkauriatza, E. J., Galarza-González, M. G., Uribe-Bujanda, A. I., Ríos-Licea, M., López-Pacheco, F., Hernández-Brenes, C. M., & Alvarez, M. M. (2008). Effect of mixing during fermentation in yogurt manufacturing. *Journal of Dairy Science, 91*(12), 4454-4465. doi:https://doi.org/10.3168/jds.2008-1140

Ahmad, S. F., Pandey, A., Kour, K., & Bani, S. (2010). Downregulation of pro-inflammatory cytokines by lupeol measured using cytometric bead array immunoassay. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 24*(1), 9-13.

Akram, M., Shahab-Uddin, A. A., Usmanghani, K., Hannan, A., Mohiuddin, E., & Asif, M. (2010). Curcuma longa and curcumin: A review article. *Romanian Journalof Biology-Plant Biology, 55*(2), 65-70.

Akulov, K., Simkovitch, R., Erez, Y., Gepshtein, R., Schwartz, T., & Huppert, D. (2014). Acid effect on photobase properties of curcumin. *The Journal of Physical Chemistry A, 118*(13), 2470-2479. doi:10.1021/jp501061p

Al Jameil, N., Tabassum, H., Fatima, S., Naiman Ali, M., Rizwana, H., & Aziz Khan, F. (2017). Ameliorating effect of Vitamin C against potassium dichromate induced oxidative stress and inflammatory response in rats. *International Journal of Pharmacology, 13*(8), 990-999. doi:10.3923/ijp.2017.990.999

Al Rehaily, A. J., El Tahir, K. E., Mossa, J. S., & Rafatullah, S. (2001). Pharmacological studies of various extracts and the major constituent, lupeol, obtained from hexane extract of Teclea nobilis in rodents. *Natural Product Sciences, 7*(3), 76-82.

Alappat, L., & Awad, A. B. (2010). Curcumin and obesity: Evidence and mechanisms. *Nutrition Reviews, 68*(12), 729-738. doi:10.1111/j.1753-4887.2010.00341.x

Algandaby, M. M., El-halawany, A. M., Abdallah, H. M., Alahdal, A. M., Nagy, A. A., Ashour, O. M., & Abdel-Naim, A. B. (2016). Gingerol protects against experimental liver fibrosis in rats via suppression of pro-inflammatory and profibrogenic mediators. *Naunyn-Schmiedeberg's Archives of Pharmacology, 389*(4), 419-428. doi:10.1007/s00210-016-1210-1

Ali, N., Rashid, S., Nafees, S., Hasan, S. K., Shahid, A., Majed, F., & Sultana, S. (2017). Protective effect of chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: An experimental approach. *Chemico-biological Interactions, 272*, 80-91.

Allgeyer, L., Miller, M., & Lee, S.Y. (2010). Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. *Journal of Dairy Science, 93*(10), 4471-4479.

Amalraj, A., Pius, A., Gopi, S., & Gopi, S. (2017). Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives–A review. *Journal of Traditional and Complementary Medicine, 7*(2), 205-233.

Amatayakul, T., Sherkat, F., & Shah, N. (2006). Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. *International Journal of Dairy Technology, 59*(3), 216-221. doi:10.1111/j.1471-0307.2006.00264.x

Amin, K. (2012). The role of mast cells in allergic inflammation. *Respiratory Medicin, 106*(1), 9-14. doi:10.1016/j.rmed.2011.09.007

Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics, 4*(6), 807-818.

Anand, P., Sundaram, C., Jhurani, S., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin and cancer: An "old-age" disease with an "age-old" solution. *Cancer Letters, 267*(1), 133-164.

Andersen, L. F., Jacobs Jr, D. R., Carlsen, M. H., & Blomhoff, R. (2006). Consumption of coffee is associated with reduced risk of death attributed to inflammatory and cardiovascular diseases in the Iowa Women's Health Study. *The American Journal of Clinical Nutrition, 83*(5), 1039-1046.

Andres, S., Pevny, S., Ziegenhagen, R., Bakhiya, N., Schafer, B., Hirsch-Ernst, K. I., & Lampen, A. (2018). Safety aspects of the use of quercetin as a dietary supplement. *Molecular Nutrition and Food Research, 62*(1). doi:10.1002/mnfr.201700447

AOAC. (2005). AOAC Official Method 947.05. Acidity of milk, titrimetric method. In *American Organization of Analytical Chemist International*.

Arciniegas, A., Apan, M. T. R., Pérez-Castorena, A. L., & de Vivar, A. R. (2004). Anti-inflammatory constituents of Mortonia greggii Gray. *Zeitschrift für Naturforschung C, 59*(3-4), 237-243.

Arvanitoyannis, I. S., & Van Houwelingen-Koukaliaroglou, M. (2005). Functional foods: A survey of health claims, pros and cons, and current legislation. *Critical Reviews in Food Science and Nutrition, 45*(5), 385-404.

Ashalatha, K., Venkateswarlu, Y., Priya, A. M., Lalitha, P., Krishnaveni, M., & Jayachandran, S. (2010). Anti inflammatory potential of Decalepis hamiltonii (Wight and Arn) as evidenced by down regulation of pro inflammatory cytokines—TNF-α and IL-2. *Journal of Ethnopharmacology, 130*(1), 167-170.

Ashina, K., Tsubosaka, Y., Nakamura, T., Omori, K., Kobayashi, K., Hori, M., . . . Murata, T. (2015). Histamine induces vascular hyperpermeability by increasing blood flow and endothelial barrier disruption in vivo. *PLoS One, 10*(7), e0132367. doi:10.1371/journal.pone.0132367

Astley, S., & Finglas, P. (2016). Nutrition and health. In *Reference Module in Food Science*.

Augustin, M. A., & Sanguansri, L. (2015). Challenges and solutions to incorporation of nutraceuticals in foods. *Annual Review of Food Science and Technology, 6*, 463-477. doi:10.1146/annurev-food-022814-015507

Azizkhani, M., & Parsaeimehr, M. (2018). Probiotics survival, antioxidant activity and sensory properties of yogurt flavored with herbal essential oils. *International Food Research Journal, 25*(3), 921-927.

Bachmeier, B., Nerlich, A., Iancu, C., Cilli, M., Schleicher, E., Vené, R., . . . Pfeffer, U. (2007). The chemopreventive polyphenol curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. *Cellular Physiology and Biochemistry, 19*(1-4), 137-152.

Badshah, H., Ali, T., Rehman, S. U., Amin, F.U., Ullah, F., Kim, T. H., & Kim, M. O. (2016). Protective effect of lupeol against lipopolysaccharide-induced neuroinflammation via the p38/c-Jun N-terminal kinase pathway in the adult mouse brain. *Journal of Neuroimmune Pharmacology, 11*(1), 48-60.

Bagdas, D., Etoz, B. C., Gul, Z., Ziyanok, S., Inan, S., Turacozen, O., . . . Tas, S. (2015). In vivo systemic chlorogenic acid therapy under diabetic conditions: Wound healing effects and cytotoxicity/genotoxicity profile. *Food and Chemical Toxicology, 81*, 54-61.

Bai, S. K., Lee, S. J., Na, H. J., Ha, K. S., Han, J. A., Lee, H., . . . Kim, Y. M. (2005). β-Carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-κB activation. *Experimental and Molecular Medicine, 37*, 323. doi:10.1038/emm.2005.42

Banerjee, M., Tripathi, L., Srivastava, V., Puri, A., & Shukla, R. (2003). Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. *Immunopharmacology and Immunotoxicology, 25*(2), 213-224.

Bani, S., Kaul, A., Khan, B., Ahmad, S. F., Suri, K., Gupta, B., . . . Qazi, G. (2006). Suppression of T lymphocyte activity by lupeol isolated from Crataeva religiosa.

Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 20(4), 279-287.

Bao, L., Li, J., Zha, D., Zhang, L., Gao, P., Yao, T., & Wu, X. (2018). Chlorogenic acid prevents diabetic nephropathy by inhibiting oxidative stress and inflammation through modulation of the Nrf2/HO-1 and NF-κB pathways. *International Immunopharmacology, 54*, 245-253.

Barzegar, A. (2012). The role of electron-transfer and H-atom donation on the superb antioxidant activity and free radical reaction of curcumin. *Food Chemistry, 135*(3), 1369-1376.

Bech-Larsen, T., & Scholderer, J. (2007). Functional foods in Europe: Consumer research, market experiences and regulatory aspects. *Trends in Food Science and Technology, 18*(4), 231-234.

Ben, P., Liu, J., Lu, C., Xu, Y., Xin, Y., Fu, J., . . . Luo, L. (2011). Curcumin promotes degradation of inducible nitric oxide synthase and suppresses its enzyme activity in RAW 264.7 cells. *International Immunopharmacology, 11*(2), 179-186.

Benly, P. (2015). Role of histamine in acute inflammation. *Journal of Pharmaceutical Sciences and Research, 7*(6), 373-376.

Betoret, E., Betoret, N., Vidal, D., & Fito, P. (2011). Functional foods development: Trends and technologies. *Trends in Food Science and Technology, 22*(9), 498-508.

Bode, A. M., & Dong, Z. (2011a). The two faces of capsaicin. *Cancer Research, 71*(8), 2809-2814. doi:10.1158/0008-5472.can-10-3756

Bode, A. M., & Dong., Z. (2011b). The amazing and mighty ginger. In I. Benzie & S. Wachtel-Galor (Eds.), *Herbal Medicine: Biomolecular and Clinical Aspects* (2 ed.). Boca Raton (FL): CRC Press/Taylor & Francis.

Bracquart, P. (1981). An agar medium for the differential enumeration of Streptococcus thermophilus and Lactobacillus bulgaricus in yoghurt. *Journal of Applied Bacteriology, 51*(2), 303-305.

Brandenburg, L. O., Kipp, M., Lucius, R., Pufe, T., & Wruck, C. J. (2010). Sulforaphane suppresses LPS-induced inflammation in primary rat microglia. *Inflammation Research, 59*(6), 443-450. doi:10.1007/s00011-009-0116-5

Buhrmann, C., Mobasheri, A., Busch, F., Aldinger, C., Stahlmann, R., Montaseri, A., & Shakibaei, M. (2011). Curcumin modulates NF-κB-mediated inflammation in human tenocytes in vitro: Role of the phosphatidylinositol 3-kinase-Akt pathway. *Journal of Biological Chemistry*, jbc. M111. 256180.

Calixto, J. B., Otuki, M. F., & Santos, A. R. (2003). Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κ B (NF-κB). *Planta Medica*, *69*(11), 973-983.

Cassandra, R. (Producer). (2016, 21st Nov 2018). The inflammation fighter: Turmeric's growth in the Vitamin and supplement industry. Retrieved from http://robinsonpharma.com/Health-Industry-Insights/The-Inflammation-Fighter-Turmerics-Growth-in-the-Vitamin-and-Supplement-Industry

Cavaillon, J. M. (2017a). Inflammation through the ages: A historical perspective In J. M. Cavaillon & M. Singer (Eds.), *Inflammation: From Molecular and Cellular Mechanisms to the Clinic* (pp. 1-16): Wiley-VCH Verlag GmbH & Co. KGaA.

Cavaillon, J. M. (2017b). Pathogen-associated molecular patterns. In J. M. Cavaillon & M. Singer (Eds.), *Inflammation: From Molecular and Cellular Mechanisms to the Clinic* (pp. 19-56): Wiley-VCH Verlag GmbH & Co. KGaA.

Cavet, M. E., Harrington, K. L., Vollmer, T. R., Ward, K. W., & Zhang, J. Z. (2011). Anti-inflammatory and anti-oxidative effects of the green tea polyphenol epigallocatechin gallate in human corneal epithelial cells. *Molecular Vision, 17*, 533-542.

Cervone, H. F. (2009). Applied digital library project management: Using Pugh matrix analysis in complex decision-making situations. *OCLC Systems and Services: International Digital Library Perspectives*, *25*(4), 228-232. doi:doi:10.1108/10650750911001815

Cha, J. W., Piao, M. J., Kim, K. C., Yao, C. W., Zheng, J., Kim, S. M., . . . Hyun, J. W. (2014). The polyphenol chlorogenic acid attenuates UVB-mediated oxidative stress in human HaCaT keratinocytes. *Biomolecules and Therapeutics, 22*(2), 136.

Chairez-Ramirez, M., Sanchez-Burgos, J., Gomes, C., Moreno-Jimenez, M., Gonzalez-Laredo, R., Bernad-Bernad, M., . . . Rocha-Guzman, N. (2015). Morphological and release characterization of nanoparticles formulated with poly (dl-lactide-co-glycolide)(PLGA) and lupeol: In vitro permeability and modulator effect on NF-κB in Caco-2 cell system stimulated with TNF-α. *Food and Chemical Toxicology, 85*, 2-9.

Chan, M. M. Y. (1995). Inhibition of tumor necrosis factor by curcumin, a phytochemical. *Biochemical Pharmacology, 49*(11), 1551-1556.

Chanput, W. (2012). *Immunomodulating effects of food compounds: A study using the THP-1 cell line*.

Chanput, W., Peters, V., & Wichers, H. (2015). THP-1 and U937 Cells. In K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, & H. Wichers (Eds.), *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models* (pp. 147-159). Cham: Springer International Publishing.

Chanrot, M., Guo, Y., Dalin, A., Persson, E., Båge, R., Svensson, A., . . . Humblot, P. (2017). Dose related effects of LPS on endometrial epithelial cell populations from dioestrus cows. *Animal Reproduction Science, 177,* 12-24.

Chauhan, P. S., Satti, N. K., Sharma, P., Sharma, V. K., Suri, K. A., & Bani, S. (2012). Differential effects of chlorogenic acid on various immunological parameters relevant to rheumatoid arthritis. *Phytotherapy Research, 26*(8), 1156-1165.

Chen, B., McClements, D. J., & Decker, E. A. (2013). Design of foods with bioactive lipids for improved health. *Annual Review of Food science and Technology, 4,* 35-56.

Chen, C., Xue, H., & Mu, S. (2014). pH dependence of reactive sites of curcumin possessing antioxidant activity and free radical scavenging ability studied using the electrochemical and ESR techniques: Polyaniline used as a source of the free radical. *Journal of Electroanalytical Chemistry, 713*, 22-27.

Chen, D., Nie, M., Fan, M. W., & Bian, Z. (2008). Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from Porphyromonas gingivalis. *Pharmacology, 82*(4), 264-269.

Chen, D., Pan, D., Tang, S., Tan, Z., Zhang, Y., Fu, Y., . . . Huang, Q. (2018a). Administration of chlorogenic acid alleviates spinal cord injury via TLR4/NF-κB and p38 signaling pathway anti-inflammatory activity. *Molecular Medicine Reports, 17*(1), 1340-1346.

Chen, J., Xie, H., Chen, D., Yu, B., Mao, X., Zheng, P., . . . He, J. (2018b). Chlorogenic acid improves intestinal development via suppressing mucosa inflammation and cell apoptosis in Weaned Pigs. *ACS Omega, 3*(2), 2211-2219.

Chen, W. P., & Wu, L. D. (2014). Chlorogenic acid suppresses interleukin-1β-induced inflammatory mediators in human chondrocytes. *International Journal of Clinical and Experimental Pathology, 7*(12), 8797.

Chen, Y., Jimmy Yu, Q., Li, X., Luo, Y., & Liu, H. (2007). Extraction and HPLC characterization of chlorogenic acid from tobacco residuals. *Separation Science and Technology, 42*(15), 3481-3492.

Chen, Y. F., Ching, C., Wu, T. S., Wu, C. R., Hsieh, W. T., & Tsai, H. Y. (2012). Balanophora spicata and lupeol acetate possess antinociceptive and anti-inflammatory activities in vivo and in vitro. *Evidence-Based Complementary and Alternative Medicine, 2012*, 371273. doi:10.1155/2012/371273

Cho, H. (2004). Modulation of the activity of pro-inflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. *Pharmacological Research, 49*(1), 37-43. doi:10.1016/s1043-6618(03)00248-2

Cho, J. W., Lee, K. S., & Kim, C. W. (2007). Curcumin attenuates the expression of IL-1β, IL-6, and TNF-α as well as cyclin E in TNF-α-treated HaCaT cells; NF-κB and MAPKs as potential upstream targets. *International Journal of Molecular Medicine, 19*(3), 469-474.

Choia, S. Y., Parkb, J. H. Y., Kimc, J. S., Kimc, M. K., Aruomae, K. I., & Sunga, M. K. (2006). Effects of quercetin and β-carotenesupplementation on azoxymethane-inducedcolon carcinogenesis and inflammatoryresponses in rats fed with high-fat diet rich inω-6 fatty acids. *BioFactors, 27*, 137–146.

Chouchouli, V., Kalogeropoulos, N., Konteles, S. J., Karvela, E., Makris, D. P., & Karathanos, V. T. (2013). Fortification of yoghurts with grape (Vitis vinifera) seed extracts. *Food Science and Technology, 53*(2), 522-529. doi:https://doi.org/10.1016/j.lwt.2013.03.008

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates–nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture, 80*(7), 1033-1043.

Coggins, P. C., Rowe, D. E., Wilson, J. C., & Kumari, S. (2010). Storage and temperature effects on appearance and textural characteristics of conventional milk yogurt. *Journal of Sensory Studies, 25*(4), 549-576. doi:10.1111/j.1745-459X.2010.00286.x

Corso, M. P., Vignoli, J. A., & Benassi, M. d. T. (2016). Development of an instant coffee enriched with chlorogenic acids. *Journal of Food Science and Technology, 53*(3), 1380-1388. doi:10.1007/s13197-015-2163-y

Corwin, E. J. (2000). Understanding cytokines Part I: Physiology and mechanism of action. *Biological Research for Nursing, 2*(1), 30-40. doi:10.1177/109980040000200104

Cui, J., Yu, B., Zhao, Y., Zhu, W., Li, H., Lou, H., & Zhai, G. (2009). Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. *International Journal of Pharmaceutics, 371*(1-2), 148-155.

Cuomo, J., Appendino, G., Dern, A. S., Schneider, E., McKinnon, T. P., Brown, M. J., . . . Dixon, B. M. (2011). Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. *Journal of Natural Products, 74*(4), 664-669.

D'Orazio, N., Gammone, M. A., Gemello, E., De Girolamo, M., Cusenza, S., & Riccioni, G. (2012). Marine bioactives: Pharmacological properties and potential applications against inflammatory diseases. *Marine Drugs, 10*(4), 812-833. doi:10.3390/md10040812

Dabija, A., Codină, G. G., Ropciuc, S., Gâtlan, A. M., & Rusu, L. (2018). Assessment of the antioxidant activity and quality attributes of yogurt enhanced with wild herbs extracts. *Journal of Food Quality,, 2018*.

Dai, S., Corke, H., & Shah, N. P. (2016). Utilization of konjac glucomannan as a fat replacer in low-fat and skimmed yogurt. *Journal of Dairy Science, 99*(9), 7063-7074. doi:https://doi.org/10.3168/jds.2016-11131

Daigneault, M., Preston, J. A., Marriott, H. M., Whyte, M. K., & Dockrell, D. H. (2010). The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. *PLoS One, 5*(1), e8668.

Day, L., Seymour, R. B., Pitts, K. F., Konczak, I., & Lundin, L. (2009). Incorporation of functional ingredients into foods. *Trends in Food Science and Technology, 20*(9), 388-395.

De Brabandere, A. G., & De Baerdemaeker, J. G. (1999). Effects of process conditions on the pH development during yogurt fermentation. *Journal of Food Engineering, 41*(3-4), 221-227.

Demirbilek, S., Ersoy, M. O., Demirbilek, S., Karaman, A., Gürbüz, N., Bayraktar, N., & Bayraktar, M. (2004). Small-dose capsaicin reduces systemic inflammatory responses in septic rats. *Anesthesia and Analgesia, 99*(5), 1501-1507. doi:10.1213/01.ane.0000132975.02854.65

Deshpande, S., Jaiswal, R., Matei, M. F., & Kuhnert, N. (2014). Investigation of acyl migration in mono-and dicaffeoylquinic acids under aqueous basic, aqueous acidic, and dry roasting conditions. *Journal of Agricultural and Food Chemistry, 62*(37), 9160-9170.

Devaraj, S., Mathur, S., Basu, A., Aung, H. H., Vasu, V. T., Meyers, S., & Jialal, I. (2008). A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. *Journal of the American College of Nutrition, 27*(2), 267-273.

Dhillon, N., Aggarwal, B. B., Newman, R. A., Wolff, R. A., Kunnumakkara, A. B., Abbruzzese, J. L., . . . Kurzrock, R. (2008). Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clinical Cancer Research, 14*(14), 4491-4499.

Dias, M. I., Ferreira, I. C., & Barreiro, M. F. (2015). Microencapsulation of bioactives for food applications. *Food and Function, 6*(4), 1035-1052. doi:10.1039/c4fo01175a

Donkor, O. N., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2006). Effect of acidification on the activity of probiotics in yoghurt during cold storage. *International Dairy Journal, 16*(10), 1181-1189. doi:https://doi.org/10.1016/j.idairyj.2005.10.008

Dönmez, Ö., Mogol, B. A., & Gökmen, V. (2017). Syneresis and rheological behaviors of set yogurt containing green tea and green coffee powders. *Journal of Dairy Science, 100*(2), 901-907. doi:https://doi.org/10.3168/jds.2016-11262

Duarte, G. S., & Farah, A. (2011). Effect of simultaneous consumption of milk and coffee on chlorogenic acids' bioavailability in humans. *Journal of Agricultural and Food Chemistry, 59*(14), 7925-7931.

Duke, J. (2001). *Handbook of Phytochemical Constituent Grass, Herbs and Other Economic Plants* (2nd ed.). New York: Routledge, Taylor & Francis Group.

Dunster, J. L. (2016). The macrophage and its role in inflammation and tissue repair: Mathematical and systems biology approaches. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *8*(1), 87-99.

Ejtahed, H., Mohtadi-Nia, J., Homayouni-Rad, A., Niafar, M., Asghari-Jafarabadi, M., Mofid, V., & Akbarian-Moghari, A. (2011). Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. *Journal of Dairy Science*, *94*(7), 3288-3294.

El-Abbadi, N. H., Dao, M. C., & Meydani, S. N. (2014). Yogurt: Role in healthy and active aging. *The American Journal of Clinical Nutrition*, *99*(5), 1263S-1270S.

Elias, R. J., Kellerby, S. S., & Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical Reviews in Food Science and Nutrition*, *48*(5), 430-441. doi:10.1080/10408390701425615

Elisia, I., Nakamura, H., Lam, V., Hofs, E., Cederberg, R., Cait, J., . . . Krystal, G. (2016). DMSO represses inflammatory cytokine production from human blood cells and reduces autoimmune arthritis. *PLoS One*, *11*(3), e0152538.

Espírito Santo, A., Perego, P., Converti, A., & Oliveira, M. N. d. (2012). Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yoghurts. *LWT-Food Science and Technology*, *47*(2), 393-399.

Estrada, J. D., Boeneke, C., Bechtel, P., & Sathivel, S. (2011). Developing a strawberry yogurt fortified with marine fish oil1. *Journal of Dairy Science*, *94*(12), 5760-5769. doi:https://doi.org/10.3168/jds.2011-4226

European Commission. (2010). *Functional foods*. Brussels, Belgium: Directorate-General for Research, FP7 cooperation – Food.

European Food Safety Authority. (2014). Refined exposure assessment for curcumin (E100). *EFSA Journal*, *12*(10), 3876.

European Food Safety Authority, Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., . . . Wright, M. (2018). Scientific opinion on the safety of green tea catechins. *European Food Safety Authority Journal*, *16*(4), e0529. doi:https://doi.org/10.2903/j.efsa.2018.5239

Fadela, C., Abderrahim, C., & Ahmed, B. (2009). Sensorial and physico-chemical characteristics of yoghurt manufactured with ewe's and skim milk. *World Journal of Dairy and Food Sciences*, *4*(2), 136-140.

Fan, X., Zhu, J. Y., Sun, Y., Luo, L., Yan, J., Yang, X., . . . Liang, H. P. (2017). Evodiamine inhibits zymosan-induced inflammation in vitro and in vivo: Inactivation of NF-κB by inhibiting IκBα phosphorylation. *Inflammation, 40*(3), 1012-1027. doi:10.1007/s10753-017-0546-0

Fang, F. C. (2004). Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nature Reviews Microbiology, 2*(10), 820-832. doi:10.1038/nrmicro1004

FAO/WHO. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Retrieved 10 May 2019, from Food and Agriculture Organization of the United Nations/World Health Organization

Farah, A., & Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology, 18*(1), 23-36.

Fazilah, N. F., Ariff, A. B., Khayat, M. E., Rios-Solis, L., & Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods, 48*, 387-399.

FDA. (2013). CFR - Code of Federal Regulations Title 21. from Department of human health and services
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=182.3013

FDA. (2018). CFR - Code of Federal Regulations Title 21. from Departmnet of health and human services
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1245

Fernández-Mar, M. I., Mateos, R., García-Parrilla, M. C., Puertas, B., & Cantos-Villar, E. (2012). Bioactive compounds in wine: Resveratrol, hydroxytyrosol and melatonin: A review. *Food Chemistry, 130*(4), 797-813. doi:https://doi.org/10.1016/j.foodchem.2011.08.023

Fernández, A., Álvarez, A., García, M. D., & Sáenz, M. T. (2001). Anti-inflammatory effect of Pimenta racemosa var. ozua and isolation of the triterpene lupeol. *Il Farmaco, 56*(4), 335-338.

Fernández, M. A., de las Heras, B., Garcia, M. D., Sáenz, M. T., & Villar, A. (2001). New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. *Journal of Pharmacy and Pharmacology, 53*(11), 1533-1539.

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., & Griendling, K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Research, 122*(6), 877-902. doi:10.1161/CIRCRESAHA.117.311401

Fox, E. S., Brower, J. S., Bellezzo, J. M., & Leingang, K. A. (1997). N-acetylcysteine and alpha-tocopherol reverse the inflammatory response in activated rat Kupffer cells. *The Journal of Immunology, 158*(11), 5418-5423.

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The Journals of Gerontology: Series A, 69*(Suppl_1), S4-S9. doi:10.1093/gerona/glu057

Fu, S., Augustin, M. A., Sanguansri, L., Shen, Z., Ng, K., & Ajlouni, S. (2016). Enhanced bioaccessibility of curcuminoids in buttermilk yogurt in comparison to curcuminoids in aqueous dispersions. *Journal of Food Science, 81*(3), H769-H776.

Fujioka, K., & Shibamoto, T. (2008). Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chemistry, 106*(1), 217-221. doi:https://doi.org/10.1016/j.foodchem.2007.05.091

Funk, C. D. (2001). Prostaglandins and leukotrienes: Advances in eicosanoid biology. *science, 294*(5548), 1871-1875.

Furrer, A., Cladis, D. P., Kurilich, A., Manoharan, R., & Ferruzzi, M. G. (2017). Changes in phenolic content of commercial potato varieties through industrial processing and fresh preparation. *Food Chemistry, 218*, 47-55.

Gad, A. S., Kholif, A. M., & Sayed, A. F. (2010). Evaluation of the nutritional value of functional yogurt resulting from combination of date palm syrup and skim milk. *American Journal of Food Technology, 5*(4), 250-259.

Gahche, J., Bailey, R., Burt, V., Hughes, J., Yetley, E., Dwyer, J., . . . Sempos, C. (2011). Dietary supplement use among US adults has increased since NHANES III (1988-1994). *NCHS Data Brief* (61), 1-8.

Galanakis, C. M. (2017). Introduction. In *Nutraceutical and Functional Food Components* (1 ed., pp. 1-14).

Ganjali, S., Sahebkar, A., Mahdipour, E., Jamialahmadi, K., Torabi, S., Akhlaghi, S., . . . Ghayour-Mobarhan, M. (2014). Investigation of the effects of curcumin on serum cytokines in obese individuals: A randomized controlled trial. *The Scientific World Journal, 2014*.

Gatto, F., Cagliani, R., Catelani, T., Guarnieri, D., Moglianetti, M., Pompa, P. P., & Bardi, G. (2017). PMA-induced THP-1 macrophage differentiation is not impaired by citrate-coated platinum nanoparticles. *Nanomaterials, 7*(10), 332.

Geetha, T., & Varalakshmi, P. (1999). Anticomplement activity of triterpenes from Crataeva nurvala stem bark in adjuvant arthritis in rats. *General Pharmacology: The Vascular System, 32*(4), 495-497.

Ghosh, S., Banerjee, S., & Sil, P. C. (2015). The beneficial role of curcumin on inflammation, diabetes and neurodegenerative disease: A recent update. *Food and Chemical Toxicology, 83*, 111-124.

Gil-Cardoso, K., Gines, I., Pinent, M., Ardevol, A., Blay, M., & Terra, X. (2016). Effects of flavonoids on intestinal inflammation, barrier integrity and changes in gut microbiota during diet-induced obesity. *Nutrition Research Reviews, 29*(2), 234-248. doi:10.1017/S0954422416000159

Gil, M., & Wianowska, D. (2017). Chlorogenic acids–their properties, occurrence and analysis. *Annales Universitatis Mariae Curie-Sklodowska, sectio AA–Chemia, 72*(1), 61.

Godbout, J. P., Berg, B. M., Krzyszton, C., & Johnson, R. W. (2005). Alpha-tocopherol attenuates NFkappaB activation and pro-inflammatory cytokine production in brain and improves recovery from lipopolysaccharide-induced sickness behavior. *Journal of Neuroimmunol, 169*(1-2), 97-105. doi:10.1016/j.jneuroim.2005.08.003

Gomaa, A. M., & Abd El-Aziz, E. A. (2016). Omega-3 fatty acids decreases oxidative stress, tumor necrosis factor-alpha, and interleukin-1 beta in hyperthyroidism-induced hepatic dysfunction rat model. *Pathophysiology, 23*(4), 295-301. doi:10.1016/j.pathophys.2016.10.001

Goncu, T., Oguz, E., Sezen, H., Kocarslan, S., Oguz, H., Akal, A., . . . Aksoy, N. (2016). Anti-inflammatory effect of lycopene on endotoxin-induced uveitis in rats. *Arquivos Brasileiros de Oftalmologia, 79*(6), 357-362. doi:10.5935/0004-2749.20160102

Gong, X. X., Su, X. S., Zhan, K., & Zhao, G. Q. (2018). The protective effect of chlorogenic acid on bovine mammary epithelial cells and neutrophil function. *Journal of Dairy Science, 101*(11), 10089-10097. doi:https://doi.org/10.3168/jds.2017-14328

Gooda Sahib, N., Saari, N., Ismail, A., Khatib, A., Mahomoodally, F., & Abdul Hamid, A. (2012). Plants' metabolites as potential antiobesity agents. *The Scientific World Journal, 2012,* 436039. doi:10.1100/2012/436039

Gordon, O. N., Luis, P. B., Sintim, H. O., & Schneider, C. (2015). Unraveling curcumin degradation: Autoxidation proceeds through spiroepoxide and vinylether intermediates en route to the main bicyclopentadione. *Journal of Biological Chemistry*, jbc. M114. 618785.

Granato, D., Nunes, D. S., & Barba, F. J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. *Trends in Food Science and Technology, 62*, 13-22.

Griesser, M., Pistis, V., Suzuki, T., Tejera, N., Pratt, D. A., & Schneider, C. (2011). Autoxidative and cyclooxygenase-2 catalyzed transformation of the dietary chemopreventive agent curcumin. *Journal of Biological Chemistry, 286*(2), 1114-1124.

Gukovsky, I., Reyes, C. N., Vaquero, E. C., Gukovskaya, A. S., & Pandol, S. J. (2003). Curcumin ameliorates ethanol and nonethanol experimental pancreatitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 284*(1), G85-G95.

Gulcubuk, A., Haktanir, D., Cakiris, A., Ustek, D., Guzel, O., Erturk, M., . . . Altunatmaz, K. (2013). Effects of curcumin on proinflammatory cytokines and tissue injury in the early and late phases of experimental acute pancreatitis. *Pancreatology*, *13*(4), 347-354.

Güler-Akın, M. B., & Akın, M. S. (2007). Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk. *Food Chemistry, 100*(2), 788-793. doi:https://doi.org/10.1016/j.foodchem.2005.10.038

Guo, M. B., Wang, D. C., Liu, H. F., Chen, L. W., Wei, J. W., Lin, Y., & Xue, H. (2018). Lupeol against high-glucose-induced apoptosis via enhancing the anti-oxidative stress in rabbit nucleus pulposus cells. *European Spine Journal, 27*(10), 2609-2620.

Guo, Y. J., Luo, T., Wu, F., Mei, Y. W., Peng, J., Liu, H., . . . Fang, Y. (2015). Involvement of TLR2 and TLR9 in the anti-inflammatory effects of chlorogenic acid in HSV-1-infected microglia. *Life Sciences, 127*, 12-18.

Gupta, S. K., Kumar, B., Nag, T. C., Agrawal, S. S., Agrawal, R., Agrawal, P., . . . Srivastava, S. (2011). Curcumin prevents experimental diabetic retinopathy in rats through its hypoglycemic, antioxidant, and anti-inflammatory mechanisms. *Journal of Ocular Pharmacology and Therapeutics, 27*(2), 123-130.

Gurol, A., Okten-Kursun, A., Kasapoglu, P., Suzergoz, F., Kucuksezer, U., Cevik, A., . . . Kucuk, M. (2016). The synergistic effect of ω3 and Vit D3 on glycemia and TNF-α in islet transplantation. *Cellular and Molecular Biology*, *62*(1), 90-98.

Ha, S. K., Park, H.-Y., Eom, H., Kim, Y., & Choi, I. (2012). Narirutin fraction from citrus peels attenuates LPS-stimulated inflammatory response through inhibition of NF-κB and MAPKs activation. *Food and Chemical Toxicology, 50*(10), 3498-3504.

Hagerman, A. E., & Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry*, *26*(4), 809-812.

Hall, G., & Wendin, K. (2008). Sensory design of foods for the elderly. *Annals of Nutrition and Metabolism, 52* (Suppl. 1), 25-28.

Han, D., Chen, W., Gu, X., Shan, R., Zou, J., Liu, G., . . . Han, B. (2017). Cytoprotective effect of chlorogenic acid against hydrogen peroxide-induced oxidative stress in MC3T3-E1 cells through PI3K/Akt-mediated Nrf2/HO-1 signaling pathway. *Oncotarget, 8*(9), 14680.

Han, S., Wu, H., Li, W., & Gao, P. (2015). Protective effects of genistein in homocysteine-induced endothelial cell inflammatory injury. *Molecular and Cellular Biochemistry, 403*(1), 43-49. doi:10.1007/s11010-015-2335-0

Hao, M. L., Pan, N., Zhang, Q. H., & Wang, X. H. (2015). Therapeutic efficacy of chlorogenic acid on cadmium-induced oxidative neuropathy in a murine model. *Experimental and therapeutic medicine, 9*(5), 1887-1894.

Hassan, A., Ibrahim, A., Mbodji, K., Coëffier, M., Ziegler, F., Bounoure, F., . . . Marion-Letellier, R. (2010). An α-Linolenic acid-rich formula reduces oxidative stress and inflammation by regulating NF-κB in rats with TNBS-induced colitis. *The Journal of Nutrition, 140*(10), 1714-1721. doi:10.3945/jn.109.119768

Hassaninasab, A., Hashimoto, Y., Tomita-Yokotani, K., & Kobayashi, M. (2011). Discovery of the curcumin metabolic pathway involving a unique enzyme in an intestinal microorganism. *Proceedings of the National Academy of Sciences, 108*(16), 6615-6620.

Hata, K., Ogihara, K., Takahashi, S., Tsuka, T., Minami, S., & Okamoto, Y. (2010). Effects of lupeol on melanoma in vitro and in vivo: Fundamental and clinical trials. In *Animal Cell Technology: Basic and Applied Aspects* (pp. 339-344): Springer.

Hatcher, H., Planalp, R., Cho, J., Torti, F., & Torti, S. (2008). Curcumin: From ancient medicine to current clinical trials. *Cellular and Molecular Life Sciences, 65*(11), 1631-1652.

He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S., & Du, Z. (2015). Curcumin, inflammation, and chronic diseases: How are they linked? *Molecules, 20*(5), 9183-9213.

Helal, A., & Tagliazucchi, D. (2018). Impact of in-vitro gastro-pancreatic digestion on polyphenols and cinnamaldehyde bioaccessibility and antioxidant activity in stirred cinnamon-fortified yogurt. *LWT, 89*, 164-170.

Helal, A., Tagliazucchi, D., Verzelloni, E., & Conte, A. (2014). Bioaccessibility of polyphenols and cinnamaldehyde in cinnamon beverages subjected to in vitro gastro-pancreatic digestion. *Journal of Functional Foods, 7*, 506-516. doi:https://doi.org/10.1016/j.jff.2014.01.005

Herrero, M., Plaza, M., Cifuentes, A., & Ibáñez, E. (2010). Green processes for the extraction of bioactives from Rosemary: Chemical and functional characterization via ultra-performance liquid chromatography-tandem mass spectrometry and in-vitro assays. *Journal of Chromatography A, 1217*(16), 2512-2520.

Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its' effects on human health. *Foods, 6*(10), 92.

Hirai, S., Kim, Y. I., Goto, T., Kang, M. S., Yoshimura, M., Obata, A., . . . Kawada, T. (2007). Inhibitory effect of naringenin chalcone on inflammatory changes in the interaction between adipocytes and macrophages. *Life Science, 81*(16), 1272-1279. doi:10.1016/j.lfs.2007.09.001

Hnizdo, E., & Vallyathan, V. (2003). Chronic obstructive pulmonary disease due to occupational exposure to silica dust: A review of epidemiological and pathological evidence. *Occupational and Environmental Medicine, 60*(4), 237-243.

Holdsworth, S. R., & Gan, P. Y. (2015). Cytokines: Names and numbers you should care about. *Clinical Journal of the American Society of Nephrology, 10*(12), 2243-2254.

Horiuchi, H., & Sasaki, Y. (2012). Short communication: Effect of oxygen on symbiosis between Lactobacillus bulgaricus and Streptococcus thermophilus. *Journal of Dairy Science, 95*(6), 2904-2909. doi:https://doi.org/10.3168/jds.2011-5147

Hwang, H., T Wecksler, A., Wagner, K., & D Hammock, B. (2013). Rationally designed multitarget agents against inflammation and pain. *Current Medicinal Chemistry, 20*(13), 1783-1799.

Hwang, S. J., Kim, Y. W., Park, Y., Lee, H. J., & Kim, K. W. (2014). Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells. *Inflammation Research, 63*(1), 81-90. doi:10.1007/s00011-013-0674-4

Izadi, Z., Nasirpour, A., Garoosi, G. A., & Tamjidi, F. (2015). Rheological and physical properties of yogurt enriched with phytosterol during storage. *Journal of Food Science and Technology, 52*(8), 5341-5346. doi:10.1007/s13197-014-1593-2

Jaiswal, R., Kiprotich, J., & Kuhnert, N. (2011). Determination of the hydroxycinnamate profile of 12 members of the Asteraceae family. *Phytochemistry, 72*(8), 781-790.

Jaiswal, R., Matei, M. F., Golon, A., Witt, M., & Kuhnert, N. (2012). Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food and Function, 3*(9), 976-984.

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry, 175*, 556-567.

Jayaraman, J., Jesudoss, V. A. S., Menon, V. P., & Namasivayam, N. (2012). Anti-inflammatory role of naringenin in rats with ethanol induced liver injury. *Toxicology Mechanisms and Methods, 22*(7), 568-576. doi:10.3109/15376516.2012.707255

Jaziri, I., Ben Slama, M., Mhadhbi, H., Urdaci, M. C., & Hamdi, M. (2009). Effect of green and black teas (Camellia sinensis L.) on the characteristic microflora of yogurt during fermentation and refrigerated storage. *Food Chemistry, 112*(3), 614-620. doi:https://doi.org/10.1016/j.foodchem.2008.06.017

Jiang, H., Wang, Z., Wang, Y., Xie, K., Zhang, Q., Luan, Q., . . . Liu, D. (2013). Antidepressant-like effects of curcumin in chronic mild stress of rats: Involvement of its anti-inflammatory action. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 47*, 33-39.

Jiang, Y., Gong, F. L., Zhao, G. B., & Li, J. (2014). Chrysin suppressed inflammatory responses and the inducible nitric oxide synthase pathway after spinal cord injury in rats. *International Journal of Molecular Sciences, 15*(7), 12270.

Jiménez-Martínez, C., Hernández-Sánchez, H., & Dávila-Ortiz, G. (2003). Production of a yogurt-like product from Lupinus campestris seeds. *Journal of the Science of Food and Agriculture, 83*(6), 515-522. doi:10.1002/jsfa.1385

Jin, C. Y., Lee, J. D., Park, C., Choi, Y. H., & Kim, G. Y. (2007). Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia 1. *Acta Pharmacologica Sinica, 28*(10), 1645-1651.

Jin, H. H., Lu, Q., & Jiang, J. G. (2016). Curcumin liposomes prepared with milk fat globule membrane phospholipids and soybean lecithin. *Journal of Dairy Science, 99*(3), 1780-1790.

Jobin, C., Bradham, C. A., Russo, M. P., Juma, B., Narula, A. S., Brenner, D. A., & Sartor, R. B. (1999). Curcumin blocks cytokine-mediated NF-κB activation and proinflammatory gene expression by inhibiting inhibitory factor I-κB kinase activity. *The Journal of Immunology, 163*(6), 3474-3483.

Joint FAO/WHO Expert Committee on Food Additives. (2004). Chemical and technical assessment compendium addendum 11/Fnp 52 Add.11/29. *Monographs, 1/417*.

Joseph, S. V., Edirisinghe, I., & Burton-Freeman, B. M. (2016). Fruit polyphenols: A review of anti-inflammatory effects in humans. *Critical Reviews in Food Science and Nutrition, 56*(3), 419-444.

Jurenka, J. S. (2009). Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: A review of preclinical and clinical research. *Alternative Medicine Review, 14*(2).

Kaminska, B. (2005). MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1754*(1-2), 253-262.

Kanai, M., Yoshimura, K., Asada, M., Imaizumi, A., Suzuki, C., Matsumoto, S., . . . Aggarwal, B. B. (2011). A phase I/II study of gemcitabine-based chemotherapy plus curcumin for patients with gemcitabine-resistant pancreatic cancer. *Cancer Chemotherapy and Pharmacology, 68*(1), 157-164. doi:10.1007/s00280-010-1470-2

Kang, I., Buckner, T., Shay, N. F., Gu, L., & Chung, S. (2016). Improvements in metabolic health with consumption of ellagic acid and subsequent conversion into Urolithins: Evidence and mechanisms. *Advances in Nutrition, 7*(5), 961-972. doi:10.3945/an.116.012575

Kangsamaksin, T., Chaithongyot, S., Wootthichairangsan, C., Hanchaina, R., Tangshewinsirikul, C., & Svasti, J. (2017). Lupeol and stigmasterol suppress tumor

angiogenesis and inhibit cholangiocarcinoma growth in mice via downregulation of tumor necrosis factor-α. *PLoS One, 12*(12), e0189628.

Kant, V., Gopal, A., Pathak, N. N., Kumar, P., Tandan, S. K., & Kumar, D. (2014). Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats. *International Immunopharmacology, 20*(2), 322-330.

Karthikesan, K., Pari, L., & Menon, V. P. (2010). Protective effect of tetrahydrocurcumin and chlorogenic acid against streptozotocin–nicotinamide generated oxidative stress induced diabetes. *Journal of Functional Foods, 2*(2), 134-142.

Kasinathan, N. K., Subramaniya, B., & Sivasithamparam, N. D. (2018). NF-κB/twist mediated regulation of colonic inflammation by lupeol in abating dextran sodium sulfate induced colitis in mice. *Journal of Functional Foods, 41*, 240-249.

Kaur, H., Patro, I., Tikoo, K., & Sandhir, R. (2015). Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy. *Neurochemistry International, 89*, 40-50.

Kaur, S., & Das, M. (2011). Functional foods: An overview. *Food Science and Biotechnology, 20*(4), 861.

Kempf, K., Herder, C., Erlund, I., Kolb, H., Martin, S., Carstensen, M., . . . Kuha, S. (2010). Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: A clinical trial. *The American Journal of Clinical Nutrition, 91*(4), 950-957.

Khajehdehi, P., Pakfetrat, M., Javidnia, K., Azad, F., Malekmakan, L., Nasab, M. H., & Dehghanzadeh, G. (2011). Oral supplementation of turmeric attenuates proteinuria, transforming growth factor-β and interleukin-8 levels in patients with overt type 2 diabetic nephropathy: A randomized, double-blind and placebo-controlled study. *Scandinavian Journal of Urology and Nephrology, 45*(5), 365-370. doi:10.3109/00365599.2011.585622

Khalatbary, A. R., & Ahmadvand, H. (2011). Anti-inflammatory effect of the epigallocatechin gallate following spinal cord trauma in rat. *Iranian Biomedical Journal, 15*(1-2), 31-37.

Khan, R. S., Grigor, J., Winger, R., & Win, A. (2013). Functional food product development–Opportunities and challenges for food manufacturers. *Trends in Food Science and Technology, 30*(1), 27-37.

Kharat, M., Du, Z., Zhang, G., & McClements, D. J. (2017). Physical and chemical stability of curcumin in aqueous solutions and emulsions: Impact of pH, temperature, and molecular environment. *Journal of Agricultural and Food Chemistry, 65*(8), 1525-1532. doi:10.1021/acs.jafc.6b04815

Kim, Lee, W., & Bae, J. S. (2011). Vascular anti-inflammatory effects of curcumin on HMGB1-mediated responses in vitro. *Inflammation Research, 60*(12), 1161-1168.

Kim, M. J., Bae, G. S., Choi, S. B., Jo, I. J., Kim, D. G., Shin, J. Y., . . . Park, S. J. (2015). Lupeol protects against cerulein-induced acute pancreatitis in mice. *Phytotherapy Research, 29*(10), 1634-1639.

Kim, S. H., Johnson, V. J., Shin, T. Y., & Sharma, R. P. (2004). Selenium attenuates lipopolysaccharide-induced oxidative stress responses through modulation of p38 MAPK and NF-κB signaling pathways. *Experimental Biology and Medicine, 229*(2), 203-213.

Kim, Y. J., Lee, H. J., & Shin, Y. (2015). Estimation of daily curcuminoid intake from commercial curry products. *Journal of the Korean Society for Applied Biological Chemistry, 58*(5), 677-684.

Kima, C. S., Kawadab, T., Kimc, B. S., Hanc, I. S., Choea, S. Y., Kuratad, T., & Yu, R. (2003). Capsaicin exhibits anti-inflammatory property by inhibiting IkB-a degradation in LPS-stimulated peritoneal macrophages. *Cellular Signalling, 15*, 299 – 306. doi:https://doi.org/10.1016/S0898-6568(02)00086-4

Klawitter, M., Quero, L., Klasen, J., Gloess, A. N., Klopprogge, B., Hausmann, O., . . . Wuertz, K. (2012). Curcuma DMSO extracts and curcumin exhibit an anti-inflammatory and anti-catabolic effect on human intervertebral disc cells, possibly by influencing TLR2 expression and JNK activity. *Journal of Inflammation, 9*(1), 29.

Kocaadam, B., & Şanlier, N. (2017). Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. *Critical Reviews in Food Science and Nutrition, 57*(13), 2889-2895.

Konishi, Y., & Kobayashi, S. (2004). Transepithelial transport of chlorogenic acid, caffeic acid, and their colonic metabolites in intestinal Caco-2 cell monolayers. *Journal of Agricultural and Food Chemistry, 52*(9), 2518-2526.

Korhonen, H., & Pihlanto, A. (2003). Food-derived bioactive peptides - Opportunities for designing future foods. *Current Pharmaceutical Design, 9*(16), 1297-1308. doi:http://dx.doi.org/10.2174/1381612033454892

Koriem, K. M., & Soliman, R. E. (2014). Chlorogenic and caftaric acids in liver toxicity and oxidative stress induced by methamphetamine. *Journal of Toxicology, 2014*.

Kotlyarov, A., Neininger, A., Schubert, C., Eckert, R., Birchmeier, C., Volk, H.-D., & Gaestel, M. (1999). MAPKAP kinase 2 is essential for LPS-induced TNF-α biosynthesis. *Nature Cell Biology, 1*, 94. doi:10.1038/10061

Krakauer, T. (2002). The polyphenol chlorogenic acid inhibits staphylococcal exotoxin-induced inflammatory cytokines and chemokines. *Immunopharmacology and Immunotoxicology, 24*(1), 113-119.

Kumar, S. (Producer). (2016, 21st Nov 2018). Curcumin: Powdered gold? Retrieved from https://www.naturalproductsinsider.com/healthy-living/curcumin-powdered-gold

Kumar, V., Abbas, A. K., & Aster, J. C. (2012a). Cell injury, cell death, and adaptations. In *Robbins Basis Pathology* (9th ed., pp. 1-28). Saunders: Elsevier.

Kumar, V., Abbas, A. K., & Aster, J. C. (2012b). Diseases of the immune system. In *Robbins Basis Pathology* (9th ed., pp. 99-159). Saunders: Elsevier.

Kumar, V., Abbas, A. K., & Aster, J. C. (2012c). Inflammation and repair. In *Robbins Basis Pathology* (9th ed., pp. 29-73). Saunders: Elsevier.

Kumari, A., & Kakkar, P. (2012a). Lupeol prevents acetaminophen-induced in vivo hepatotoxicity by altering the Bax/Bcl-2 and oxidative stress-mediated mitochondrial signaling cascade. *Life Sciences, 90*(15-16), 561-570.

Kumari, A., & Kakkar, P. (2012b). Lupeol protects against acetaminophen-induced oxidative stress and cell death in rat primary hepatocytes. *Food and Chemical Toxicology, 50*(5), 1781-1789.

Lafay, S., Gil-Izquierdo, A., Manach, C., Morand, C., Besson, C., & Scalbert, A. (2006). Chlorogenic acid is absorbed in its intact form in the stomach of rats. *The Journal of Nutrition, 136*(5), 1192-1197.

Laine, L. (2001). Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient. *Gastroenterology, 120*(3), 594-606.

Lambertini, E., Lampronti, I., Penolazzi, L., Khan, M. T. H., Ather, A., Giorgi, G., . . . Piva, R. (2005). Expression of estrogen receptor α gene in breast cancer cells treated with transcription factor decoy is modulated by Bangladeshi natural plant extracts. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics, 15*(2), 69-79.

Lamothe, S., Azimy, N., Bazinet, L., Couillard, C., & Britten, M. (2014). Interaction of green tea polyphenols with dairy matrices in a simulated gastrointestinal environment. *Food and Function, 5*(10), 2621-2631. doi:10.1039/C4FO00203B

Larrosa, M., Yañéz-Gascón, M. J., Selma, M. V., González-Sarrías, A., Toti, S., Cerón, J. J., . . . Espín, J. C. (2009). Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *Journal of Agricultural and Food Chemistry, 57*(6), 2211-2220. doi:10.1021/jf803638d

Laureati, M., Pagliarini, E., Calcinoni, O., & Bidoglio, M. (2006). Sensory acceptability of traditional food preparations by elderly people. *Food Quality and Preference, 17*(1-2), 43-52.

Lee, C., Lee, J. W., Seo, J. Y., Hwang, S. W., Im, J. P., & Kim, J. S. (2016). Lupeol inhibits LPS-induced NF-kappa B signaling in intestinal epithelial cells and macrophages, and attenuates acute and chronic murine colitis. *Life Sciences, 146*, 100-108. doi:10.1016/j.lfs.2016.01.001

Lee, C. W., Won, T. J., Kim, H. R., Lee, D. H., Hwang, K. W., & Park, S. Y. (2011). Protective effect of chlorogenic acid against Aβ-induced neurotoxicity. *Biomolecules and Therapeutics, 19*(2), 181-186.

Lee, T. K., Poon, R. T. P., Wo, J. Y., Ma, S., Guan, X. Y., Myers, J. N., . . . Yuen, A. P. W. (2007). Lupeol suppresses cisplatin-induced nuclear factor-κB activation in head and neck squamous cell carcinoma and inhibits local invasion and nodal metastasis in an orthotopic nude mouse model. *Cancer Research, 67*(18), 8800-8809. doi:10.1158/0008-5472.can-07-0801

Lee, W. J., & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on the physical and sensory properties of stirred yogurts. *Journal of Dairy Science, 89*(7), 2374-2385. doi:https://doi.org/10.3168/jds.S0022-0302(06)72310-4

Lee, W. J., & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Australasian Journal of Animal Sciences, 23*(9), 1127-1136.

Lee, W. J., Ou, H. C., Hsu, W. C., Chou, M. M., Tseng, J. J., Hsu, S. L., . . . Sheu, W. H. (2010). Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation, and inflammation in human endothelial cells. *Journal of Vascular Surgery, 52*(5), 1290-1300. doi:10.1016/j.jvs.2010.04.085

Lev-Ari, S., Strier, L., Kazanov, D., Elkayam, O., Lichtenberg, D., Caspi, D., & Arber, N. (2005). Curcumin synergistically potentiates the growth-inhibitory and pro-apoptotic effects of celecoxib in osteoarthritis synovial adherent cells. *Rheumatology, 45*(2), 171-177. doi:10.1093/rheumatology/kei132

Li, C., Ma, J., Peng, X., Chen, S., & Zhang, X. (2011). Expression of IL-18, IL-6 and oxidative stress in rat peritoneal mesothelial cells stimulated by lipopolysaccharide. *Chinese journal of cellular and molecular immunology, 27*(6), 688-690.

Li, T., Gao, D., Du, M., Cheng, X., & Mao, X. (2018). Casein glycomacropeptide hydrolysates inhibit PGE2 production and COX2 expression in LPS-stimulated RAW 264.7 macrophage cells via Akt mediated NF-kappaB and MAPK pathways. *Food and Function, 9*(4), 2524-2532. doi:10.1039/c7fo01989k

Li, W., Suwanwela, N. C., & Patumraj, S. (2017). Curcumin prevents reperfusion injury following ischemic stroke in rats via inhibition of NF-κB, ICAM-1, MMP-9 and caspase-3 expression. *Molecular Medicine Reports, 16*(4), 4710-4720.

Li, X., Udagawa, N., Takami, M., Sato, N., Kobayashi, Y., & Takahashi, N. (2003). p38 Mitogen-activated protein kinase is crucially involved in osteoclast differentiation but not in cytokine production, phagocytosis, or dendritic cell differentiation of bone marrow macrophages. *Endocrinology, 144*(11), 4999-5005.

Li, Y. J., Zhang, C. F., Ding, G., Huang, W. Z., Wang, Z. Z., Bi, Y. A., & Xiao, W. (2015). Investigating the thermal stability of six caffeoylquinic acids employing rapid-resolution liquid chromatography with quadrupole time-of-flight tandem mass spectrometry. *European Food Research and Technology, 240*(6), 1225-1234.

Liang, N., & Kitts, D. (2018). Amelioration of oxidative stress in Caco-2 cells treated with pro-inflammatory proteins by chlorogenic acid isomers via activation of the Nrf2-Keap1-ARE-signaling pathway. *Journal of Agricultural and Food Chemistry*.

Liang, N., & Kitts, D. (2015). Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients, 8*(1), 16.

Liang, N., Sang, Y., Liu, W., Yu, W., & Wang, X. (2018). Anti-Inflammatory effects of gingerol on lipopolysaccharide-stimulated RAW 264.7 cells by inhibiting NF-κB signaling pathway. *Inflammation, 41*(3), 835-845. doi:10.1007/s10753-018-0737-3

Liang, Z., Wu, R., Xie, W., Geng, H., Zhao, L., Xie, C., . . . Zhu, M. (2015). Curcumin suppresses MAPK pathways to reverse tobacco smoke-induced gastric epithelial–mesenchymal transition in mice. *Phytotherapy Research, 29*(10), 1665-1671.

Liby, K. T., Yore, M. M., & Sporn, M. B. (2007). Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. *Nature Reviews Cancer, 7*(5), 357.

Lim, H. D., Kim, Y. S., Ko, S. H., Yoon, I. J., Cho, S. G., Chun, Y. H., . . . Kim, E. C. (2012). Cytoprotective and anti-inflammatory effects of melatonin in hydrogen peroxide-stimulated CHON-001 human chondrocyte cell line and rabbit model of osteoarthritis via the SIRT1 pathway. *Journal of Pineal Research, 53*(3), 225-237. doi:https://doi.org/10.1111/j.1600-079X.2012.00991.x

Lima, L. M., Perazzo, F. F., Carvalho, J. C. T., & Bastos, J. K. (2007). Anti-inflammatory and analgesic activities of the ethanolic extracts from Zanthoxylum riedelianum (Rutaceae) leaves and stem bark. *Journal of Pharmacy and Pharmacology, 59*(8), 1151-1158.

Lin, C. C., Lin, H. Y., Chi, M. H., Shen, C. M., Chen, H. W., Yang, W. J., & Lee, M. H. (2014). Preparation of curcumin microemulsions with food-grade soybean oil/lecithin and their cytotoxicity on the HepG2 cell line. *Food Chemistry, 154*, 282-290.

Lin, J. Y., & Tang, C. Y. (2008). Strawberry, loquat, mulberry, and bitter melon juices exhibit prophylactic effects on LPS-induced inflammation using murine peritoneal macrophages. *Food Chemistry, 107*(4), 1587-1596.

Liu, W., Zhai, Y., Heng, X., Che, F. Y., Chen, W., Sun, D., & Zhai, G. (2016). Oral bioavailability of curcumin: Problems and advancements. *Journal of Drug Targeting, 24*(8), 694-702.

Liu, Y. N., Pan, S. L., Liao, C. H., Huang, D. Y., Guh, J. H., Peng, C. Y., . . . Teng, C. M. (2009). Evodiamine represses hypoxia-induced inflammatory proteins expression and hypoxia-inducible factor 1alpha accumulation in RAW264.7. *Shock, 32*(3), 263-269. doi:10.1097/SHK.0b013e31819940cb

Long, F., Yang, H., Xu, Y., Hao, H., & Li, P. (2015). A strategy for the identification of combinatorial bioactive compounds contributing to the holistic effect of herbal medicines. *Scientific Reports, 5*, 12361.

Lopez-Garcia, E., van Dam, R. M., Qi, L., & Hu, F. B. (2006). Coffee consumption and markers of inflammation and endothelial dysfunction in healthy and diabetic women. *The American Journal of Clinical Mutrition, 84*(4), 888-893.

Louis, K. S., & Siegel, A. C. (2011). Cell viability analysis using trypan blue: Manual and automated methods. In M. J. Stoddart (Ed.), *Mammalian Cell Viability: Methods and Protocols* (pp. 7-12). Totowa, NJ: Humana Press.

Lozada-García, M. C., Enríquez, R. G., Ramírez-Apán, T. O., Nieto-Camacho, A., Palacios-Espinosa, J. F., Custodio-Galván, Z., . . . Pérez-Villanueva, J. (2017). Synthesis of curcuminoids and evaluation of their cytotoxic and antioxidant properties. *Molecules, 22*(4), 633.

Lucetti, D. L., Lucetti, E. C., Bandeira, M. A. M., Veras, H. N., Silva, A. H., Leal, L. K. A., . . . Viana, G. B. (2010). Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from Himatanthus drasticus (Mart.) Plumel. *Journal of Inflammation, 7(1), 60*. doi:https://doi.org/10.1186/1476-9255-7-60

Lucey, J. A. (2002). Formation and physical properties of milk protein gGels. *Journal of Dairy Science, 85*(2), 281-294. doi:https://doi.org/10.3168/jds.S0022-0302(02)74078-2

Lucey, J. A., Munro, P. A., & Singh, H. (1998). Whey separation in acid skim milk gels made with glucono-δ-lactone: Effects of heat treatment and gelation temperature. *Journal of Texture Studies, 29*(4), 413-426. doi:10.1111/j.1745-4603.1998.tb00813.x

Ludwig, I. A., Mena, P., Calani, L., Cid, C., Del Rio, D., Lean, M. E. J., & Crozier, A. (2014). Variations in caffeine and chlorogenic acid contents of coffees: What are we drinking? *Food and Function, 5*(8), 1718-1726. doi:10.1039/C4FO00290C

Lund, M. E., To, J., O'Brien, B. A., & Donnelly, S. (2016). The choice of phorbol 12-myristate 13-acetate differentiation protocol influences the response of THP-1 macrophages to a pro-inflammatory stimulus. *Journal of Immunological Methods, 430*, 64-70.

Lutchman, D., Pillay, S., Naidoo, R., Shangase, N., Nayak, R., & Rughoobeer, A. (2006). Evaluation of the buffering capacity of powdered cow's, goat's and soy milk and non-prescription antacids in the treatment of non-ulcer dyspepsia. *South African Medical Journal, 96*(1), 57-61.

Ma, F., Liu, F., Ding, L., You, M., Yue, H., Zhou, Y., & Hou, Y. (2017). Anti-inflammatory effects of curcumin are associated with down regulating microRNA-155 in LPS-treated macrophages and mice. *Pharmaceutical Biology, 55*(1), 1263-1273.

Mahdian, E., & Tehrani, M. M. (2007). Evaluation the effect of milk total solids on the relationship between growth and activity of starter cultures and quality of concentrated yoghurt. *Journal Agriculture and Environmental Science, 2*(5), 587-592.

Mahmoud, M. F., El-Nagar, M., & El-Bassossy, H. M. (2012). Anti-inflammatory effect of atorvastatin on vascular reactivity and insulin resistance in fructose fed rats. *Archives of Pharmacal Research, 35*(1), 155-162.

Mahran, R. I., Hagras, M. M., Sun, D., & Brenner, D. E. (2017). Bringing curcumin to the clinic in cancer prevention: A review of strategies to enhance bioavailability and efficacy. *The AAPS Journal, 19*(1), 54-81.

Mansouri, M. T., Hemmati, A. A., Naghizadeh, B., Mard, S. A., Rezaie, A., & Ghorbanzadeh, B. (2015). A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. *Indian Journal of Pharmacology, 47*(3), 292-298. doi:10.4103/0253-7613.157127

Marchiani, A., Rozzo, C., Fadda, A., Delogu, G., & Ruzza, P. (2014). Curcumin and curcumin-like molecules: From spice to drugs. *Current Medicinal Chemistry, 21*(2), 204-222.

Marini, H., Polito, F., Adamo, E. B., Bitto, A., Squadrito, F., & Benvenga, S. (2012). Update on genistein and thyroid: An overall message of safety. *Frontiers in Endocrinology, 3*, 94. doi:10.3389/fendo.2012.00094

Martineza, J., & Morenoa, J. J. (2000). Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. *Biochemical Pharmacology, 59*(7), 865-870. doi:https://doi.org/10.1016/S0006-2952(99)00380-9

Matei, M. F., Jaiswal, R., & Kuhnert, N. (2012). Investigating the chemical changes of chlorogenic acids during coffee brewing: Conjugate addition of water to the olefinic moiety of chlorogenic acids and their quinides. *Journal of Agricultural and Food Chemistry, 60*(49), 12105-12115.

Mei, Y. Z., Liu, R. X., Wang, D. P., Wang, X., & Dai, C. C. (2015). Biocatalysis and biotransformation of resveratrol in microorganisms. *Biotechnology Letters, 37*(1), 9-18. doi:10.1007/s10529-014-1651-x

Meirer, K., Steinhilber, D., & Proschak, E. (2014). Inhibitors of the arachidonic acid cascade: Interfering with multiple pathways. *Basic and Clinical Pharmacology and Toxicology, 114*(1), 83-91.

Meirow, Y., & Baniyash, M. (2017). Immune biomarkers for chronic inflammation related complications in non-cancerous and cancerous diseases. *Cancer Immunology, Immunotherapy, 66*(8), 1089-1101. doi:10.1007/s00262-017-2035-6

Meng, F., & Lowell, C. A. (1997). Lipopolysaccharide (LPS)-induced macrophage activation and signal transduction in the absence of Src-family kinases Hck, Fgr, and Lyn. *Journal of Experimental Medicine, 185*(9), 1661-1670.

Meng, Z., Yan, C., Deng, Q., Gao, D. F., & Niu, X. L. (2013). Curcumin inhibits LPS-induced inflammation in rat vascular smooth muscle cells in vitro via ROS-relative TLR4-MAPK/NF-κB pathways. *Acta Pharmacologica Sinica, 34*(7), 901.

Mirahmadi, S. M., Shahmohammadi, A., Rousta, A. M., Azadi, M. R., Fahanik-Babaei, J., Baluchnejadmojarad, T., & Roghani, M. (2018). Soy isoflavone genistein attenuates lipopolysaccharide-induced cognitive impairments in the rat via exerting anti-oxidative and anti-inflammatory effects. *Cytokine, 104*, 151-159. doi:10.1016/j.cyto.2017.10.008

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal, 20*(7), 1126-1167. doi:10.1089/ars.2012.5149

Moon, J. K., Yoo, H. S., & Shibamoto, T. (2009). Role of roasting conditions in the level of chlorogenic acid content in coffee beans: Correlation with coffee acidity. *Journal of Agricultural and Food Chemistry, 57*(12), 5365-5369.

Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research, 41*(6), 457-500.

Morikawa, K., Nonaka, M., Narahara, M., Torii, I., Kawaguchi, K., Yoshikawa, T., . . . Morikawa, S. (2003). Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. *Life Sciences, 74*(6), 709-721. doi:10.1016/j.lfs.2003.06.036

Mosiyani, Z. G., Pourahmad, R., & Eshaghi, M. R. (2017). Investigating the effect of aqueous extracts of basil and savory on antioxidant activity, microbial and sensory properties of probiotic yogurt. *Acta Scientiarum Polonorum Technologia Alimentaria, 16*(3), 311-320.

Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., & Hu, F. B. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. *New England Journal of Medicine, 364*(25), 2392-2404.

Mudgil, D., Barak, S., & Khatkar, B. (2017). Texture profile analysis of yogurt as influenced by partially hydrolyzed guar gum and process variables. *Journal of Food Science and Technology, 54*(12), 3810-3817.

Mueller, M., Hobiger, S., & Jungbauer, A. (2010). Anti-inflammatory activity of extracts from fruits, herbs and spices. *Food Chemistry, 122*(4), 987-996.

Mullaicharam, A., & Maheswaran, A. (2012). Pharmacological effects of curcumin. *International Journal of Nutrition, Pharmacology, Neurological Diseases, 2*(2), 92.

Muniandy, P., Shori, A. B., & Baba, A. S. (2016). Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packaging and Shelf Life, 8*, 1-8.

Murakami, Y., Ishii, H., Takada, N., Tanaka, S., Machino, M., Ito, S., & Fujisawa, S. (2008). Comparative anti-inflammatory activities of curcumin and tetrahydrocurcumin based on the phenolic OH bond dissociation enthalpy, ionization potential and quantum chemical descriptor. *Anticancer Research, 28*(2A), 699-707.

Murakami, Y., Kawata, A., Ito, S., Katayama, T., & Fujisawa, S. (2015). Radical-scavenging and anti-inflammatory activity of quercetin and related compounds and their combinations against RAW264.7 cells stimulated with porphyromonas gingivalis fimbriae. Relationships between anti-inflammatory activity and quantum chemical parameters. *In Vivo, 29*, 701-710.

Naik, R., Mujumdar, A., & Ghaskadbi, S. (2004). Protection of liver cells from ethanol cytotoxicity by curcumin in liver slice culture in vitro. *Journal of Ethnopharmacology, 95*(1), 31-37.

Narataruksa, P., Pichitvittayakarn, W., Heggs, P. J., & Tia, S. (2010). Fouling behavior of coconut milk at pasteurization temperatures. *Applied Thermal Engineering, 30*(11), 1387-1395. doi:https://doi.org/10.1016/j.applthermaleng.2010.02.028

Nardini, M., Cirillo, E., Natella, F., & Scaccini, C. (2002). Absorption of phenolic acids in humans after coffee consumption. *Journal of Agricultural and Food Chemistry, 50*(20), 5735-5741.

Narita, Y., & Inouye, K. (2013). Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate on its stability under alkaline conditions. *Journal of Agricultural and Food Chemistry, 61*(4), 966-972.

Naso, L. G., Valcarcel, M., Roura-Ferrer, M., Kortazar, D., Salado, C., Lezama, L., . . . Ferrer, E. G. (2014). Promising antioxidant and anticancer (human breast cancer) oxidovanadium (IV) complex of chlorogenic acid. Synthesis, characterization and spectroscopic examination on the transport mechanism with bovine serum albumin. *Journal of Inorganic Biochemistry, 135*, 86-99.

National Institutes of Health USA. (2018). Omega-3 Fatty Acids. Retrieved 7 Oct 2018, from Department of health and human services https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/

Naveed, M., Hejazi, V., Abbas, M., Kamboh, A. A., Khan, G. J., Shumzaid, M., . . . Modarresi-Ghazani, F. (2018). Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomedicine and Pharmacotherapy, 97*, 67-74.

Ndife, J., Idoko, F., & Garba, R. (2014). Production and quality assessment of functional yoghurt enriched with coconut. *International Journal of Nutrition and Food Sciences, 3*(6), 545-550.

Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The Essential medicinal chemistry of curcumin. *Journal of Medicinal Chemistry, 60*(5), 1620-1637. doi:10.1021/acs.jmedchem.6b00975

Neurath, M. F. (2014). Cytokines in inflammatory bowel disease. *Nature Reviews Immunology, 14*(5), 329.

Ng, E. W., Yeung, M., & Tong, P. S. (2011). Effects of yogurt starter cultures on the survival of Lactobacillus acidophilus. *International Journal of Food Microbiology, 145*(1), 169-175. doi:https://doi.org/10.1016/j.ijfoodmicro.2010.12.006

Ngkelo, A., Meja, K., Yeadon, M., Adcock, I., & Kirkham, P. A. (2012). LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and G i α dependent PI-3kinase signalling. *Journal of Inflammation, 9*(1), 1.

Nguemfo, E., Dimo, T., Dongmo, A., Azebaze, A., Alaoui, K., Asongalem, A., . . . Kamtchouing, P. (2009). Anti-oxidative and anti-inflammatory activities of some isolated constituents from the stem bark of Allanblackia monticola Staner LC (Guttiferae). *Inflammopharmacology, 17*(1), 37-41.

Nguyen, M. A., Staubach, P., Tamai, I., & Langguth, P. (2015). High-dose short-term administration of naringin did not alter talinolol pharmacokinetics in humans. *European Journal of Pharmaceutical Sciences, 68*, 36-42. doi:10.1016/j.ejps.2014.12.001

Nishio, K., Horie, M., Akazawa, Y., Shichiri, M., Iwahashi, H., Hagihara, Y., . . . Niki, E. (2013). Attenuation of lipopolysaccharide (LPS)-induced cytotoxicity by tocopherols and tocotrienols. *Redox Biology, 1*(1), 97-103.

Noorafshan, A., & Ashkani-Esfahani, S. (2013). A review of therapeutic effects of curcumin. *Current Pharmaceutical Design, 19*(11), 2032-2046.

O'Sullivan, A. M., O'Grady, M. N., O'Callaghan, Y. C., Smyth, T. J., O'Brien, N. M., & Kerry, J. P. (2016). Seaweed extracts as potential functional ingredients in yogurt. *Innovative*

Food Science and Emerging Technologies, 37, 293-299. doi:https://doi.org/10.1016/j.ifset.2016.07.031

Ohama, H., Ikeda, H., & Moriyama, H. (2008). Health foods and foods with health claims in Japan. In *Nutraceutical and Functional Food Regulations in the United States and around the World* (pp. 249-280): Elsevier.

Ohkawara, T., Takeda, H., & Nishihira, J. (2017). Protective effect of chlorogenic acid on the inflammatory damage of pancreas and lung in mice with l-arginine-induced pancreatitis. *Life Sciences, 190*, 91-96.

Onakpoya, I. J., Spencer, E. A., Thompson, M. J., & Heneghan, C. J. (2014). The effect of chlorogenic acid on blood pressure: A systematic review and meta-analysis of randomized clinical trials. *Journal Of Human Hypertension, 29*, 77. doi:10.1038/jhh.2014.46 https://www.nature.com/articles/jhh201446#supplementary-information

Onwulata, C. I. (2013). Microencapsulation and functional bioactive foods. *Journal of Food Processing and Preservation, 37*(5), 510-532. doi:10.1111/j.1745-4549.2012.00680.x

Onyeneho, S. N., & Hettiarachchy, N. S. (1993). Antioxidant activity, fatty acids and phenolic acids compositions of potato peels. *Journal of the Science of Food and Agriculture, 62*(4), 345-350.

Özbilgin, S., Ergene, B., Altun, M. L., Yilmaz, B. S., Saltan, G., & Yüksel, E. (2015). HPLC method for the analysis of chlorogenic acid of Viburnum tinus L. and Viburnum orientale Pallas. *Turkish Journal of Pharmaceutical Sciences, 12*(2).

Palócz, O., Pászti-Gere, E., Gálfi, P., & Farkas, O. (2016). Chlorogenic acid combined with Lactobacillus plantarum 2142 reduced LPS-induced intestinal inflammation and oxidative stress in IPEC-J2 cells. *PLoS One, 11*(11), e0166642.

Pan, M. H., Lin-Shiau, S. Y., & Lin, J. K. (2000). Comparative studies on the suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of IκB kinase and NFκB activation in macrophages. *Biochemical Pharmacology, 60*(11), 1665-1676.

Panahi, Y., Badeli, R., Karami, G. R., & Sahebkar, A. (2015). Investigation of the efficacy of adjunctive therapy with bioavailability-boosted curcuminoids in major depressive disorder. *Phytotherapy Research, 29*(1), 17-21. doi:10.1002/ptr.5211

Panahi, Y., Ghanei, M., Bashiri, S., Hajihashemi, A., & Sahebkar, A. (2015). Short-term curcuminoid supplementation for chronic pulmonary complications due to sulfur mustard intoxication: Positive results of a randomized double-blind placebo-controlled Trial. *Drug Research (Stuttg), 65*(11), 567-573. doi:10.1055/s-0034-1389986

Panahi, Y., Hosseini, M. S., Khalili, N., Naimi, E., Majeed, M., & Sahebkar, A. (2015). Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: A randomized controlled trial and an updated meta-analysis. *Clinical Nutrition, 34*(6), 1101-1108.

Panahi, Y., Hosseini, M. S., Khalili, N., Naimi, E., Simental-Mendía, L. E., Majeed, M., & Sahebkar, A. (2016). Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial. *Biomedicine and Pharmacotherapy, 82*, 578-582.

Park, E., Jung, H., Yang, H., Yoo, M., Kim, C., & Kim, K. (2007). Optimized THP-1 differentiation is required for the detection of responses to weak stimuli. *Inflammation Research, 56*(1), 45-50.

Park, S. Y., Kim, H. S., Cho, E. K., Kwon, B. Y., Phark, S., Hwang, K. W., & Sul, D. (2008). Curcumin protected PC12 cells against beta-amyloid-induced toxicity through the inhibition of oxidative damage and tau hyperphosphorylation. *Food and Chemical Toxicology, 46*(8), 2881-2887.

Parthiban, M., Jaiganesh, R., Aruni, W. S. K., Meignanalakshmi, S., & Ramadass, P. (2007). Rapid detection of Mycobacterium bovis in clinical samples using THP-1 cells. *Veterinarski arhiv, 77*(3), 265-270.

Paseephol, T., Small, D. M., & Sherkat, F. (2008). Rheology and texture of set yogurt as affected by inulin addition. *Journal of Texture Studies, 39*(6), 617-634. doi:10.1111/j.1745-4603.2008.00161.x

Patil, P. (2018). A review on Lupeol: Superficial triterpenoid from horticulture crops. *International Journal of Communication Systems, 6*(3), 3301-3305.

Pavlica, S., & Gebhardt, R. (2005). Protective effects of ellagic and chlorogenic acids against oxidative stress in PC12 cells. *Free Radical Research, 39*(12), 1377-1390. doi:10.1080/09670260500197660

Payton, F., Sandusky, P., & Alworth, W. L. (2007). NMR study of the solution structure of curcumin. *Journal of Natural Products, 70*(2), 143-146.

Pei, Z., & Cheung, R. T. F. (2004). Pretreatment with melatonin exerts anti-inflammatory effects against ischemia/reperfusion injury in a rat middle cerebral artery occlusion stroke model. *Journal of Pineal Research, 37*(2), 85-91. doi:https://doi.org/10.1111/j.1600-079X.2004.00138.x

Peram, M. R., Jalalpure, S. S., Palkar, M. B., & Diwan, P. V. (2017). Stability studies of pure and mixture form of curcuminoids by reverse phase-HPLC method under various experimental stress conditions. *Food Science and Biotechnology, 26*(3), 591-602.

Pereira Beserra, F., Xue, M., Maia, G., Leite Rozza, A., Helena Pellizzon, C., & Jackson, C. (2018). Lupeol, a pentacyclic triterpene, promotes migration, wound Closure, and contractile effect in vitro: Possible involvement of PI3K/Akt and p38/ERK/MAPK pathways. *Molecules, 23*(11), 2819.

Perrone, D., Donangelo, R., Donangelo, C. M., & Farah, A. (2010). Modeling weight loss and chlorogenic acids content in coffee during roasting. *Journal of Agricultural and Food Chemistry, 58*(23), 12238-12243.

Petrotos, K. B., Karkanta, F. K., Gkoutsidis, P. E., Giavasis, I., Papatheodorou, K. N., & Ntontos, A. C. (2012). Production of novel bioactive yogurt enriched with olive fruit polyphenols. *World Academy of Science, Engineering and Technology, 64*, 867-872.

Pham, T. H., Kim, M. S., Le, M. Q., Song, Y. S., Bak, Y., Ryu, H. W., . . . Yoon, D. Y. (2017). Fargesin exerts anti-inflammatory effects in THP-1 monocytes by suppressing PKC-dependent AP-1 and NF-κB signaling. *Phytomedicine, 24*, 96-103.

Phelan, M., & Kerins, D. (2011). The potential role of milk-derived peptides in cardiovascular disease. *Food and Function, 2*(3-4), 153-167. doi:10.1039/C1FO10017C

Phillips, D. R., Rasbery, J. M., Bartel, B., & Matsuda, S. P. (2006). Biosynthetic diversity in plant triterpene cyclization. *Current Opinion in Plant Biology, 9*(3), 305-314.

Pitchai, D., Roy, A., & Ignatius, C. (2014). In vitro evaluation of anticancer potentials of lupeol isolated from Elephantopus scaber L. on MCF-7 cell line. *Journal of Advanced Pharmaceutical Technology and Research, 5*(4), 179.

Popovich, D. G., & Kitts, D. D. (2002). Structure–function relationship exists for ginsenosides in reducing cell proliferation and inducing apoptosis in the human leukemia (THP-1) cell line. *Archives of Biochemistry and Biophysics, 406*(1), 1-8.

Prabhala, H. K., Pai, C., & Prabhala, R. H. (2013). Chapter 21 - Anti-Inflammatory Natural Foods. In R. R. Watson & V. R. Preedy (Eds.), *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (pp. 279-303). San Diego: Academic Press.

Prabhu, B., Balakrishnan, D., & Sundaresan, S. (2016). Antiproliferative and anti-inflammatory properties of diindolylmethane and lupeol against N-butyl-N-(4-hydroxybutyl) nitrosamine induced bladder carcinogenesis in experimental rats. *Human and Experimental Toxicology, 35*(6), 685-692.

Prasad, N., Sabarwal, A., Yadav, U. C., & Singh, R. P. (2018). Lupeol induces S-phase arrest and mitochondria-mediated apoptosis in cervical cancer cells. *Journal of Biosciences*, 1-13.

Prasad, S., & Aggarwal, B. (2014). Chronic diseases caused by chronic inflammation require chronic treatment: Anti-inflammatory role of dietary spices. *Journal of Clinical and Cellular Immunolog, 5*(238), 10.4172.

Prasad, S., Gupta, S. C., Tyagi, A. K., & Aggarwal, B. B. (2014). Curcumin, a component of golden spice: From bedside to bench and back. *Biotechnology Advances, 32*(6), 1053-1064.

Prasad, S., Kalra, N., & Shukla, Y. (2007). Induction of apoptosis by lupeol and mango extract in mouse prostate and LNCaP cells. *Nutrition and Cancer, 60*(1), 120-130. doi:10.1080/01635580701613772

Preetha, S., Kanniappan, M., Selvakumar, E., Nagaraj, M., & Varalakshmi, P. (2006). Lupeol ameliorates aflatoxin B1-induced peroxidative hepatic damage in rats. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 143*(3), 333-339.

Priyadarsini, K. I. (2014). The chemistry of curcumin: From extraction to therapeutic agent. *Molecules, 19*(12), 20091-20112. doi:10.3390/molecules191220091

PubChem Open Chemistry Data Base. (2018). PubChem Compound Database. Retrieved 20th Oct 2018, from National Center for Biotechnology Information https://pubchem.ncbi.nlm.nih.gov/compound/969516

Qi, T., Xu, F., Yan, X., Li, S., & Li, H. (2016). Sulforaphane exerts anti-inflammatory effects against lipopolysaccharide-induced acute lung injury in mice through the Nrf2/ARE pathway. *International Journal of Molecular Medicine, 37*(1), 182-188. doi:10.3892/ijmm.2015.2396

Qin, H. D., Shi, Y. Q., Liu, Z. H., Li, Z. G., Wang, H. S., Wang, H., & Liu, Z. P. (2010). Effect of chlorogenic acid on mast cell-dependent anaphylactic reaction. *International Immunopharmacology, 10*(9), 1135-1141.

Qin, X., Jiang, X., Jiang, X., Wang, Y., Miao, Z., He, W., . . . Zheng, Y. (2016). Micheliolide inhibits LPS-induced inflammatory response and protects mice from LPS challenge. *Scientific Reports, 6*, 23240.

Quitschke, W. W. (2008). Differential solubility of curcuminoids in serum and albumin solutions: Implications for analytical and therapeutic applications. *BMC Biotechnology, 8*(1), 84.

Raetz, C. R., & Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual Review of Biochemistry, 71*(1), 635-700.

Rafi, M. M., Yadav, P. N., & Reyes, M. (2007). Lycopene inhibits LPS-induced proinflammatory mediator inducible nitric oxide synthase in mouse macrophage cells. *Journal of Food Science, 72*(1), S069-S074. doi:https://doi.org/10.1111/j.1750-3841.2006.00219.x

Raina, V., Srivastava, S., & Syamsundar, K. (2005). Rhizome and leaf oil composition of Curcuma longa from the lower Himalayan region of northern India. *Journal of Essential Oil Research, 17*(5), 556-559.

Rajagopal, S. N., & Sandine, W. E. (1990). Associative growth and proteolysis of Streptococcus thermophilus and Lactobacillus bulgaricus in skim milk. *Journal of Dairy Science, 73*(4), 894-899. doi:https://doi.org/10.3168/jds.S0022-0302(90)78745-0

Ramirez, C. N., Li, W., Zhang, C., Wu, R., Su, S., Wang, C., . . . Kong, A. N. T. (2018). In vivo dose response of ursolic Acid, Sulforaphane, PEITC, and curcumin in cancer prevention. *The AAPS journal, 20*(1), 19.

Rege, S. A., & Momin, S. A. (2017). Pro-and antioxidant activity of curcuminoids with lecithin in sunflower oil. *Ukrainian Food Journal, 6*(3).

Ringman, J. M., Frautschy, S. A., Teng, E., Begum, A. N., Bardens, J., Beigi, M., . . . Cole, G. M. (2012). Oral curcumin for Alzheimer's disease: Tolerability and efficacy in a 24-week randomized, double blind, placebo-controlled study. *Alzheimer's Research and Therapy, 4*(5), 43. doi:10.1186/alzrt146

Roberfroid, M. B. (2000). Concepts and strategy of functional food science: The European perspective. *The American Journal of Clinical Nutrition, 71*(6), 1660S-1664S.

Rodríguez-Roque, M. J., de Ancos, B., Sánchez-Moreno, C., Cano, M. P., Elez-Martínez, P., & Martín-Belloso, O. (2015). Impact of food matrix and processing on the in vitro bioaccessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods, 14*, 33-43.

Rodríguez-Roque, M. J., de Ancos, B., Sánchez-Vega, R., Sánchez-Moreno, C., Cano, M. P., Elez-Martínez, P., & Martín-Belloso, O. (2016). Food matrix and processing influence on carotenoid bioaccessibility and lipophilic antioxidant activity of fruit juice-based beverages. *Food and Function, 7*(1), 380-389.

Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O. (2014a). In vitro bioaccessibility of health-related compounds as affected by the formulation of fruit juice-and milk-based beverages. *Food Research International, 62*, 771-778.

Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O. (2014b). In vitro bioaccessibility of health-related compounds from a blended fruit juice–soymilk beverage: Influence of the food matrix. *Journal of Functional Foods, 7*, 161-169.

Roome, T., Aziz, S., Razzak, A., Aslam, Z., Jamali, K. S., Sikandar, B., . . . Faizi, S. (2019). Opuntioside, opuntiol and its metallic nanoparticles attenuate adjuvant-induced arthritis: Novel suppressors of Toll-like receptors-2 and-4. *Biomedicine and pharmacotherapy, 112*, 108624.

Rysstad, G., Knutsen, W., & Abrahamsen, R. (1990). Effect of threonine and glycine on acetaldehyde formation in goats' milk yogurt. *Journal of Dairy Research, 57*(3), 401-411. doi:10.1017/S0022029900027059

Sah, B., Vasiljevic, T., McKechnie, S., & Donkor, O. (2016). Physicochemical, textural and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel powder during refrigerated storage. *LWT-Food Science and Technology, 65*, 978-986.

Sahan, N., Yasar, K., & Hayaloglu, A. A. (2008). Physical, chemical and flavour quality of non-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage. *Food Hydrocolloids, 22*(7), 1291-1297. doi:https://doi.org/10.1016/j.foodhyd.2007.06.010

Saint-Eve, A., Lévy, C., Martin, N., & Souchon, I. (2006). Influence of proteins on the perception of flavored stirred yogurts. *Journal of Dairy Science, 89*(3), 922-933.

Saleem, M. (2009). Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Letters, 285*(2), 109-115.

Saleem, M., Afaq, F., Adhami, V. M., & Mukhtar, H. (2004). Lupeol modulates NF-κB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. *Oncogene, 23*(30), 5203.

Saleem, M., Kweon, M. H., Yun, J. M., Adhami, V. M., Khan, N., Syed, D. N., & Mukhtar, H. (2005). A novel dietary triterpene Lupeol induces fas-mediated apoptotic death of androgen-sensitive prostate cancer cells and inhibits tumor growth in a xenograft model. *Cancer Research, 65*(23), 11203-11213.

Saleh, I. A., Vinatoru, M., Mason, T., Abdel-Azim, N., Aboutabl, E., & Hammouda, F. (2016). A possible general mechanism for ultrasound-assisted extraction (UAE) suggested from the results of UAE of chlorogenic acid from Cynara scolymus L.(artichoke) leaves. *Ultrasonics Sonochemistry, 31*, 330-336.

Salminen, A., Lehtonen, M., Suuronen, T., Kaarniranta, K., & Huuskonen, J. (2008). Terpenoids: Natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential. *Cellular and Molecular Life Sciences, 65*(19), 2979-2999.

Salvador, A., & Fiszman, S. M. (2004). Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. *Journal of Dairy Science, 87*(12), 4033-4041. doi:https://doi.org/10.3168/jds.S0022-0302(04)73544-4

Sanful, R. E. (2009). Promotion of coconut in the production of yoghurt. *African Journal of Food Science, 3*(5), 147-149.

Santana-Gálvez, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2017). Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. *Molecules, 22*(3), 358.

Santeramo, F. G., Carlucci, D., De Devitiis, B., Seccia, A., Stasi, A., Viscecchia, R., & Nardone, G. (2018). Emerging trends in European food, diets and food industry. *Food Research International, 104*, 39-47.

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., . . . Iseki, K. (2011). In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *International Journal of Pharmaceutics, 403*(1-2), 136-138.

Schwende, H., Fitzke, E., Ambs, P., & Dieter, P. (1996). Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1, 25-dihydroxyvitamin D3. *Journal of Leukocyte Biology, 59*(4), 555-561.

Sciberras, J. N., Galloway, S. D., Fenech, A., Grech, G., Farrugia, C., Duca, D., & Mifsud, J. (2015). The effect of turmeric (Curcumin) supplementation on cytokine and inflammatory marker responses following 2 hours of endurance cycling. *Journal of the International Society of Sports Nutrition, 12*(1), 5.

Seo, J. A., Kim, B., Dhanasekaran, D. N., Tsang, B. K., & Song, Y. S. (2016). Curcumin induces apoptosis by inhibiting sarco/endoplasmic reticulum Ca2+ ATPase activity in ovarian cancer cells. *Cancer Letters, 371*(1), 30-37.

Shacter, E., & Weitzman, S. A. (2002). Chronic inflammation and cancer. *Oncology-Williston Park then Huntington, 16*(2), 217-229.

Shakerian, M., Razavi, S. H., Ziai, S. A., Khodaiyan, F., Yarmand, M. S., & Moayedi, A. (2015). Proteolytic and ACE-inhibitory activities of probiotic yogurt containing non-viable bacteria as affected by different levels of fat, inulin and starter culture. *Journal of Food Science and Technology, 52*(4), 2428-2433. doi:10.1007/s13197-013-1202-9

Shakibaei, M., Mobasheri, A., & Buhrmann, C. (2011). Curcumin synergizes with resveratrol to stimulate the MAPK signaling pathway in human articular chondrocytes in vitro. *Genes and Nutrition, 6*(2), 171.

Shalova, I. N., Saha, S., & Biswas, S. K. (2017). Monocytes and macrophages. In J. M. Cavaillon & M. Singer (Eds.), *Inflammation: From Molecular and Cellular Mechanisms to the Clinic* (pp. 219-252): Wiley-VCH Verlag GmbH & Co. KGaA.

Shan, J., Fu, J., Zhao, Z., Kong, X., Huang, H., Luo, L., & Yin, Z. (2009). Chlorogenic acid inhibits lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264. 7 cells through suppressing NF-κB and JNK/AP-1 activation. *International Immunopharmacology, 9*(9), 1042-1048.

Shana, Sridhar, R., Roopa, B. S., Varadaraj, M. C., & Vijayendra, S. V. N. (2015). Optimization of a novel coconut milk supplemented dahi - A fermented milk product of Indian subcontinent. *Journal of Food Science and Technology, 52*(11), 7486-7492. doi:10.1007/s13197-015-1825-0

Shao-Ling, W., Ying, L., Ying, W., Yan-Feng, C., Li-Xin, N., Song-Tao, L., & Chang-Hao, S. (2009). Curcumin, a potential inhibitor of up-regulation of TNF-alpha and IL-6 induced by

palmitate in 3T3-L1 adipocytes through NF-kappaB and JNK pathway. *Biomedical and Environmental Sciences, 22*(1), 32-39.

Shao, W., Yu, Z., Chiang, Y., Yang, Y., Chai, T., Foltz, W., . . . Jin, T. (2012). Curcumin prevents high fat diet induced insulin resistance and obesity via attenuating lipogenesis in liver and inflammatory pathway in adipocytes. *PLoS One, 7*(1), e28784.

Sharma, D., & Singh Saharan, B. (2014). Simultaneous production of biosurfactants and bacteriocins by probiotic Lactobacillus casei MRTL3. *International Journal of Microbiology, 2014*.

Shen, L., & Ji, H.-F. (2007). Theoretical study on physicochemical properties of curcumin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 67*(3-4), 619-623.

Shen, W., Qi, R., Zhang, J., Wang, Z., Wang, H., Hu, C., . . . Fu, Y. (2012). Chlorogenic acid inhibits LPS-induced microglial activation and improves survival of dopaminergic neurons. *Brain Research Bulletin, 88*(5), 487-494.

Shi, A., Shi, H., Wang, Y., Liu, X., Cheng, Y., Li, H., . . . Dong, L. (2018). Activation of Nrf2 pathway and inhibition of NLRP3 inflammasome activation contribute to the protective effect of chlorogenic acid on acute liver injury. *International Immunopharmacology, 54*, 125-130.

Shi, H., Dong, L., Jiang, J., Zhao, J., Zhao, G., Dang, X., . . . Jia, M. (2013). Chlorogenic acid reduces liver inflammation and fibrosis through inhibition of toll-like receptor 4 signaling pathway. *Toxicology, 303*, 107-114.

Shi, H., Guo, Y., Liu, Y., Shi, B., Guo, X., Jin, L., & Yan, S. (2016). The in vitro effect of lipopolysaccharide on proliferation, inflammatory factors and antioxidant enzyme activity in bovine mammary epithelial cells. *Animal Nutrition, 2*(2), 99-104.

Shin, H. S., Satsu, H., Bae, M. J., Zhao, Z., Ogiwara, H., Totsuka, M., & Shimizu, M. (2015). Anti-inflammatory effect of chlorogenic acid on the IL-8 production in Caco-2 cells and the dextran sulphate sodium-induced colitis symptoms in C57BL/6 mice. *Food Chemistry, 168*, 167-175.

Siddique, H. R., & Saleem, M. (2011). Beneficial health effects of lupeol triterpene: A review of preclinical studies. *Life Sciences, 88*(7-8), 285-293. doi:10.1016/j.lfs.2010.11.020

Siebert, K. J., Troukhanova, N. V., & Lynn, P. Y. (1996). Nature of polyphenol−protein interactions. *Journal of Agricultural and Food Chemistry, 44*(1), 80-85. doi:10.1021/jf9502459

Silva, B. V., Barreira, J. C., & Oliveira, M. B. P. (2016). Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies. *Trends in Food Science and Technology, 50*, 144-158.

Singh, P., Arora, D., & Shukla, Y. (2017). Enhanced chemoprevention by the combined treatment of pterostilbene and lupeol in B[a]P-induced mouse skin tumorigenesis. *Food and Chemical Toxicology, 99*, 182-189.

Singh, S., & Aggarwal, B. B. (1995). Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane). *Journal of Biological Chemistry, 270*(42), 24995-25000.

Singh, T. (2018). *Bioactivity of food-grade curcuminoids and their incorporation into coconut yogurt.* (Master), Massey University, Albany.

Siro, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite, 51*(3), 456-467.

Small, A., Lansdown, N., Al-Baghdadi, M., Quach, A., & Ferrante, A. (2018). Facilitating THP-1 macrophage studies by differentiating and investigating cell functions in polystyrene test tubes. *Journal of Immunological Methods, 461*, 73-77.

Sohi, H., Sultana, Y., & Khar, R. K. (2004). Taste masking technologies in oral pharmaceuticals: Recent developments and approaches. *Drug Development and Industrial Pharmacy, 30*(5), 429-448.

Soufli, I., Toumi, R., Rafa, H., & Touil-Boukoffa, C. (2016). Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases. *World Journal of Gastrointestinal Pharmacology and Therapeutics, 7*(3), 353.

Srivastava, A. K., Mishra, S., Ali, W., & Shukla, Y. (2016). Protective effects of lupeol against mancozeb-induced genotoxicity in cultured human lymphocytes. *Phytomedicine, 23*(7), 714-724.

Starr, T., Bauler, T. J., Malik-Kale, P., & Steele-Mortimer, O. (2018). The phorbol 12-myristate-13-acetate differentiation protocol is critical to the interaction of THP-1 macrophages with Salmonella Typhimurium. *PLoS One, 13*(3), e0193601.

Strober, W. (1997). Trypan blue exclusion test of cell viability. *Current Protocols in Immunology, 21*(1), A. 3B. 1-A. 3B. 2.

Sudhahar, V., Kumar, S. A., Varalakshmi, P., & Sujatha, V. (2008). Protective effect of lupeol and lupeol linoleate in hypercholesterolemia associated renal damage. *Molecular and Cellular Biochemistry, 317*(1-2), 11.

Sun-Waterhouse, D., & Wadhwa, S. S. (2013). Industry-relevant approaches for minimising the bitterness of bioactive compounds in functional foods: A review. *Food and Bioprocess Technology, 6*(3), 607-627.

Sun-Waterhouse, D. (2011). The development of fruit-based functional foods targeting the health and wellness market: A review. *International Journal of Food Science and Technology, 46*(5), 899-920.

Sun, D., Zhuang, X., Xiang, X., Liu, Y., Zhang, S., Liu, C., . . . Zhang, H. G. (2010). A novel nanoparticle drug delivery system: The anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Molecular Therapy, 18*(9), 1606-1614.

Sunitha, S., Nagaraj, M., & Varalakshmi, P. (2001). Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats. *Fitoterapia, 72*(5), 516-523.

Supavititpatana, P., Wirjantoro, T. I., & Raviyan, P. (2010). Characteristics and shelf-life of corn milk yogurt. *Chiang Mai Univeersity Journal of Natural Sciences, 9*(1), 133-149.

Surh, Y. J., Chun, K. S., Cha, H. H., Han, S. S., Keum, Y. S., Park, K. K., & Lee, S. S. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: Down-regulation of COX-2 and iNOS through suppression of NF-κB activation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 480*, 243-268.

Swindle, E. J., & Metcalfe, D. D. (2007). The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. *Immunological Reviews, 217*(1), 186-205.

Szakály, Z., Szente, V., Kövér, G., Polereczki, Z., & Szigeti, O. (2012). The influence of lifestyle on health behavior and preference for functional foods. *Appetite, 58*(1), 406-413.

Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. *European Journal of Nutrition, 56*(7), 2215-2244.

Takashiba, S., Van Dyke, T. E., Amar, S., Murayama, Y., Soskolne, A. W., & Shapira, L. (1999). Differentiation of monocytes to macrophages primes cells for lipopolysaccharide stimulation via accumulation of cytoplasmic nuclear factor κB. *Infection and Immunity, 67*(11), 5573-5578.

Takeuchi, H., Sakurai, C., Noda, R., Sekine, S., Murano, Y., Wanaka, K., . . . Kondo, K. (2007). Antihypertensive effect and safety of dietary alpha-linolenic acid in subjects with high-normal blood pressure and mild hypertension. *Journal of Oleo Science, 56*(7), 347-360. doi:10.5650/jos.56.347

Tamime, A. Y., & Robinson, R. K. (2007). *Tamime and Robinson's Yoghurt: Science and Technology*: Elsevier.

Tan, G., & Korel, F. (2007). Quality of flavored yogurt containing added coffee and sugar. *Journal of Food Quality, 30*(3), 342-356. doi:10.1111/j.1745-4557.2007.00125.x

Tan, P. Y., Tan, T. B., Chang, H. W., Tey, B. T., Chan, E. S., Lai, O. M., . . . Tan, C. P. (2018). Effects of storage and yogurt matrix on the stability of tocotrienols encapsulated in

chitosan-alginate microcapsules. *Food Chemistry, 241*, 79-85. doi:https://doi.org/10.1016/j.foodchem.2017.08.075

Tansakul, A., & Chaisawang, P. (2006). Thermophysical properties of coconut milk. *Journal of Food Engineering, 73*(3), 276-280. doi:https://doi.org/10.1016/j.jfoodeng.2005.01.035

Tapping, R. I., & Tobias, P. S. (1997). Cellular binding of soluble CD14 requires lipopolysaccharide (LPS) and LPS-binding protein. *Journal of Biological Chemistry, 272*(37), 23157-23164.

Taram, F., Winter, A. N., & Linseman, D. A. (2016). Neuroprotection comparison of chlorogenic acid and its metabolites against mechanistically distinct cell death-inducing agents in cultured cerebellar granule neurons. *Brain Research, 1648*, 69-80. doi:https://doi.org/10.1016/j.brainres.2016.07.028

Tavakoli, M., Habibi Najafi, M. B., & Mohebbi, M. (2019). Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. *Heliyon, 5*(2), e01204. doi:https://doi.org/10.1016/j.heliyon.2019.e01204

Taylor, M., Porter, R., & Gonzalez, M. (2014). Intense pulsed light may improve inflammatory acne through TNF-α down-regulation. *Journal of Cosmetic and Laser Therapy, 16*(2), 96-103.

Tedesco, S., De Majo, F., Kim, J., Trenti, A., Trevisi, L., Fadini, G. P., . . . Vitiello, L. (2018). Convenience versus biological significance: Are PMA-differentiated THP-1 cells a reliable substitute for blood-derived macrophages when studying in vitro polarization? *Frontiers in Pharmacology, 9*, 71.

Thimmappa, R., Geisler, K., Louveau, T., O'Maille, P., & Osbourn, A. (2014). Triterpene biosynthesis in plants. *Annual Review of Plant Biology, 65*.

Thomson, C., Bloch, A., Hasler, C., Kubena, K., Earl, R., & Heins, J. (1999). Position of the American Dietetic Association: Functional foods. *Journal of the American Dietetic Association, 99*(10), 1278-1285.

Tien, M.-T., Girardin, S. E., Regnault, B., Le Bourhis, L., Dillies, M.-A., Coppée, J.-Y., . . . Pédron, T. (2006). Anti-inflammatory effect of Lactobacillus casei on Shigella-infected human intestinal epithelial cells. *The Journal of Immunology, 176*(2), 1228-1237.

Tomé-Carneiro, J., Larrosa, M., González-Sarrías, A., Tomás-Barberán, F. A., García-Conesa, M. T., & Espín, J. C. (2013). Resveratrol and clinical trials: The crossroad from in vitro studies to human evidence. *Current Pharmaceutical Design, 19*(34), 6064-6093. doi:10.2174/13816128113199990407

Torres-Fuentes, C., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2015). A natural solution for obesity: Bioactives for the prevention and treatment of weight gain. A review. *Nutritional Neuroscience, 18*(2), 49-65. doi:10.1179/1476830513Y.0000000099

Tošović, J., Marković, S., Marković, J. M. D., Mojović, M., & Milenković, D. (2017). Antioxidative mechanisms in chlorogenic acid. *Food Chemistry, 237*, 390-398.

Trumbo, P. R. (2005). Are there adverse effects of lycopene exposure? *The Journal of Nutrition, 135*(8), 2060S-2061S. doi:10.1093/jn/135.8.2060S

Tsai, F. S., Lin, L. W., & Wu, C. R. (2016). Lupeol and its role in chronic diseases. In *Drug Discovery from Mother Nature* (pp. 145-175): Springer.

Tsuda, T. (2018). Curcumin as a functional food-derived factor: Degradation products, metabolites, bioactivity, and future perspectives. *Food and Function, 9*(2), 705-714. doi:10.1039/c7fo01242j

Turgut, T., & Cakmakci, S. (2018). Probiotic strawberry yogurts: Microbiological, chemical and sensory properties. *Probiotics and Antimicrobial Proteins, 10*(1), 64-70. doi:10.1007/s12602-017-9278-6

Upadhyay, R., & Mohan Rao, L. J. (2013). An outlook on chlorogenic acids—occurrence, chemistry, technology, and biological activities. *Critical Reviews in Food Science and Nutrition, 53*(9), 968-984.

van Meerloo, J., Kaspers, G. J. L., & Cloos, J. (2011). Cell sensitivity assays: The MTT assay. In I. A. Cree (Ed.), *Cancer Cell Culture: Methods and Protocols* (pp. 237-245). Totowa, NJ: Humana Press.

Vareltzis, P., Adamopoulos, K., Stavrakakis, E., Stefanakis, A., & Goula, A. M. (2016). Approaches to minimise yoghurt syneresis in simulated tzatziki sauce preparation. *International Journal of Dairy Technology, 69*(2), 191-199. doi:10.1111/1471-0307.12238

Vasconcelos, J. F., Teixeira, M. M., Barbosa-Filho, J. M., Lúcio, A. S. S. C., Almeida, J. R. G. D. S., De Queiroz, L., . . . Soares, M. B. P. (2008). The triterpenoid lupeol attenuates allergic airway inflammation in a murine model. *International Immunopharmacology, 8*(9), 1216-1221.

Vasiljevic, T., Kealy, T., & Mishra, V. K. (2007). Effects of β-Glucan addition to a probiotic containing yogurt. *Journal of Food Science, 72*(7), C405-C411. doi:10.1111/j.1750-3841.2007.00454.x

Vicentini, A., Liberatore, L., & Mastrocola, D. (2016). Functional foods: Treands and development of the global market. *Italian Journal of Food Science, 28*(2).

Victor, V. V., Guayerbas, N., Puerto, M., Medina, S., & Fuente, M. D. l. (2000). Ascorbic acid modulates in vitro the function of macrophages from mice with endotoxic shock. *Immunopharmacology, 46*, 89–101.

Vidya, L., Lenin, M., & Varalakshmi, P. (2002). Evaluation of the effect of triterpenes on urinary risk factors of stone formation in pyridoxine deficient hyperoxaluric rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 16*(6), 514-518.

Vijayendra, S. V. N., & Gupta, R. C. (2012). Assessment of probiotic and sensory properties of dahi and yoghurt prepared using bulk freeze-dried cultures in buffalo milk. *Annals of Microbiology, 62*(3), 939-947.

Vinson, J. A., Burnham, B. R., & Nagendran, M. V. (2012). Randomized, double-blind, placebo-controlled, linear dose, crossover study to evaluate the efficacy and safety of a green coffee bean extract in overweight subjects. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 5*, 21.

Vors, C., Couillard, C., Paradis, M. E., Gigleux, I., Marin, J., Vohl, M. C., . . . Lamarche, B. (2018). Supplementation with resveratrol and curcumin does not affect the inflammatory rsponse to a high-fat meal in older adults with abdominal obesity: A randomized, placebo-controlled crossover trial. *The Journal of Nutrition, 148*(3), 379-388.

Wal, A., Srivastava, R., Wal, P., Rai, A., & Sharma, S. (2015). Lupeol as a magical drug. *Pharmaceutical and Biological Evaluations, 2*(5), 142-151.

Wal, P., Wal, A., Sharma, G., & Rai, A. (2011). Biological activities of lupeol. *Systematic Reviews in Pharmacy, 2*(2), 96-96.

Wang, F., Gao, Q., Guo, S., Cheng, J., Sun, X., Li, Q., . . . Tian, Y. (2013). The sonodynamic effect of curcumin on THP-1 cell-derived macrophages. *BioMed Research International, 2013*.

Wang, M. S., Boddapati, S., Emadi, S., & Sierks, M. R. (2010). Curcumin reduces α-synuclein induced cytotoxicity in Parkinson's disease cell model. *BMC Neuroscience, 11*(1), 57.

Wang, S., Moustaid-Moussa, N., Chen, L., Mo, H., Shastri, A., Su, R., . . . Shen, C. L. (2014). Novel insights of dietary polyphenols and obesity. *The Journal of Nutritional Biochemistry, 25*(1), 1-18. doi:10.1016/j.jnutbio.2013.09.001

Wang, Y. J., Pan, M. H., Cheng, A. L., Lin, L. I., Ho, Y. S., Hsieh, C. Y., & Lin, J. K. (1997). Stability of curcumin in buffer solutions and characterization of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis, 15*(12), 1867-1876.

Wang, Z., Zhang, Q., Yuan, L., Wang, S., Liu, L., Yang, X., . . . Liu, D. (2014). The effects of curcumin on depressive-like behavior in mice after lipopolysaccharide administration. *Behavioural Brain Research, 274*, 282-290.

Wanninger, S., Lorenz, V., Subhan, A., & Edelmann, F. T. (2015). Metal complexes of curcumin–synthetic strategies, structures and medicinal applications. *Chemical Society Reviews, 44*(15), 4986-5002.

Ward, P. A. (2010). The inflammatory response- An overview. In *Fundamentals of Inflammation* (pp. 1-16). Cambridge: Cambridge University Press.

Watanabe, T., Arai, Y., Mitsui, Y., Kusaura, T., Okawa, W., Kajihara, Y., & Saito, I. (2006). The blood pressure-lowering effect and safety of chlorogenic acid from green coffee bean extract in essential hypertension. *Clinical and Experimental Hypertension, 28*(5), 439-449.

Weerathilake, W., Rasika, D., Ruwanmali, J., & Munasinghe, M. (2014). The evolution, processing, varieties and health benefits of yogurt. *International Journal of Scientific and Research Publications, 4*(4), 1-10.

Weisberg, S. P., Leibel, R., & Tortoriello, D. V. (2008). Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. *Endocrinology, 149*(7), 3549-3558.

Weldon, S. M., Mullen, A. C., Loscher, C. E., Hurley, L. A., & Roche, H. M. (2007). Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *Journal of Nutritional Biochemistry, 18*(4), 250-258. doi:10.1016/j.jnutbio.2006.04.003

WHO. (2003). *Diet, nutrition and the prevention of chronic diseases*. Geneva, Switzerland. Retrieved from http://www.fao.org/docrep/005/AC911E/AC911E00.HTM#Contents

WHO. (2014). *Global status report on noncommunicable diseases 2014*. Geneva, Switzerland. Retrieved from http://apps.who.int/iris/bitstream/handle/10665/148114/9789241564854_eng.pdf;jsessionid=3F13174CDFC31C4E5D31507686412205?sequence=1

Wilken, R., Veena, M. S., Wang, M. B., & Srivatsan, E. S. (2011). Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Molecular Cancer, 10*(1), 12.

Woo, H. M., Kang, J. H., Kawada, T., Yoo, H., Sung, M. K., & Yu, R. (2007). Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. *Life Sciences, 80*(10), 926-931.

Xaus, J., Comalada, M., Valledor, A. F., Lloberas, J., López-Soriano, F., Argilés, J. M., . . . Celada, A. (2000). LPS induces apoptosis in macrophages mostly through the autocrine production of TNF-α. *Blood, 95*(12), 3823-3831.

Xu, J. G., Hu, Q. P., & Liu, Y. (2012). Antioxidant and DNA-protective activities of chlorogenic acid isomers. *Journal of Agricultural and Food Chemistry, 60*(46), 11625-11630.

Xue, M., Shi, H., Zhang, J., Liu, Q. Q., Guan, J., Zhang, J. Y., & Ma, Q. (2016). Stability and degradation of caffeoylquinic acids under different storage conditions studied by high-performance liquid chromatography with photo diode array detection and high-performance liquid chromatography with electrospray ionization collision-induced dissociation tandem mass spectrometry. *Molecules, 21*(7), 948.

Yaakob, H., Ahmed, N. R., Daud, S. K., Malek, R. A., & Rahman, R. A. (2012). Optimization of ingredient and processing levels for the production of coconut yogurt using response surface methodology. *Food Science and Biotechnology, 21*(4), 933-940. doi:10.1007/s10068-012-0123-0

Yadav, S. K., Sah, A. K., Jha, R. K., Sah, P., & Shah, D. K. (2013). Turmeric (curcumin) remedies gastroprotective action. *Pharmacognosy Reviews, 7*(13), 42.

Yamashita, K., Yatsuya, H., Muramatsu, T., Toyoshima, H., Murohara, T., & Tamakoshi, K. (2012). Association of coffee consumption with serum adiponectin, leptin, inflammation and metabolic markers in Japanese workers: A cross-sectional study. *Nutrition and Diabetes, 2*(4), e33.

Yang, C. W., Chang, C. L., Lee, H. C., Chi, C. W., Pan, J. P., & Yang, W. C. (2012). Curcumin induces the apoptosis of human monocytic leukemia THP-1 cells via the activation of JNK/ERK pathways. *BMC Complementary and Alternative Medicine, 12*(1), 22.

Yang, J. Y., Zhong, X., Kim, S. J., Kim, D. H., Kim, H. S., Lee, J. S., . . . Surh, Y. J. (2018). Comparative effects of curcumin and tetrahydrocurcumin on dextran sulfate sodium-induced colitis and inflammatory signaling in mice. *Journal of Cancer Prevention, 23*(1), 18.

Yen, Y. H., Pu, C. M., Liu, C. W., Chen, Y. C., Chen, Y. C., Liang, C. J., . . . Chen, Y. L. (2018). Curcumin accelerates cutaneous wound healing via multiple biological actions: The involvement of TNF-α, MMP-9, α-SMA, and collagen. *International Wound Journal*.

Yokoe, I., Azuma, K., Hata, K., Mukaiyama, T., Goto, T., Tsuka, T., . . . Osaki, T. (2015). Clinical systemic lupeol administration for canine oral malignant melanoma. *Molecular and Clinical Oncology, 3*(1), 89-92.

You, Y. J., Nam, N. H., Kim, Y., Bae, K. H., & Ahn, B. Z. (2003). Antiangiogenic activity of lupeol from Bombax ceiba. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 17*(4), 341-344.

You, Z., Luo, C., Zhang, W., Chen, Y., He, J., Zhao, Q., . . . Wu, Y. (2011). Pro-and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: involvement in depression. *Behavioural Brain Research, 225*(1), 135-141.

Youn, G. S., Kwon, D.-J., Ju, S. M., Choi, S. Y., & Park, J. (2013). Curcumin ameliorates TNF-α-induced ICAM-1 expression and subsequent THP-1 adhesiveness via the induction of heme oxygenase-1 in the HaCaT cells. *BMB Reports, 46*(8), 410.

Yu, J., Bi, X., Yu, B., & Chen, D. (2016). Isoflavones: Anti-inflammatory benefit and possible caveats. *Nutrients, 8*(6). doi:10.3390/nu8060361

Yuliana, N., Rangga, A., & Rakhmiati. (2010). Manufacture of fermented coco milk-drink containing lactic acid bacteria cultures. *African Journal of Food Science, 4*(9), 558-562.

Yun, N., Kang, J. W., & Lee, S. M. (2012). Protective effects of chlorogenic acid against ischemia/reperfusion injury in rat liver: Molecular evidence of its antioxidant and anti-inflammatory properties. *The Journal of Nutritional Biochemistry, 23*(10), 1249-1255.

Zainoldin, K. H., & Baba, A. S. (2009). The effect of Hylocereus polyrhizus and Hylocereus undatus on physicochemical, proteolysis, and antioxidant activity in yogurt. *World Academy of Science, Engineering and Technology, 60*, 361-366.

Zampelas, A., Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., & Stefanadis, C. (2004). Associations between coffee consumption and inflammatory markers in healthy persons: The ATTICA study. *The American Journal of Clinical Nutrition, 80*(4), 862-867.

Zaręba, D., & Ziarno, M. (2017). The viability of yogurt bacteria in selected plant beverages. *Zesz. Probl. Post. Nauk Roln, 591*, 87-96.

Zatorski, H., Sałaga, M., Zielińska, M., Piechota-Polańczyk, A., Owczarek, K., Kordek, R., . . . Fichna, J. (2015). Experimental colitis in mice is attenuated by topical administration of chlorogenic acid. *Naunyn-Schmiedeberg's Archives of Pharmacology, 388*(6), 643-651.

Zeng, C., Zhong, P., Zhao, Y., Kanchana, K., Zhang, Y., Khan, Z. A., . . . Liang, G. (2015). Curcumin protects hearts from FFA-induced injury by activating Nrf2 and inactivating NF-κB both in vitro and in vivo. *Journal of Molecular and Cellular Cardiology, 79*, 1-12.

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science, 8*, 33-42.

Zhang, J. M., & An, J. (2007). Cytokines, inflammation and pain. *International Anesthesiology Clinics, 45*(2), 27.

Zhang, N., Li, H., Jia, J., & He, M. (2015). Anti-inflammatory effect of curcumin on mast cell-mediated allergic responses in ovalbumin-induced allergic rhinitis mouse. *Cellular Immunology, 298*(1-2), 88-95.

Zhang, X., Huang, H., Yang, T., Ye, Y., Shan, J., Yin, Z., & Luo, L. (2010). Chlorogenic acid protects mice against lipopolysaccharide-induced acute lung injury. *Injury, 41*(7), 746-752. doi:10.1016/j.injury.2010.02.029

Zhang, Y., Miao, L., Zhang, H., Wu, G., Zhang, Z., & Lv, J. (2018). Chlorogenic acid against palmitic acid in endoplasmic reticulum stress-mediated apoptosis resulting in protective effect of primary rat hepatocytes. *Lipids in Health and Disease, 17*(1), 270. doi:10.1186/s12944-018-0916-0

Zhao, G., Etherton, T. D., Martin, K. R., Vanden Heuvel, J. P., Gillies, P. J., West, S. G., & Kris-Etherton, P. M. (2005). Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. *Biochemical and Biophysical Research Communications, 336*(3), 909-917. doi:10.1016/j.bbrc.2005.08.204

Zhao, Y., Wang, J., Ballevre, O., Luo, H., & Zhang, W. (2012). Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertension Research, 35*(4), 370.

Zhao, Z., Shin, H. S., Satsu, H., Totsuka, M., & Shimizu, M. (2008). 5-Caffeoylquinic acid and caffeic acid down-regulate the oxidative stress-and TNF-α-induced secretion of interleukin-8 from Caco-2 cells. *Journal of Agricultural and Food Chemistry, 56*(10), 3863-3868.

Zheng, Z., Sheng, Y., Lu, B., & Ji, L. (2015). The therapeutic detoxification of chlorogenic acid against acetaminophen-induced liver injury by ameliorating hepatic inflammation. *Chemico-Biological Interactions, 238*, 93-101.

Zheng, Z., Shi, L., Sheng, Y., Zhang, J., Lu, B., & Ji, L. (2016). Chlorogenic acid suppresses monocrotaline-induced sinusoidal obstruction syndrome: The potential contribution of NFκB, Egr1, Nrf2, MAPKs and PI3K signals. *Environmental Toxicology and Pharmacology, 46*, 80-89.

Zhong, W., Qian, K., Xiong, J., Ma, K., Wang, A., & Zou, Y. (2016). Curcumin alleviates lipopolysaccharide induced sepsis and liver failure by suppression of oxidative stress-related inflammation via PI3K/AKT and NF-kappaB related signaling. *Biomed Pharmacother, 83*, 302-313. doi:10.1016/j.biopha.2016.06.036

Zhu, F., Du, B., & Xu, B. (2018). Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Critical Reviews in Food Science and Nutrition, 58*(8), 1260-1270. doi:10.1080/10408398.2016.1251390

Zhu, Y., Li, X., Chen, J., Chen, T., Shi, Z., Lei, M., . . . Fei, X. (2016). The pentacyclic triterpene Lupeol switches M1 macrophages to M2 and ameliorates experimental inflammatory bowel disease. *International Immunopharmacology, 30*, 74-84.

Zoidou, E., Magiatis, P., Melliou, E., Constantinou, M., Haroutounian, S., & Skaltsounis, A. L. (2014). Oleuropein as a bioactive constituent added in milk and yogurt. *Food Chemistry, 158*, 319-324. doi:https://doi.org/10.1016/j.foodchem.2014.02.137

Zou, L., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2015). Utilizing food matrix effects to enhance nutraceutical bioavailability: Increase of curcumin bioaccessibility using excipient emulsions. *Journal of Agricultural and Food Chemistry, 63*(7), 2052-2062.

Appendices

Appendix 1: Permission to Reproduce Images

Appendix 2: Protocol for Counting Cells using Haemocytometer

Appendix 3: qRT-PCR Experimental Design

Appendix 4: Documents for Consumer Sensory Test

Appendix 5: Raw and Statistically Analysed Data for *In Vitro* Study

Appendix 6: Raw and Statistically Analysed Data for qRT-PCR

Appendix 7: Standard Curve for Chlorogenic Acid and Curcumin

Appendix 8: Raw and Statistically Analysed Data for Product Development

Appendix 1

Permission to Reproduce Images

BA

Bisht, Akshay
Wed 31/10/2018 6:20 PM
mwalters@umn.edu

Respected Author,

I am Akshay Bisht, a masters student at Massey University, New Zealand. I am working with curcumin for my master's project.

I would like to request you to grant me permission to reproduce an image published in your paper, details as follows, in chapter 2 (literature review) of my thesis.

> **Paper Detail:** Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The Essential Medicinal Chemistry of Curcumin. *J Med Chem, 60*(5), 1620 1637. doi:10.1021/acs.jmedchem.6b00975
>
> **Figure Detail:** *Figure 5.* Major chemical degradation pathways of compound 1 *(curcumin).* (A) Solvolysis under alkaline pH in buffered aqueous solution rapidly leads to multiple fragmentation byproducts. (B) Autoxidation in buffered medium creates a bicyclopentadione that is the major degradation product in aqueous conditions. (C) Photodegradation of 1 can occur when in crystalline form and dissolved inorganic solvent. (D) When dissolved in certain organic solvents (like isopropanol), photodegradation can include reaction with the solvent as a substrate.

Looking forward to a positive reply from your end. Thanks in anticipation.

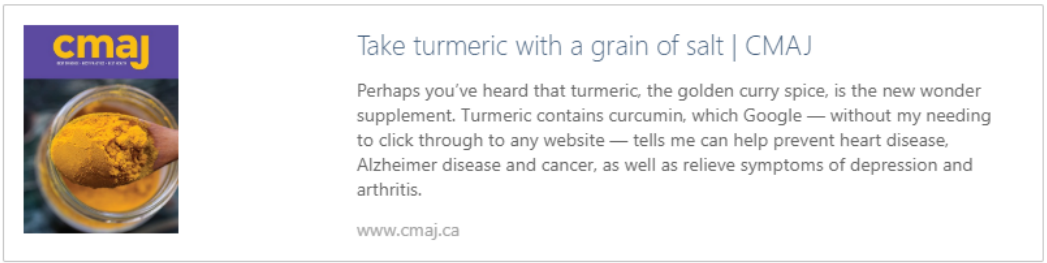
Regards,
Akshay Bisht
Masters (Food Technology)
Massey University (Auckland)
Student Id: 16420298
Phone Number: +64-0276087945

MW

Michael Walters <mwalters@umn.edu>
Thu 1/11/2018 2:09 AM

You have my permission to use this figure solely in your thesis.

You might also be interested in this new study on curcumin. http://www.cmaj.ca/content/190/43/E1270



Best of luck with your work!

Mike

Please excuse brevity. Sent from my iPhone

...

Reproduced as: Figure 2.4: Degradation of curcumin (a) at alkaline pH; (b) autoxidation in solvent; (c) photo-oxidation when in crystalline or aqueous state; and (d) photo-oxidation when is specific solvent like isopropanol. Chapter 2, Literature Review, Section 2.5.1

ELSEVIER LICENSE
TERMS AND CONDITIONS

Nov 26, 2018

This Agreement between Mr. Akshay Bisht ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	4472101224324
License date	Nov 18, 2018
Licensed Content Publisher	Elsevier
Licensed Content Publication	Life Sciences
Licensed Content Title	Beneficial health effects of lupeol triterpene: A review of preclinical studies
Licensed Content Author	Hifzur Rahman Siddique,Mohammad Saleem
Licensed Content Date	Feb 14, 2011
Licensed Content Volume	88
Licensed Content Issue	7-8
Licensed Content Pages	9
Start Page	285
End Page	293
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Original figure numbers	Fig. 1. (a) Chemical structure of lupeol and (b) schematic representation of the critical steps of the biosynthesis of lupeol in plants
Title of your thesis/dissertation	Anti-inflammatory potential of a mixture of curcumin, lupeol and chlorogenic acid in coconut yogurt
Expected completion date	Jul 2019
Estimated size (number of pages)	150
Requestor Location	Mr. Akshay Bisht 42, Forrest Hill Road Forest Hill, Auckland Auckland, 0620 New Zealand Attn: Mr. Akshay Bisht
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD
Terms and Conditions	

Reproduced as: *Figure 2.7: (a) Chemical structure of lupeol, and (b) key steps of mevalonate (MVA) biosynthesis pathway of lupeol in plant cells.* Chapter 2, Literature Review, Section 2.5.3

Appendix 2

Protocol for Counting Cells using Haemocytometer

Step 1: Mix equal volume of trypan blue and cell solution and let it rest for 2-3 minutes at room temperature.

Step 2: Load both the chambers of haemocytometer by pipetting 10 µl of mixture under the coverslip.

Step 3: Place the haemocytometer under the microscope.

Step 4: *Counting the cells.* Each chamber is divided into grid pattern consisting of 9 large square box (Figure A1.1). Each box can hold equal volume of 10^{-4} ml. Count both, viable and non-viable cells in all large corner square (A, B, C and D in Figure A1.1) of each chamber.

Note: The dead cell are stained blue as trypan blue can penetrate them while viable cell restrict trypan blue and appear unstained.

Step 5: *Calculations.* Let there were *x* total viable and *y* total non-viable cells counted in 4 squares (A, B, C and D). Therefore:

a) Percentage of viable cells: $\frac{x}{x+y} \times 100$

b) Average number of cells per square: $\frac{x}{Number\ of\ boxes\ counted}$

(4 boxes counted in this case)

c) Dilution factor: $\frac{Final\ volume\ of\ mixture}{Volume\ of\ cell\ solution}$

(Since equal volume of trypan blue and cell solution were mixed in this case so dilution factor is 2)

d) Concentration of viable cells/ml:

$$average\ number\ of viable\ cells\ \times \text{dilution factor}\ \times 10^4$$

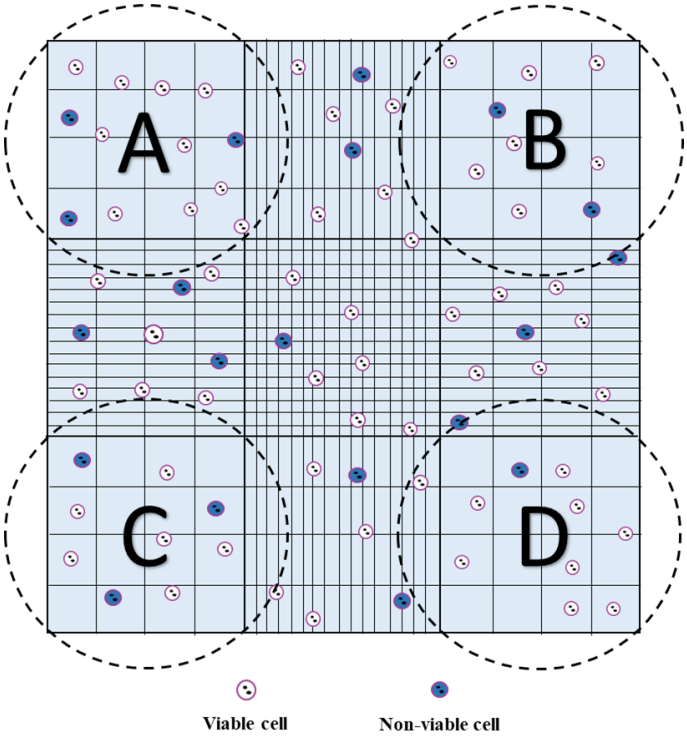
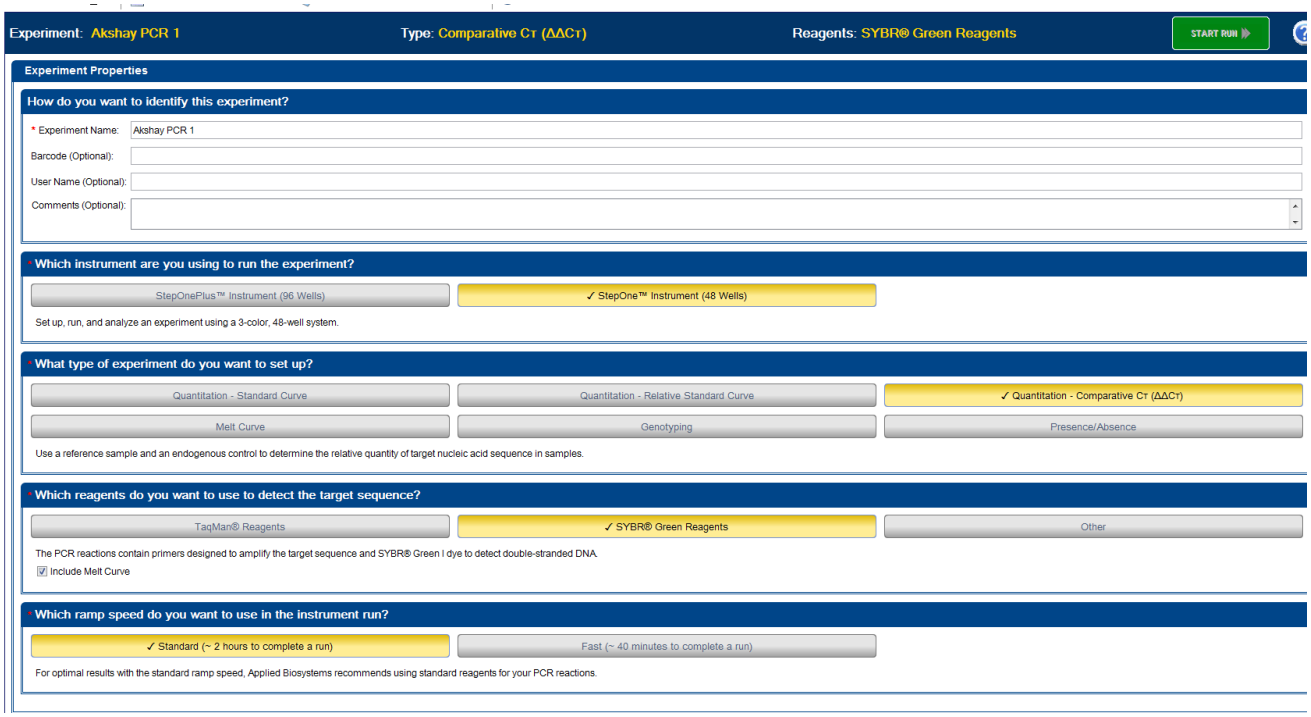


Figure A2.1: Grid pattern in haemocytometer with viable and non-viable cells.

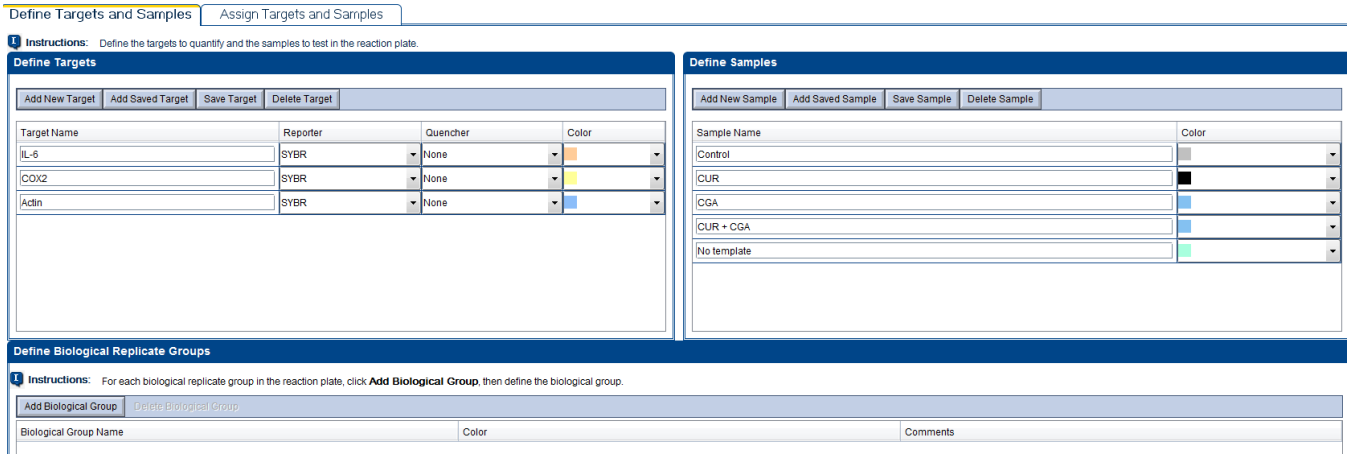
Appendix 3

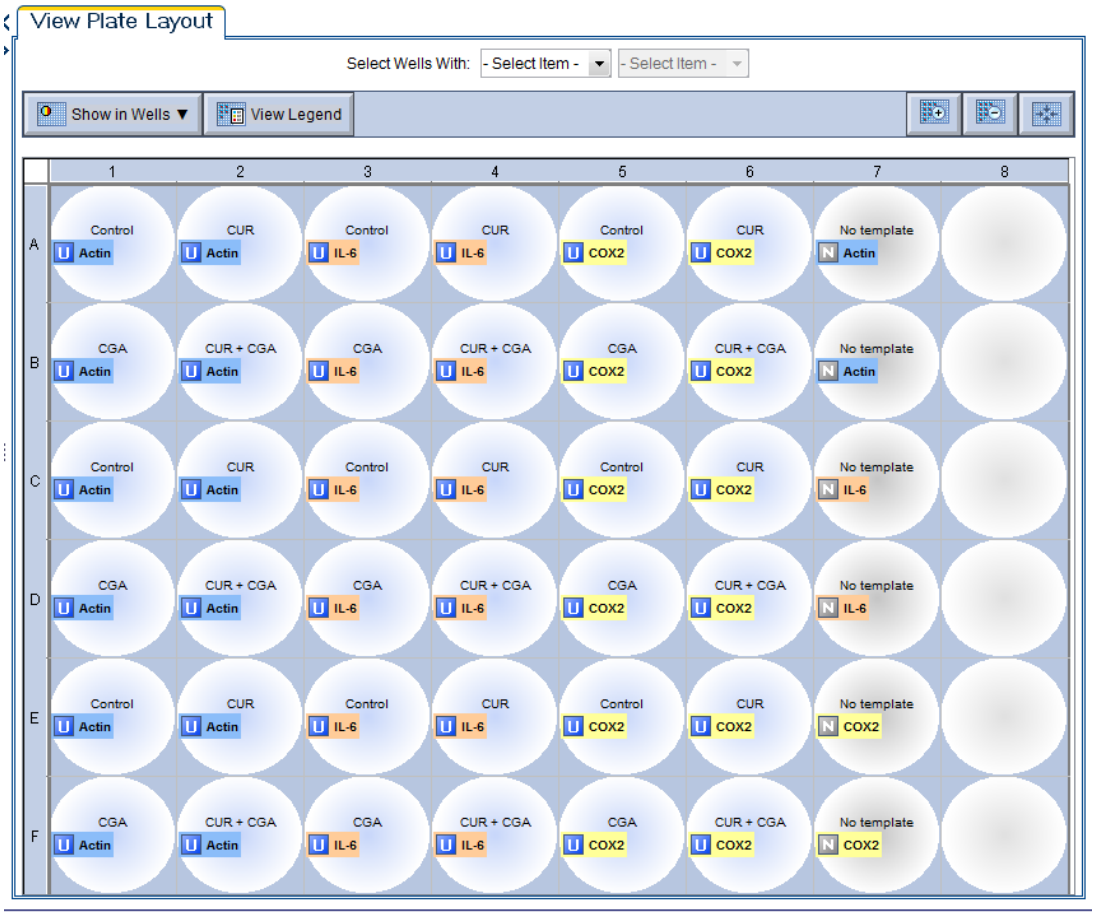
qRT-PCR Experimental Design

I. Experimental Properties

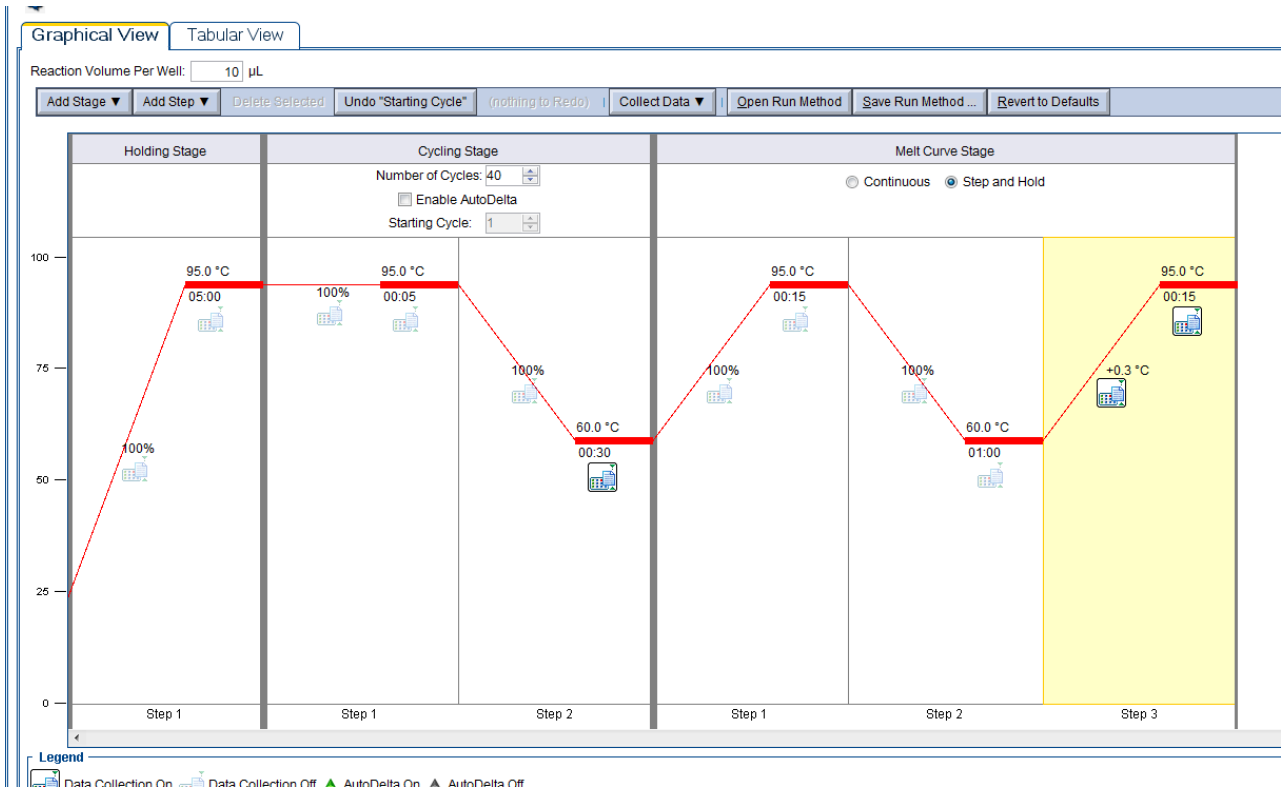


II. Plate Setup





III. Run Method



IV. Reaction Setup

Reaction Volume Per Well: 10 µL Excess Reaction Volume: 10 %

Reactions for IL-6

Master Mix Concentration: 2.0 X

Forward Primer Starting Concentration 20.0 pmol/µL Forward Primer Final Concentration 200.0 nM

Reverse Primer Starting Concentration 20.0 pmol/µL Reverse Primer Final Concentration 200.0 nM

Component	Volume (µL) for 1 Reaction
Master Mix (2.0X)	5.00
Forward Primer	0.10
Reverse Primer	0.10
Sample (10X)	1.00
H_2O	3.80
Total Volume	10.00

Appendix 4

Documents for Consumer Sensory Test

PARTICIPANT INFORMATION SHEET

Project Title: **Development of coconut yogurt supplemented with curcumin (turmeric) and coffee**

Locality: **Massey University, Auckland**

Lead investigator: **Akshay Bisht** Email: **bishtakshay51@gmail.com**

Researcher Introduction

My name is Akshay Bisht, a Master of Food Technology student at School of Food and Advanced Technology (SFAT), Massey University. My supervisors are Dr. Tony Mutukumira, A/Prof. Kay Rutherfurd, Dr. Martin Dickens and Dr. Rohith Thota

Project Description and Procedure

The main aim of this study is to develop a coconut yogurt supplemented with food-grade curcumin (turmeric) and coffee. Specific objectives of this research include optimising the concentration of turmeric and coffee in yogurt and evaluation of physico-chemical, microbiological and sensory properties of the final formulation.

You are invited to participate in consumer sensory test to help us determine the acceptance of coconut yogurt enriched with curcumin and coffee. An ideal participant for this study would be person consuming any type of yogurt at least once a week. The participant is required to taste and evaluate the yogurt samples. Including information sheet, this will take you about 10-15 minutes to complete the task.

The food you will be tasting contains coconut cream, turmeric, coffee, honey and live probiotic culture (*Lactobacillus* and *Streptococcus*). You **should not participate** if you are allergic to or may be affected by the consumption of any of the ingredients mentioned above. Please provide the researcher, of any, potential cultural, religious or ethical beliefs which may prevent you from consuming the samples under considerations.

The information collected in this study will be used toward partial fulfilment of the Masters in Food Technology. Participation in this research is completely voluntary and the participant is under no obligation to accept the invitation of this survey. However, if you decide to participate, you have right to:

- Withdraw from this study at any time;
- Decline to answer any particular question;
- Ask any further questions regarding this study at any time during participation;
- Provide information on the understanding that your name will be kept confidential until and unless you give permission to the researcher; and
- Ask for the summary and conclusion of the project once the research project has concluded.

If you have any questions, concerns or complaints about the study at any stage, you can contact the following researchers involved in the study:

- Akshay Bisht, Master student: bishtakshay51@gmail.com
- Dr. Tony Mutukumira, Chief Supervisor: A.N.Mutukumira@massey.ac.nz
- A/Prof. Kay Rutherfurd, Co-Supervisor: K.J.Rutherfurd@massey.ac.nz
- Dr. Martin Dickens, Co-supervisor: M.Dickens@massey.ac.nz
- Dr. Rohith Thota, Co-supervisor: R.Thota@massey.ac.nz

Disclaimer: This project has been evaluated by peer review and judged to be low risk. Consequently it has not been reviewed by one of the University's Human Ethics Committees (**Application no.: 4000020553**). The researcher(s) named in this document are responsible for the ethical conduct of this research. If you have any concerns about the conduct of this research that you want to raise with someone other than the researcher(s), please contact Professor Craig Johnson, Director (Research Ethics), email humanethics@massey.ac.nz.

PARTICIPANT CONSENT FORM

Development of coconut yogurt supplemented with curcumin (turmeric) and coffee

- I have read and understood the Information Sheet and have details of the study explained to me. My questions have been answered to satisfaction, and I understand that I may ask further questions at any time.
- I agree to voluntarily participate in this study under the conditions set out in the Information Sheet.
- I understand that I have the right to withdraw from the study at any time and to decline to answer any particular question.
- I have discussed and advised the researcher of any potentially relevant cultural, religious or ethical beliefs that may prevent me from consuming the Food under consideration.

Participants Signature:……

Participants Full Name:……..

Date:……...

SENSORY ACCEPTANCE TEST

You will be given two coded samples. Please taste the samples and evaluate them on following characteristics by selecting the appropriate attribute (✓) the best reflects your feelings about the respective property of the product. You may taste the sample more than once and also provide additional comments regarding the sample. **Note: Each sample must be evaluated on a separate form.**

Name: **Sample No.:**

1. How would you rate the **APPEARANCE** of this product?

Dislike extremely	Dislike very much	Dislike moderately	Disliked slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

2. How would you rate the **COLOUR** of this product?

Dislike extremely	Dislike very much	Dislike moderately	Disliked slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

3. How would you rate the **TEXTURE** of this product?

Dislike extremely	Dislike very much	Dislike moderately	Disliked slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

4. How would you rate the **FLAVOUR** of this product?

Dislike extremely	Dislike very much	Dislike moderately	Disliked slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

5. How would you rate the **OVERALL ACCEPTANCE** of this product?

Dislike extremely	Dislike very much	Dislike moderately	Disliked slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Comment(s):……

Appendix 5

Raw and Statistically Analysed Data for *In Vitro* Study

I) Optimisation of PMA concentration

PMA concentration (nM)	Replicate	Absorbance (blank subtracted) at 595 nm	Average absorbance	Standard deviation	% Cell adherence (with respect to 200 nM dose)	Average % cell adherence	Standard deviation
0	1	0.006	0.003	0.006	1.22	0.623	1.28
		-0.006			-1.12		
		0.004			0.77		
	2	-0.002			-0.45		
		0.002			0.41		
		0.014			2.89		
5	1	0.395	0.401	0.059	80.61	81.18	8.47
		0.438			82.02		
		0.512			99.03		
	2	0.333			74.66		
		0.358			74.74		
		0.368			76.03		
25	1	0.438	0.414	0.053	89.39	83.84	6.63
		0.467			87.45		
		0.483			93.42		
	2	0.335			75.11		
		0.374			78.08		
		0.385			79.55		
50	1	0.433	0.501	0.044	88.37	102.48	13.11
		0.471			88.20		
		0.476			92.07		
	2	0.53			118.83		
		0.542			113.15		
		0.553			114.26		
100	1	0.542	0.484	0.092	110.61	97.71	14.02
		0.585			109.55		
		0.593			114.70		
	2	0.37			82.96		
		0.399			83.29		
		0.412			85.12		
200	1	0.49	0.492	0.028	100	100	0
		0.534			100		
		0.517			100		
	2	0.446			100		
		0.479			100		
		0.484			100		

Statistical Analysis

% Cell adherence (with respect to 200 nM dose)

ANOVA

Adherence

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45013.794	5	9002.759	92.688	.000
Within Groups	2913.892	30	97.130		
Total	47927.687	35			

Adherence

Duncan[a]

		Subset for alpha = 0.05		
PMA	N	1	2	3
0 nM	6	.6227		
5 nM	6		81.1839	
25 nM	6		83.8336	
100 nM	6			97.7075
200 nM	6			100.0000
50 nM	6			102.4803
Sig.		1.000	.645	.436

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

II) Data points for TNF-α standard curve

TNF-α concentration (pg/ml)	Absorbance (blank subtracted) at 450 nm	Average absorbance	Standard deviation
500	1.848	1.773	0.088
	1.822		
	1.65		
250	0.951	1.043	0.066
	1.097		
	1.082		
125	0.499	0.513	0.009
	0.52		
	0.52		
62.5	0.247	0.302	0.045
	0.357		
	0.303		
31.3	0.125	0.147	0.019
	0.17		
	0.145		
15.6	0.067	0.071	0.004
	0.076		
	0.07		
7.8	0.043	0.04	0.004
	0.035		
	0.043		

III) Optimisation of TNF-α dilution factor for ELISA

Dilution Factor	Replicate	Absorbance (blank subtracted) at 450 nm	Average absorbance	Standard deviation	TNF-α levels (without multiplication with dilution factor)	Average TNF-α levels ± Standard deviation (without multiplication with dilution factor)	TNF-α levels (After multiplication with dilution factor)	Average TNF-α levels ± Standard deviation (After multiplication with dilution factor)
No dilution	1	3.124	3.18	0.272	Above standard curve detection limit	-	-	-
		3.071						
	2	3.628						
		2.898						
10 times	1	2.481	2.39	0.116	Above standard curve detection limit	-	-	-
		2.526						
	2	2.299						
		2.254						
50 times	1	0.849	0.898	0.111	220.83	236.62±32.25	11041.5	11831.13±1612.34
		0.872			230.59		11529.5	
	2	0.789			204.9		10245	
		1.082			290.17		14508.5	
100 times	1	0.509	0.505	0.052	121.13	120.32±14.71	12113	12031.5±1471.36
		0.555			132.92		13292	
	2	0.42			96.05		9605	
		0.537			131.16		13116	
200 times	1	0.246	0.258	0.014	44.89	49.07±4.37	8978	9814±873.897
		0.245			44.89		8978	
	2	0.261			51.45		10290	
		0.279			55.05		11010	

Statistical Analysis

TNF-α levels (After multiplication with dilution factor)

ANOVA

TNF-α levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12034999.542	2	6017499.771	2.449	.142
Within Groups	22112943.688	9	2456993.743		
Total	34147943.229	11			

TNF-α levels

Duncan[a]

		Subset for alpha = 0.05
dilution	N	1
200 time	4	9814.0000
50 time	4	11831.1250
100 time	4	12031.5000
Sig.		.088

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

IV) Optimisation of LPS dose for treatment of THP-1 cells

LPS concentration (ng/ml)	Replicate	MTT assay				ELISA			
		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
200	1	0.083	40.097	46.243	8.240	0.849	10495.5	10685.25	1161.248
		0.081	36.161			0.872	10834		
	2	0.136	54.839			0.732	9072.5		
		0.146	53.875			0.978	12339		
100	1	0.113	54.589	55.528	1.035	0.589	7066	9996.00	3258.849
		0.123	54.912			1.02	7988		
	2	0.142	57.258			1.188	15437.5		
		0.15	55.351			0.736	9492.5		
50	1	0.118	57.005	59.467	4.123	0.52	6351	5052	820.527
		0.121	54.018			0.357	4213		
	2	0.159	64.113			0.423	5135		
		0.17	62.731			0.381	4509		
25	1	0.117	56.522	63.113	6.5739	0.014	low	741.3333	66.7861
		0.127	56.696			0.06	658		
	2	0.176	70.968			0.07	821.5		
		0.185	68.266			0.065	744.5		
5	1	0.179	86.473	72.064	14.045	0.059	Below standard curve detection limit	-	-
		0.192	85.714			0.06			
	2	0.146	58.871			0.013			
		0.155	57.196			0.006			
0	1	0.207	100	100	-	-0.005	Below standard curve detection limit	-	-
		0.224	100			-0.003			
	2	0.248	100			-0.012			
		0.271	100			-0.007			

Statistical Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6975.171	5	1395.034	19.230	.000
Within Groups	1305.787	18	72.544		
Total	8280.958	23			

Cell viability

Duncan[a]

		Subset for alpha = 0.05			
LPS	N	1	2	3	4
200 ng/ml	4	46.2426			
100 ng/ml	4	55.5272	55.5272		
50 ng/ml	4	59.4666	59.4666	59.4666	
25 ng/ml	4		63.1129	63.1129	
5 ng/ml	4			72.0636	
0 ng/ml	4				100.0000
Sig.		.051	.248	.062	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	222918232.317	3	74306077.439	16.160	.000
Within Groups	50580822.917	11	4598256.629		
Total	273499055.233	14			

TNF-alpha

Duncan[a,b]

		Subset for alpha = 0.05		
LPS	N	1	2	3
25 ng/ml	3	741.3333		
50 ng/ml	4		5052.0000	
100 ng/ml	4			9996.0000
200 ng/ml	4			10685.2500
Sig.		1.000	1.000	.671

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.692.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

V) Optimisation of LPS incubation time for THP-1 cells

Incubation time (h)	Replicate	MTT assay				ELISA			
		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
6	1	0.236	38.562	35.703	2.917	0.257	3070.25	3212.325	171.3376
		0.196	32.026			0.286	3303.65		
	2	0.206	33.660			0.295	3447		
		0.236	38.562			0.274	3028.4		
4	1	0.3	49.019	52.696	2.199	0.287	3351.55	3188.425	314.3544
		0.33	53.922			0.275	3303.65		
	2	0.325	53.105			0.293	3447.15		
		0.335	54.739			0.228	2651.35		
2	1	0.311	50.817	55.678	4.611	0.187	2093.2	1709.813	319.6801
		0.336	54.902			0.162	1813.45		
	2	0.329	53.758			0.102	1208.95		
		0.387	63.235			0.157	1723.65		
1	1	0.357	58.333	63.358	4.199	0.173	391.43	371.655	12.26826
		0.428	69.935			0.166	372.26		
	2	0.379	61.928			0.15	362.68		
		0.387	63.235			0.151	360.25		
0.5	1	0.51	83.333	75.939	4.609	0.002	Below standard curve detection limit	-	-
		0.442	72.222			-0.008			
	2	0.44	71.895			0.002			
		0.467	76.307			-0.002			
0	1	0.559	91.339	100.00	7.295	-0.006	Below standard curve detection limit	-	-
		0.577	94.281			-0.008			
	2	0.644	105.228			0.003			
		0.666	108.824			0.001			

Statistical Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9723.122	5	1944.624	69.062	.000
Within Groups	506.836	18	28.158		
Total	10229.959	23			

Cell viability

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
6 h	4	35.7026				
4 h	4		52.6961			
2 h	4		55.6781	55.6781		
1 h	4			63.3578		
0.5 h	4				75.9395	
0 h	4					99.9183
Sig.		1.000	.437	.056	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22238674.724	3	7412891.575	96.471	.000
Within Groups	922084.538	12	76840.378		
Total	23160759.263	15			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05		
time	N	1	2	3
1 h	4	371.6550		
2 h	4		1709.8125	
4 h	4			3188.4250
6 h	4			3212.3250
Sig.		1.000	1.000	.905

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

VI) Treatment of cells with curcumin

Concentration of curcumin (µM)	Replicate	MTT assay				ELISA			
		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
25	1	0.183	65.125	73.824	7.456	0.21	1824.55	1701.933	82.296
		0.188	66.904			0.192	1774.4		
		0.189	67.26			0.181	1623.9		
	2	0.221	78.648			0.2	1724.25		
		0.198	70.462			0.199	1623.9		
		0.213	75.801			0.175	1623.9		
	3	0.198	70.462			0.175	1623.9		
		0.226	80.427			0.195	1674.05		
		0.251	89.324			0.2	1824.55		
10	1	0.197	70.108	71.609	3.803	0.563	4721.25	4620.95	66.867
		0.189	67.26			0.534	4570.8		
		0.204	72.598			0.545	4620.95		
	2	0.194	69.039			0.538	4520.65		
		0.192	68.327			0.546	4620.95		
		0.191	67.972			0.557	4721.25		
	3	0.208	74.021			0.553	4671.1		
		0.219	77.936			0.532	4570.8		
		0.217	77.224			0.546	4570.8		
5	1	0.2	71.174	78.806	8.131	0.749	6439	6373.722	174.941
		0.215	76.512			0.74	6288.75		
		0.198	70.462			0.729	6244.85		
	2	0.208	74.021			0.761	6683.75		
		0.206	73.31			0.764	6539.6		
		0.209	74.377			0.75	6489.4		
	3	0.241	85.765			0.744	6389.1		
		0.261	92.882			0.71	6094.35		
		0.255	90.747			0.726	6194.7		
2.5	1	0.245	87.189	93.673	5.431	0.914	8012.8	7667.406	153.678
		0.266	94.662			0.871	7668.15		
		0.23	81.85			0.867	7618		
	2	0.271	96.441			0.871	7718.3		
		0.271	96.441			0.885	7762		
		0.264	93.95			0.858	7567.8		
	3	0.263	93.594			0.844	7423.6		
		0.279	99.288			0.851	7567.85		
		0.28	99.644			0.857	7668.15		
1	1	0.239	85.053	88.177	5.523	0.918	8041.5	7724.606	215.473
		0.225	80.071			0.855	7467.5		
		0.23	81.85			0.906	8107.05		
	2	0.24	85.409			0.854	7517.65		
		0.247	87.901			0.879	7771.5		
		0.248	88.256			0.872	7668.15		
	3	0.26	92.527			0.884	7812.4		
		0.273	97.153			0.854	7567.85		
		0.268	95.374			0.858	7567.85		
0	1	0.228	100	100	0	0.868	7718.3	7972.322	209.113
		0.243	100			0.939	8257.5		
		0.249	100			0.928	8257.5		

	2	0.304	100			0.92	8155		
		0.332	100			0.888	7862.5		
		0.331	100			0.876	7668.15		
	3	0.267	100			0.91	8013		
		0.29	100			0.887	7812.35		
		0.286	100			0.911	8006.6		

Statically Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5853.253	5	1170.651	31.831	.000
Within Groups	1765.316	48	36.777		
Total	7618.569	53			

Cell viability

Duncan[a]

		Subset for alpha = 0.05			
Curcumin	N	1	2	3	4
10 µM	9	71.6093			
25 µM	9	73.8236	73.8236		
5 µM	9		78.8059		
1 µM	9			88.1771	
2.5 µM	9			93.6734	
0 µM	9				100.0000
Sig.		.442	.088	.060	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	271428893.150	5	54285778.630	1860.436	.000
Within Groups	1400595.116	48	29179.065		
Total	272829488.265	53			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05				
Curcumin	N	1	2	3	4	5
25 µM	9	1701.9333				
10 µM	9		4620.9500			
5 µM	9			6373.7222		
2.5 µM	9				7667.4056	
1 µM	9				7724.6056	
0 µM	9					7972.3222
Sig.		1.000	1.000	1.000	.481	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

VII) Treatment of cells with lupeol

Concentration of lupeol (µM)	Replicate	MTT assay				ELISA			
		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
25	1	0.147	62.025	66.98	2.951	0.321	2313.6	2334.556	111.431
		0.153	64.557			0.331	2313.6		
		0.152	64.135			0.328	2507.95		
	2	0.155	65.401			0.324	2313.6		
		0.163	68.777			0.333	2413.95		
		0.163	68.777			0.297	2213.3		
	3	0.163	68.777			0.343	2507.95		
		0.161	67.933			0.313	2213.75		
		0.171	72.152			0.296	2213.3		
10	1	0.181	76.371	78.997	7.394	0.404	3003.3	2610.639	170.068
		0.194	81.857			0.349	2608.3		
		0.195	82.279			0.354	2618.75		
	2	0.161	67.933			0.373	2752.5		
		0.168	70.886			0.345	2558.15		
		0.168	70.886			0.354	2574.1		
	3	0.204	86.076			0.34	2508.45		
		0.199	83.966			0.344	2508.45		
		0.215	90.717			0.333	2363.75		
5	1	0.197	83.122	91.374	6.507	0.315	2363.75	2816.722	220.796
		0.2	84.388			0.423	3146.25		
		0.192	81.013			0.411	3002.3		
	2	0.217	91.561			0.353	2574.1		
		0.224	94.515			0.397	2953.15		
		0.22	92.827			0.374	2752.5		
	3	0.233	98.312			0.39	2802.65		
		0.234	98.734			0.401	2903		
		0.232	97.890			0.393	2852.8		
2.5	1	0.189	79.7465	87.904	5.541	0.388	2852.85	3175.298	307.534
		0.197	83.122			0.385	2852		
		0.201	84.810			0.405	3003.4		
	2	0.2	84.388			0.483	3592.7		
		0.206	86.92			0.489	3636.55		
		0.21	88.608			0.475	3542.5		
		0.213	89.873			0.414	3047.2		
		0.23	97.0461			0.396	2902.985		
		0.229	96.625			0.422	3147.5		
1	1	0.238	100.422	98.453	9.152	0.442	3341.9	3535.539	189.262
		0.245	103.375			0.491	3636.55		
		0.24	101.266			0.521	3887.35		
	2	0.196	82.700			0.469	3492.2		
		0.211	89.029			0.49	3736.9		
		0.208	87.764			0.471	3492.35		
	3	0.242	102.109			0.446	3247.85		
		0.26	109.705			0.477	3592.7		
		0.26	109.705			0.447	3392.05		
0	1	0.211	100	100	0	0.516	3887.35	3918.022	179.301
		0.223	100			0.517	3887.4		
		0.228	100			0.544	4081.75		

	2	0.198	100			0.535	3981.4		
		0.208	100			0.539	4031.6		
		0.212	100			0.549	4131.9		
	3	0.271	100			0.486	3636.55		
		0.287	100			0.538	4031.6		
		0.292	100			0.478	3592.65		

Statically Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7072.057	5	1414.411	34.263	.000
Within Groups	1981.480	48	41.281		
Total	9053.538	53			

Cell viability

Duncan[a]

		Subset for alpha = 0.05			
Lupeol	N	1	2	3	4
25 µM	9	66.9480			
10 µM	9		78.9967		
2.5 µM	9			87.9044	
5 µM	9			91.3737	
1 µM	9				98.4529
0 µM	9				100.0000
Sig.		1.000	1.000	.258	.612

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

Production of TNF-α

ANOVA

TNF –alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15865689.061	5	3173137.812	66.987	.000
Within Groups	2273732.875	48	47369.435		
Total	18139421.937	53			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05				
Lupeol	N	1	2	3	4	5
25 µM	9	2334.5556				
10 µM	9		2610.6389			
5 µM	9		2816.7222			
2.5 µM	9			3175.2983		
1 µM	9				3535.5389	
0 µM	9					3918.0222
Sig.		1.000	.050	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 9.000.

VIII) Treatment of cells with chlorogenic acid (CGA)

Concentration of CGA (µM)	Replicate	MTT assay				ELISA			
		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
25	1	0.173	153.097	140.153	14.549	0.25	2213.3	2615.281	397.506
		0.185	146.825			0.27	2457.8		
		0.191	156.557			0.262	2363.75		
	2	0.413	136.304			0.278	2558.15		
		0.439	135.494			0.285	2658.45		
		0.442	137.267			0.271	2413.95		
	3	0.333	156.338			0.262	2370.2		
		0.351	153.947			0.267	2425.6		
		0.355	153.017			0.258	2327.95		
	4	0.245	142.442			0.394	3299.8		
		0.264	141.936			0.419	3543.95		
		0.26	140.541			0.407	3408.85		
	5	0.197	111.299			0.318	2661.8		
		0.207	110.106			0.315	2621.1		
		0.208	111.23			0.345	2892.9		
	6	0.2	144.928			0.275	2280.95		
		0.203	145			0.283	2336.3		
		0.205	146.429			0.269	2240.25		
10	1	0.157	138.938	133.011	14.253	0.289	2658.45	2710.544	393.737
		0.168	133.333			0.286	2508		
		0.159	130.328			0.275	2508		
	2	0.407	134.323			0.27	2467.7		
		0.429	132.407			0.266	2398.65		
		0.433	134.472			0.267	2412.1		
	3	0.281	131.925			0.288	2622.65		
		0.309	135.526			0.256	2296		
		0.299	128.879			0.275	2496.35		
	4	0.261	151.744			0.426	3571.6		
		0.279	150			0.424	3556.95		
		0.276	149.189			0.421	3543.95		
	5	0.187	105.65			0.312	2593.45		
		0.194	103.192			0.315	2606.45		
		0.198	105.88			0.332	2769.2		
	6	0.192	139.13			0.319	2647.15		
		0.201	143.571			0.301	2499		
		0.204	145.714			0.317	2634.15		
5	1	0.145	128.319	122.505	11.379	0.267	2413.95	2724.931	357.876
		0.166	131.746			0.247	2213.3		
		0.153	125.41			0.274	2414.4		
	2	0.39	128.713			0.288	2609.15		
		0.437	134.877			0.277	2496.3		
		0.433	134.472			0.305	2748.9		
	3	0.264	123.944			0.299	2693.35		
		0.287	125.877			0.3	2706.85		
		0.282	121.552			0.304	2748.9		
	4	0.218	126.744			0.412	3462.55		
		0.237	127.419			0.411	3449.5		
		0.239	129.189			0.408	3434.9		

	5	0.173	97.7401			0.3	2499.05		
		0.183	97.3404			0.289	2390		
		0.187	100			0.306	2552.75		
	6	0.168	121.739			0.329	2743.2		
		0.176	125.714			0.339	2824.55		
		0.174	124.285			0.318	2647.15		
2.5	1	0.144	127.434	123.594	12.092	0.31	2752.5	2904.506	311.795
		0.157	124.603			0.328	2953.15		
		0.153	125.41			0.316	2852.85		
	2	0.379	125.083			0.322	2890.4		
		0.408	125.926			0.312	2819.6		
		0.408	126.708			0.313	2833.15		
	3	0.273	128.169			0.308	2777.55		
		0.295	129.386			0.306	2764.1		
		0.293	126.293			0.297	2693.35		
	4	0.25	145.349			0.413	3462.55		
		0.262	140.86			0.426	3571.6		
		0.261	141.081			0.401	3366.55		
	5	0.183	103.39			0.358	2987.3		
		0.192	102.128			0.37	3096.35		
		0.192	102.674			0.366	3055.65		
	6	0.158	114.493			0.304	2530.05		
		0.164	117.143			0.303	2525.1		
		0.166	118.571			0.285	2349.3		
1	1	0.377	124.422	119.002	13.055	0.254	2313.6	2494.236	272.752
		0.399	123.148			0.255	2263.45		
		0.402	124.845			0.25	2263.45		
	2	0.262	123.005			0.252	2299.3		
		0.281	123.246			0.268	2398.65		
		0.278	119.828			0.247	2243.75		
	3	-	-			0.261	2356.55		
		-	-			0.278	2524.95		
		-	-			0.271	2440.75		
	4	0.23	133.721			0.284	2349.3		
		0.247	132.796			0.279	2295.6		
		0.253	136.757			0.359	3000.35		
	5	0.17	96.045			0.367	3068.7		
		0.181	96.277			0.361	3028		
		0.175	93.583			0.337	2811.55		
	6	0.159	115.217			0.282	2349.3		
		0.167	119.286			0.286	2376.95		
		0.172	122.857			0.3	2512.05		
0	1	0.113	100	100	0	0.225	2018.9	2371.653	372.529
		0.126	100			0.219	1968.75		
		0.122	100			0.213	1868.45		
	2	0.303	100			0.236	2146.05		
		0.324	100			0.237	2111		
		0.322	100			0.248	2230.25		
	3	0.213	100			0.236	2130.9		
		0.228	100			0.252	2299.3		
		0.232	100			0.234	2103.95		
	4	0.172	100			0.27	2240.25		
		0.186	100			0.274	2254.9		
		0.185	100			0.412	3449.55		
	5	0.177	100			0.329	2743.2		
		0.188	100			0.322	2687.85		
		0.187	100			0.298	2471.35		
	6	0.138	100			0.324	2615.5		

		0.14	100			0.321	2687.8		
		0.14	100			0.316	2661.8		

Statically Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16870.127	5	3374.025	22.290	.000
Within Groups	14985.349	99	151.367		
Total	31855.476	104			

Cell viability

Duncan[a,b]

		Subset for alpha = 0.05		
CGA	N	1	2	3
0 µM	18	100.0000		
1 µM	15		119.0021	
5 µM	18		122.5045	
2.5 µM	18		123.5944	
10 µM	18			133.0114
25 µM	18			140.1532
Sig.		1.000	.304	.090

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 17.419.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3167317.446	5	633463.489	4.777	.001
Within Groups	13527031.012	102	132617.951		
Total	16694348.457	107			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05		
CGA	N	1	2	3
0 µM	18	2371.6528		
1 µM	18	2494.2361	2494.2361	
25 µM	18	2615.2806	2615.2806	
10 µM	18		2710.5444	2710.5444
5 µM	18		2724.9306	2724.9306
2.5 µM	18			2904.5056
Sig.		.060	.085	.135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 18.000.

IX) Treatment of THP-1 cells with DMSO

Concentration of DMSO (%)	Replicate	MTT assay				ELISA			
		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
0.2	1	0.184	95.833	90.716	4.076	0.479	5178	4904.167	309.646
		0.181	93.299			0.484	5243		
		0.178	92.708			0.48	5205		
	2	0.184	92			0.436	4701.5		
		0.186	84.546			0.425	4574		
		0.189	85.909			0.42	4523.5		
0.1	1	0.184	95.833	93.875	3.775	0.457	4918	4821.583	151.468
		0.191	98.454			0.454	4893		
		0.188	97.917			0.459	4956.5		
	2	0.183	91.5			0.428	4606		
		0.194	88.182			0.429	4612.5		
		0.201	91.364			0.458	4943.5		
0.05	1	0.18	93.75	90.242	5.788	0.474	5115.5	5093.25	208.629
		0.187	96.392			0.461	4988		
		0.185	96.354			0.481	5198.5		
	2	0.179	89.5			0.493	5351		
		0.182	82.727			0.482	5211		
		0.182	82.727			0.437	4695.5		
0.01	1	0.174	90.625	95.78	3.109	0.523	5683	5365.333	286.661
		0.183	94.33			0.485	5243		
		0.181	94.271			0.534	5803.5		
	2	0.2	100			0.49	5300		
		0.213	96.818			0.461	4982.5		
		0.217	98.636			0.479	5180		
0.001	1	0.193	100.52	96.448	4.117	0.513	5555.5	5393.917	214.373
		0.192	98.969			0.498	5402.5		
		0.195	101.563			0.473	5096.5		
	2	0.188	94			0.506	5472.5		
		0.205	93.182			0.476	5141		
		0.199	90.455			0.524	5695.5		
0	1	0.192	100	100	0	0.529	5740	5604.25	122.586
		0.194	100			0.526	5721		
		0.192	100			0.525	5702		
	2	0.2	100			0.511	5555.5		
		0.22	100			0.501	5434.5		
		0.22	100			0.505	5472.5		

Statically Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	411.163	5	82.233	4.520	.003
Within Groups	545.801	30	18.193		
Total	956.963	35			

Cell viability

Duncan[a]

		Subset for alpha = 0.05			
DMSO	N	1	2	3	4
0.05%	6	90.2417			
0.2%	6	90.7158	90.7158		
0.1%	6	93.8750	93.8750	93.8750	
0.01%	6		95.7800	95.7800	95.7800
0.001%	6			96.4482	96.4482
0%	6				100.0000
Sig.		.173	.060	.333	.115

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2822507.417	5	564501.483	9.239	.000
Within Groups	1833037.833	30	61101.261		
Total	4655545.250	35			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05		
DMSO	N	1	2	3
0.1%	6	4821.5833		
0.2%	6	4904.1667		
0.05%	6	5093.2500	5093.2500	
0.01%	6		5365.3333	5365.3333
0.001%	6		5393.9167	5393.9167
0%	6			5604.2500
Sig.		.081	.054	.123

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

(X) Treatment of THP-1 cells with DMSO + Ethanol

Combination		Replicate	MTT assay				ELISA			
DMSO (%)	Ethanol (%)		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
0.2	0.2	1	0.29	90.342	91.615	1.492	0.537	5854.5	5749.5	301.594
			0.294	91.304			0.56	6109.5		
			0.301	94.357			0.563	6141		
		2	0.292	90.402			0.503	5466		
			0.295	90.491			0.498	5402.5		
			0.296	92.79			0.508	5523.5		
0.1	0.1	1	0.289	90.031	88.965	1.845	0.522	5670	5956	355.666
			0.281	87.267			0.558	6084.5		
			0.281	88.088			0.492	5326		
		2	0.279	86.377			0.555	6045.5		
			0.295	90.491			0.583	6383		
			0.292	91.536			0.582	6227		
0.05	0.05	1	0.286	89.097	89.492	1.8181	0.535	5810	6145.667	358.592
			0.295	91.615			0.533	5791		
			0.292	91.536			0.551	6020.5		
		2	0.288	89.164			0.624	6861.5		
			0.281	86.196			0.567	6198.5		
			0.285	89.342			0.566	6192.5		
0.01	0.01	1	0.252	78.505	87.083	7.313	0.588	6434.5	6444.083	286.26
			0.259	80.435			0.612	6721		
			0.261	81.818			0.624	6861.5		
		2	0.288	89.164			0.567	6186		
			0.311	95.399			0.588	6434.5		
			0.31	97.179			0.552	6027		
0.075	0.05	1	0.296	92.212	91.817	0.751	0.503	5466	6141.583	648.336
			0.298	92.547			0.502	5447.5		
			0.296	92.79			0.514	5574.5		
		2	0.294	91.022			0.616	6759.5		
			0.296	90.798			0.624	6874.5		
			0.292	91.536			0.61	6727.5		
0.0375	0.0125	1	0.279	86.916	88.503	2.212	0.474	5122.5	6089.25	443.288
			0.278	86.335			0.589	6440.5		
			0.285	89.342			0.58	6358		
		2	0.279	86.378			0.56	6128.5		
			0.292	89.57			0.57	6243		
			0.295	92.477			0.57	6243		
0	0	1	0.321	100	100	0	0.571	6237	6525.417	483.247
			0.322	100			0.546	5957		
			0.319	100			0.565	6150.5		
		2	0.323	100			0.591	6472.5		
			0.326	100			0.641	7053		
			0.319	100			0.661	7282.5		

Statically Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	660.031	6	110.005	9.453	.000
Within Groups	407.279	35	11.637		
Total	1067.310	41			

Cell viability

Duncan[a]

		Subset for alpha = 0.05		
Combination	N	1	2	3
0.01% DMSO + 0.01% ethanol	6	87.0833		
0.0375% DMSO + 0.0125% ethanol	6	88.5030	88.5030	
0.1% DMSO + 0.1% ethanol	6	88.9650	88.9650	
0.05% DMSO + 0.05% ethanol	6	89.4917	89.4917	
0.2% DMSO + 0.2% ethanol	6		91.6143	
0.075% DMSO + 0.05% ethanol	6		91.8175	
0% DMSO + 0% ethanol	6			100.0000
Sig.		.274	.142	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2575432.738	6	429238.790	1.959	.099
Within Groups	7670166.333	35	219147.610		
Total	10245599.071	41			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05	
Combination	N	1	2
0.2% DMSO + 0.2% ethanol	6	5749.5000	
0.1% DMSO + 0.1% ethanol	6	5956.0000	5956.0000
0.0375% DMSO + 0.0125% ethanol	6	6089.2500	6089.2500
0.075% DMSO + 0.05% ethanol	6	6141.5833	6141.5833
0.05% DMSO + 0.05% ethanol	6	6145.6667	6145.6667
0.01% DMSO + 0.01% ethanol	6		6444.0833
0% DMSO + 0% ethanol	6		6525.4167
Sig.		.200	.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

(XI) Treatment of THP-1 cells with curcumin + lupeol

Combination		Replicate	MTT assay				ELISA			
Lupeol (µM)	Curcumin (µM)		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
0	0	1	0.224	100	100	0	0.869	9839.05	6506.778	3094.755
			0.242	100			0.859	9729		
			0.236	100			0.761	8498.65		
		2	0.323	100			0.861	9784.4		
			0.332	100			0.827	9381.25		
			0.341	100			0.858	9766.45		
		3	0.21	100			0.858	9746.65		
			0.226	100			0.859	9746.7		
			0.228	100			0.861	9766.45		
		4	0.24	100			0.494	3387.95		
			0.255	100			0.501	3464.15		
			0.249	100			0.511	3525.7		
		5	0.284	100			0.507	3491.45		
			0.301	100			0.52	3690.75		
			0.307	100			0.55	3775.25		
		6	0.194	100			0.454	3135.35		
			0.205	100			0.44	3035.2		
			0.21	100			0.487	3357.6		
2.5	0	1	0.2	89.286	90.969	5.775	0.684	7506.65	5577.338	2465.967
			0.217	89.669			0.671	7397.3		
			0.212	89.831			0.696	7672.3		
		2	0.302	93.498			0.729	8085.5		
			0.287	86.446			0.702	7804.15		
			0.306	89.736			0.708	7888.2		
		3	0.193	91.905			0.751	8407.45		
			0.208	92.035			0.752	8407.75		
			0.205	89.912			0.798	8997.35		
		4	0.212	88.333			0.456	3142.65		
			0.217	85.098			0.473	3257.5		
			0.223	89.558			0.461	3173		
		5	0.244	85.915			0.442	3050.83		
			0.257	85.382			0.475	3264.85		
			0.256	83.388			0.441	3033.05		
		6	0.199	102.577			0.433	2972.4		
			0.214	104.39			0.445	3081		
			0.211	100.476			0.472	3250.15		
5	0	1	0.195	87.054	85.097	9.933	0.705	7857.45	5044.011	2145.331
			0.204	84.298			0.671	7397.3		
			0.203	86.017			0.673	7410.75		
		2	0.285	88.235			0.622	6792.9		
			0.282	84.94			0.622	6792.9		
			0.29	85.044			0.646	7103.45		
		3	0.185	88.095			0.618	6751.65		
			0.191	84.513			0.647	7128.9		
			0.2	87.719			0.665	7326.65		
		4	0.212	88.333			0.442	3058.15		
			0.222	87.059			0.442	3058.15		

			0.224	89.96			0.429	2959		
		5	0.186	65.493			0.421	2890.1		
			0.199	66.113			0.418	2881.8		
			0.197	64.169			0.444	3058.2		
		6	0.193	99.485			0.415	2881.8		
			0.203	99.024			0.396	2752.3		
			0.202	96.19			0.388	2690.75		
10	0	1	0.185	82.59	80.456	4.523	0.619	6755.5	4743.089	1992.573
			0.196	80.992			0.611	6645.45		
			0.187	79.237			0.596	6401.6		
		2	0.233	72.136			0.605	6590.4		
			0.243	73.193			0.582	6263.7		
			0.255	74.78			0.582	6263.7		
		3	0.175	83.333			0.613	6700.45		
			0.191	84.513			0.641	7030.65		
			0.197	86.404			0.692	7680.7		
		4	0.201	83.75			0.437	3020.5		
			0.211	82.745			0.45	3119.7		
			0.209	83.936			0.44	3035.2		
		5	0.22	77.465			0.336	2346.25		
			0.223	74.086			0.348	2429.9		
			0.233	75.896			0.354	2468.45		
		6	0.163	84.021			0.408	2805.6		
			0.171	83.414			0.444	3058.2		
			0.18	85.714			0.4	2759.65		
0	5	1	0.17	79.893	78.901	6.174	0.618	6708.85	5329.914	2207.025
			0.183	79.62			0.673	7415.8		
			0.175	78.153			0.641	7030.65		
		2	0.235	82.755			0.69	7635.9		
			0.246	78.097			0.698	7692.9		
			0.264	81.419			0.697	7692.9		
		3	0.162	81.143			0.713	7994.2		
			0.171	79.664			0.688	7618.25		
			0.172	79.439			0.705	7857.45		
		4	0.208	86.667			0.474	3273.1		
			0.224	87.843			0.478	3287.8		
			0.225	90.362			0.476	3280.45		
		5	0.193	67.958			0.416	2890.1		
			0.202	67.11			0.429	2966.35		
			0.209	68.078			0.407	2821.2		
		6	0.152	78.351			0.464	3195.95		
			0.156	76.098			0.477	3296.1		
			0.164	78.095			0.476	3280.5		
2.5	5	1	0.147	69.625	75.527	5.823	0.581	6063.7	4302.172	1565.974
			0.157	68.876			0.577	6095		
			0.163	73.068			0.586	5915.3		
		2	0.225	73.659			0.555	5902.75		
			0.238	75.687			0.575	5905.25		
			0.246	76.141			0.553	5785.1		
		3	0.149	74.952			0.551	5547.7		
			0.164	76.566			0.524	5543.3		
			0.158	73.298			0.558	5857.75		
		4	0.202	86.166			0.464	3095.95		
			0.19	76.51			0.481	3119.05		
			0.206	84.731			0.468	3111.55		
		5	0.188	66.197			0.425	2843.35		
			0.204	69.774			0.425	29843.35		
			0.198	66.495			0.421	2804.8		

		6	0.16	84.474			0.348	2329.95		
			0.159	79.561			0.347	2329.75		
			0.164	80.095			0.351	2345.5		
5	5	1	0.097	47.304	63.271	10.613	0.455	4747	3868.186	1256.384
			0.106	47.802			0.484	5050		
			0.102	47.22			0.477	4994.5		
		2	0.193	63.752			0.472	4957.55		
			0.198	63.639			0.521	5405.34		
			0.2	62.651			0.488	5087.4		
		3	0.105	54			0.497	5197.5		
			0.11	52.673			0.49	5160.05		
			0.112	53.123			0.506	5298.75		
		4	0.161	71.083			0.417	2774.45		
			0.159	66.353			0.416	2774.45		
			0.168	71.47			0.418	2774.45		
		5	0.183	68.437			0.328	2199.45		
			0.186	65.794			0.331	2215.1		
			0.187	64.912			0.336	2253.65		
		6	0.145	78.742			0.442	2966.45		
			0.158	81.073			0.434	2889.3		
			0.153	78.857			0.431	2881.95		
10	5	1	0.11	53.107	56.419	7.064	0.463	4245.25	3239.136	1178.218
			0.117	52.347			0.459	4416.7		
			0.117	53.576			0.47	4444.45		
		2	0.116	48.913			0.451	4532.65		
			0.118	49.566			0.458	4603.25		
			0.123	40.07			0.434	4337.6		
		3	0.109	55.905			0.448	4505.7		
			0.108	52.787			0.427	4067		
			0.113	54.561			0.451	4532.65		
		4	0.143	63.583			0.395	2036.7		
			0.156	65.177			0.399	2075.25		
			0.138	59.422			0.36	2114.4		
		5	0.16	60.338			0.34	2083.95		
			0.171	50.811			0.348	2029.9		
			0.162	56.769			0.361	2121.75		
		6	0.129	66.495			0.374	1798.9		
			0.128	64.439			0.37	2167.7		
			0.142	67.619			0.373	2190.65		

Statically Analysis
Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25095.180	7	3585.026	69.971	.000
Within Groups	6968.071	136	51.236		
Total	32063.251	143			

Cell viability

Duncan[a]

		Subset for alpha = 0.05						
Combination	N	1	2	3	4	5	6	7
10 Lup + 5 Cur	18	56.4158						
5 Lup + 5 Cur	18		63.2714					
2.5 Lup + 5 Cur	18			75.3264				
0 Lup + 5 Cur	18			78.9303	78.9303			
10 Lup + 0 Cur	18				80.4558	80.4558		
5 Lup + 0 Cur	18					85.0967		
2.5 Lup + 0 Cur	18						90.9686	
0 Lup + 0 Cur	18							100.0000
Sig.		1.000	1.000	.133	.524	.054	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 18.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26234.118	7	3747.731	37.446	.000
Within Groups	13611.212	136	100.082		
Total	39845.330	143			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05						
Combination	N	1	2	3	4	5	6	7
10 Lup + 5 Cur	18	53.3296						
5 Lup + 5 Cur	18		65.4913					
2.5 Lup + 5 Cur	18		70.7517	70.7517				
10 Lup + 0 Cur	18			75.9015	75.9015			
5 Lup + 0 Cur	18				80.1201	80.1201		
0 Lup + 5 Cur	18					85.4033	85.4033	
2.5 Lup + 0 Cur	18						87.7738	
0 Lup + 0 Cur	18							100.0000
Sig.		1.000	.117	.125	.208	.115	.478	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 18.000.

(XII) Treatment of THP-1 cells with curcumin + CGA

Combination		Replicate	MTT assay				ELISA			
CGA (µM)	Curcumin (µM)		Absorbance (blank subtracted) at 595 nm	Cell viability (%)	Average cell viability (%)	Standard deviation	Absorbance (blank subtracted) at 450 nm	TNF-α levels	Average TNF-α levels	Standard deviation
0	0	1	0.195	100	100	0	0.409	3114.35	3979.467	1797.045
			0.207	100			0.384	2926.95		
			0.219	100			0.427	3243.7		
		2	0.175	100			0.346	2655.9		
			0.183	100			0.393	2991.2		
			0.189	100			0.385	2940.1		
		3	0.189	100			0.428	3256.05		
			0.2	100			0.44	3353.65		
			0.202	100			0.434	3294.8		
		4	0.198	100			0.265	2479.3		
			0.208	100			0.263	2450.15		
			0.212	100			0.227	2119.8		
		5	0.189	100			0.269	2513.7		
			0.191	100			0.298	2780.55		
			0.199	100			0.254	2391.9		
		6	0.22	100			0.225	2105.25		
			0.23	100			0.252	2362.75		
			0.236	100			0.241	2256.15		
		7	0.28	100			0.774	6406.55		
			0.297	100			0.746	6158.95		
			0.297	100			0.791	6565.9		
		8	0.217	100			0.756	6258.5		
			0.228	100			0.83	6952.85		
			0.229	100			0.812	6764.95		
		9	0.24	100			0.736	6079.5		
			0.245	100			0.784	6506.1		
			0.246	100			0.787	6516.05		
2.5	0	1	0.212	108.718	109.708	4.335	0.446	3459.25	4516.17	2120.256
			0.229	110.628			0.454	3519.45		
			0.226	103.196			0.45	3511.15		
		2	0.205	117.143			0.459	3563.05		
			0.211	115.301			0.463	3601.75		
			0.218	115.344			0.443	3339.9		
		3	0.194	102.645			0.46	3640.45		
			0.22	110			0.459	3584.3		
			0.209	103.465			0.466	3611.6		
		4	0.232	117.172			0.301	2819.6		
			0.241	115.865			0.318	2979.8		
			0.238	112.264			0.317	2960.55		
		5	0.194	102.646			0.31	2902.3		
			0.219	114.66			0.307	2873.2		
			0.219	110.05			0.273	2542.8		
		6	0.242	110			0.25	2314.4		
			0.243	105.652			0.206	1925.2		
			0.243	102.966			0.258	2401.8		
		7	0.309	110.357			0.877	7370.95		
			0.316	106.397			0.877	7370.95		

			0.322	108.418			0.967	8209.7		
		8	0.236	108.756			0.966	8209.7		
			0.25	109.649			0.961	8169.9		
			0.254	110.917			0.961	8169.9		
		9	0.261	108.75			0.756	6278.4		
			0.269	109.796			0.752	6228.6		
			0.274	111.382			0.768	6377.95		
5	0	1	0.251	128.718	112.901	7.663	0.434	3430.5	4197.765	1967.17
			0.236	114.01			0.445	3457.95		
			0.244	111.416			0.441	3453.6		
		2	0.232	132.571			0.363	2957		
			0.216	118.033			0.363	2979.55		
			0.232	122.751			0.371	3031.45		
		3	0.214	113.228			0.438	3431.2		
			0.224	112			0.458	3541.8		
			0.209	103.465			0.441	3451.15		
		4	0.233	117.677			0.287	2683.8		
			0.231	111.058			0.278	2586.5		
			0.229	108.019			0.268	2513.7		
		5	0.204	107.937			0.251	2348.2		
			0.197	103.141			0.256	2391.9		
			0.194	97.487			0.265	2464.75		
		6	0.243	110.455			0.247	2309.15		
			0.246	106.957			0.24	2246.25		
			0.244	103.39			0.248	2319.1		
		7	0.316	112.857			0.842	7042.45		
			0.319	107.407			0.776	6446.4		
			0.328	110.438			0.868	7281.45		
		8	0.237	109.217			0.968	8219.65		
			0.273	119.737			0.866	7281.35		
			0.259	113.1			0.866	7281.35		
		9	0.273	113.75			0.758	6288.45		
			0.295	120.408			0.69	5672.4		
			0.293	119.106			0.751	6228.6		
10	0	1	0.251	128.718	127.081	17.483	0.41	3136.3	4359.637	2128.376
			0.273	131.884			0.41	3136.3		
			0.273	124.658			0.424	3233.15		
		2	0.265	151.429			0.408	3032		
			0.288	157.377			0.413	3143.3		
			0.27	142.857			0.463	3649.3		
		3	0.263	139.153			0.46	3640.3		
			0.279	139.5			0.461	3644.7		
			0.273	135.149			0.457	3577.3		
		4	0.253	127.778			0.275	2557.4		
			0.251	120.673			0.268	2503.8		
			0.257	121.226			0.281	2620.3		
		5	0.234	123.809			0.271	2532.9		
			0.239	125.131			0.275	2562.05		
			0.241	121.106			0.253	2358.1		
		6	0.247	112.273			0.246	2289.95		
			0.247	107.391			0.248	2314.4		
			0.259	109.746			0.256	2382		
		7	0.297	106.071			0.787	6536		
			0.32	107.744			0.921	7787.8		
			0.325	109.428			0.943	8010.6		
		8	0.342	157.604			0.876	7361		
			0.36	157.895			0.761	6308.25		
			0.353	154.149			0.921	7787.8		

		9	0.251	104.583			0.893	7519.05		
			0.265	108.163			0.924	7827.65		
			0.26	105.691			0.755	6258.5		
0	5	1	0.166	85.128	81.003	5.984	0.31	2425.95	3304.278	1541.866
			0.161	77.778			0.309	2408		
			0.157	71.69			0.337	2664.35		
		2	0.153	87.429			0.311	2435.2		
			0.136	74.317			0.309	2408		
			0.161	85.185			0.32	2501.6		
		3	0.168	88.889			0.32	2501.6		
			0.159	79.5			0.333	2651.2		
			0.155	76.733			0.334	2673.2		
		4	0.158	79.798			0.221	2096.25		
			0.166	79.808			0.207	1979.15		
			0.168	79.245			0.206	1955.25		
		5	0.16	84.656			0.243	2312.75		
			0.158	82.723			0.233	2202.15		
			0.167	83.92			0.24	2243.55		
		6	0.164	74.546			0.2	1898.2		
			0.182	79.13			0.201	1911.45		
			0.182	77.119			0.203	1938.7		
		7	0.243	86.786			0.56	4540		
			0.249	83.838			0.563	4559.9		
			0.256	86.195			0.542	4360.8		
		8	0.195	89.862			0.611	4986.75		
			0.204	89.474			0.597	4837.4		
			0.205	89.52			0.612	4996.7		
		9	0.171	71.25			0.802	6685.3		
			0.175	71.429			0.784	6506.1		
			0.175	71.138			0.788	6536		
2.5	5	1	0.175	89.744	96.677	5.276	0.365	2791.4	3547.331	1413.846
			0.191	92.271			0.379	2888.2		
			0.193	88.128			0.388	2965.65		
		2	0.187	106.857			0.307	2358.5		
			0.182	99.454			0.317	2449.1		
			0.183	96.825			0.307	2358.5		
		3	0.188	99.471			0.424	3224.3		
			0.197	98.5			0.411	3133.7		
			0.198	98.02			0.423	3217.35		
		4	0.199	100.505			0.241	2246.25		
			0.212	101.923			0.236	2211.85		
			0.211	99.528			0.238	2217.1		
		5	0.198	104.762			0.29	2707.7		
			0.206	107.853			0.269	2508.45		
			0.204	102.513			0.255	2382		
		6	0.202	91.818			0.247	2309.15		
			0.211	91.739			0.234	2188		
			0.204	86.441			0.226	2129.7		
		7	0.257	91.786			0.686	5612.65		
			0.275	92.593			0.673	5513.1		
			0.277	93.266			0.667	5463.35		
		8	0.21	96.775			0.647	5295.35		
			0.222	97.368			0.665	5433.45		
			0.221	96.507			0.65	5305.3		
		9	0.229	95.417			0.678	5562.9		
			0.233	95.102			0.684	5622.6		
			0.234	95.122			0.692	5682.35		
5	5	1	0.182	93.333	96.311	4.867	0.303	2339.15	2952.63	1173.996

			0.183	88.406			0.299	2300.4		
			0.22	100.457			0.301	2325.95		
		2	0.193	110.286			0.301	2325.95		
			0.166	90.7104			0.297	2294.2		
			0.193	102.116			0.289	2229.15		
		3	0.199	105.291			0.327	2507.2		
			0.2	100			0.329	2604		
			0.209	103.465			0.338	2681.45		
		4	0.193	97.475			0.202	1856.15		
			0.202	97.115			0.197	1807.2		
			0.199	93.868			0.19	1783.95		
		5	0.174	92.063			0.237	2211.85		
			0.19	99.476			0.237	2211.85		
			0.189	94.975			0.244	2270.7		
		6	0.212	96.364			0.181	1702		
			0.223	96.957			0.173	1609.35		
			0.225	95.339			0.168	1570.35		
		7	0.264	94.286			0.572	4629.55		
			0.27	90.909			0.576	4669.4		
			0.269	90.572			0.57	4609.7		
		8	0.205	94.47			0.567	4599.75		
			0.223	97.807			0.557	4530		
			0.216	94.323			0.524	4232.65		
		9	0.229	95.417			0.564	4569.85		
			0.226	92.245			0.567	4589.8		
			0.228	92.683			0.574	4659.45		
			0.235	120.513			0.383	2920.7		
		1	0.257	124.155			0.406	3108.15		
			0.255	116.438			0.388	2965.65		
		2	0.218	124.571			0.413	3153.05		
			0.232	126.776			0.399	3043		
			0.26	137.566			0.405	3101.2		
		3	0.227	120.106			0.371	2823.95		
			0.237	118.5			0.387	2946.3		
			0.235	116.337			0.387	2946.3		
		4	0.242	122.222			0.285	2649.5		
			0.241	115.865			0.258	2406.5		
			0.239	112.733			0.242	2256.15		
		5	0.214	113.228			0.262	2435.6		
10	5		0.227	118.848	114.572	11.856	0.242	2256.15	3692.774	1540.294
			0.223	112.06			0.217	2032.4		
		6	0.232	105.455			0.199	1857.6		
			0.242	105.217			0.237	2207.2		
			0.237	100.424			0.246	2294.6		
		7	0.268	95.7143			0.723	5950		
			0.288	96.97			0.727	5999.65		
			0.29	97.643			0.724	5959.85		
		8	0.281	129.493			0.751	6228.6		
			0.3	131.579			0.688	5652.45		
			0.3	131.004			0.689	5672.4		
		9	0.243	101.25			0.654	5343.85		
			0.24	97.959			0.692	5682.35		
			0.248	100.813			0.708	5811.75		

Statically Analysis

Cell viability

ANOVA

Cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	38043.411	7	5434.773	68.521	.000
Within Groups	16497.667	208	79.316		
Total	54541.077	215			

Cell viability

Duncan[a]

		Subset for alpha = 0.05			
Combbination	N	1	2	3	4
0 CGA + 5 Cur	27	81.0032			
5 CGA + 5 Cur	27		96.3114		
2.5 CGA + 5 Cur	27		96.6773		
0 CGA + 0 Cur	27		100.0000		
2.5 CGA + 0 Cur	27			109.7088	
5 CGA + 0 Cur	27			112.9012	
10 CGA + 5 Cur	27			114.5718	
10 CGA + 0 Cur	27				127.0810
Sig.		1.000	.153	.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 27.000.

Production of TNF-α

ANOVA

TNF-alpha

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30452.118	7	4350.303	54.053	.000
Within Groups	16740.248	208	80.482		
Total	47192.366	215			

TNF-alpha

Duncan[a]

		Subset for alpha = 0.05					
Combination	N	1	2	3	4	5	6
5 CGA + 5 Cur	27	76.0027					
0 CGA + 5 Cur	27		83.7358				
2.5 CGA + 5 Cur	27			91.3723			
10 CGA + 5 Cur	27			94.4210			
0 CGA + 0 Cur	27				100.0000		
5 CGA + 0 Cur	27					105.2731	
10 CGA + 0 Cur	27					108.3954	
2.5 CGA + 0 Cur	27						113.3373
Sig.		1.000	1.000	.213	1.000	.202	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 27.000.

Appendix 6

Raw and Statistically Analysed Data for qRT-PCR

I) Ct and ΔΔCt for inflammatory biomarkers

Replicate	Control		CGA		Curcumin		Curcumin + CGA	
	Ct	ΔΔCt	Ct	ΔΔCt	Ct	ΔΔCt	Ct	ΔΔCt
IL-6								
1	21.068	0.95	19.754	1.653	26.176	0.032	29.171	0.004
2	20.379	1.219	21.379	0.85	26.997	0.017	30.946	0.001
3	20.972	0.864	20.905	0.856	25.596	0.041	30.158	0.002
TNF-α								
1	15.707	0.88	15.202	0.987	17.122	0.42	18.257	0.143
2	15.181	1.109	15.527	1.355	17.114	0.363	18.753	0.124
3	15.355	1.025	15.381	0.738	17.047	0.403	18.669	0.107
IL-10								
1	21.458	0.916	21.248	0.899	25.35	0.081	29.3	0.004
2	21.277	1.117	21.365	1.205	26.027	0.049	28.181	0.01
3	21.152	0.978	21.107	0.914	24.726	0.104	29.097	0.005
NF-*k*B								
1	18.644	0.732	17.868	0.99	19.281	0.598	20.199	0.237
2	17.765	1.178	18.448	1.14	19.15	0.563	20.6	0.22
3	17.847	1.16	17.815	0.87	18.917	0.702	20.229	0.231
COX-2								
1	17.74	1.088	16.997	1.275	19.804	0.298	21.434	0.087
2	17.33	1.151	17.858	1.113	19.836	0.275	21.788	0.084
3	17.952	0.799	17.795	0.843	19.739	0.272	21.544	0.069
iNOS								
1	30.232	1.14	30.374	0.876	29.925	1.853	28.442	4.161
2	30.588	0.958	31.187	0.725	29.917	1.788	28.696	3.606
3	30.335	0.916	30.127	0.958	29.717	1.773	28.558	3.921
TLR-4								
1	22.778	1.099	22.583	0.845	24.19	0.479	24.858	0.241
2	22.626	1.045	23.235	0.889	24.153	0.415	24.833	0.277
3	22.732	0.871	22.492	0.863	23.592	0.54	24.474	0.321
I*k*B-β-kinase								
1	20.562	0.904	19.955	1.002	21.271	0.633	21.764	0.381
2	19.894	1.039	20.571	0.959	21.233	0.721	21.779	0.407
3	19.93	1.064	19.849	1.02	20.995	0.625	21.579	0.42
I*k*B-α								
1	17.304	1.126	16.881	1.1	17.044	1.543	17.881	0.731
2	17.054	0.969	18.55	0.507	17.018	1.744	17.901	0.778
3	17.206	0.916	17.01	0.951	16.913	1.378	17.73	0.787

Statically Analysis

IL-6

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.321	3	1.107	17.850	.001
Within Groups	.496	8	.062		
Total	3.818	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
Curcumin + CGA	3	.00212415	
Curcumin	3	.02982505	
Control	3		1.01086146
CGA	3		1.11952599
Sig.		.895	.608

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TNF-a

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.823	3	.608	21.915	.000
Within Groups	.222	8	.028		
Total	2.045	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
Curcumin + CGA	3	.12471638	
Curcumin	3	.39498847	
Control	3		1.00454827
CGA	3		1.02648059
Sig.		.082	.876

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

IL-10

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.788	3	.929	90.283	.000
Within Groups	.082	8	.010		
Total	2.870	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
Curcumin + CGA	3	.00620845	
Curcumin	3	.07777789	
Control	3		1.00346090
CGA	3		1.00587621
Sig.		.413	.977

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

NF-*k*B

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.262	3	.421	19.289	.001
Within Groups	.175	8	.022		
Total	1.437	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05		
Treatment	N	1	2	3
Curcumin + CGA	3	.22928832		
Curcumin	3		.62099485	
CGA	3			.99966885
Control	3			1.02322541
Sig.		1.000	1.000	.850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

COX-2

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.307	3	.769	37.024	.000
Within Groups	.166	8	.021		
Total	2.473	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
Curcumin + CGA	3	.07957984	
Curcumin	3	.28138599	
Control	3		1.01246141
CGA	3		1.07662119
Sig.		.125	.600

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

iNOS

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.669	3	5.890	219.832	.000
Within Groups	.214	8	.027		
Total	17.883	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05		
Treatment	N	1	2	3
CGA	3	.85305914		
Control	3	1.00451691		
Curcumin	3		1.80469533	
Curcumin + CGA	3			3.89576234
Sig.		.290	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TLR-4

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.017	3	.339	67.119	.000
Within Groups	.040	8	.005		
Total	1.058	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05			
Treatment	N	1	2	3	4
Curcumin + CGA	3	.27952114			
Curcumin	3		.47783552		
CGA	3			.86562709	
Control	3				1.00490946
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

I*k*B-β-kinase

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.754	3	.251	86.176	.000
Within Groups	.023	8	.003		
Total	.778	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05		
Treatment	N	1	2	3
Curcumin + CGA	3	.40232226		
Curcumin	3		.65963791	
CGA	3			.99375968
Control	3			1.00255011
Sig.		1.000	1.000	.847

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

I*k*B-α

ANOVA

ΔΔCt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.131	3	.377	10.648	.004
Within Groups	.283	8	.035		
Total	1.414	11			

ΔΔCt

Duncan[a]

		Subset for alpha = 0.05	
Treatment	N	1	2
Curcumin + CGA	3	.76561532	
CGA	3	.85230404	
Control	3	1.00386433	
Curcumin	3		1.55501043
Sig.		.175	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 7

Standard Curve for Chlorogenic Acid and Curcumin

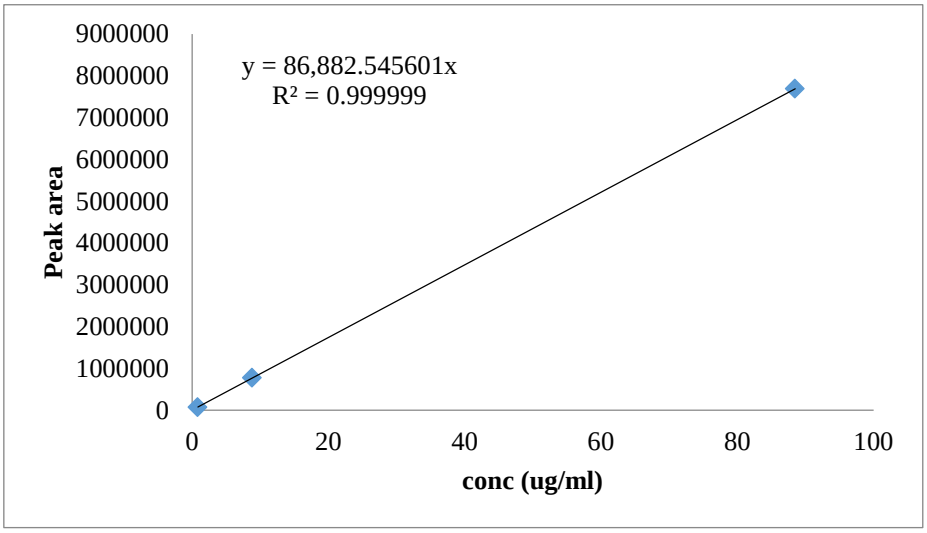


Figure A7.1: Standard curve for chlorogenic acid

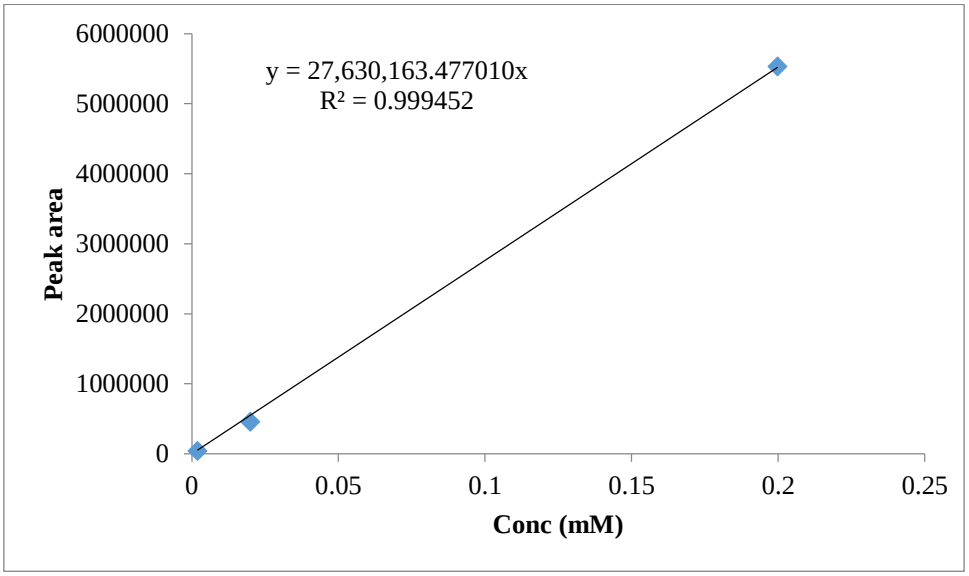


Figure A7.2: Standard curve for curcumin

Appendix 8

Raw and Statistically Analysed Data for Product Development

I) Effect of curcumin and CGA on fermentation process

Sample	Incubation time (h)	Replicate	pH	TA	LB count	ST count	L*	a*	b*
Control	0	1	5.84	0.180	6.74	6.93	96.71	0.39	1.8
		2	5.83	0.176	6.59	6.87	96.66	0.34	1.79
		3	5.89	0.189	6.81	6.96	96.98	0.4	1.81
	2	1	5.67	0.212	6.89	8.4	96.82	0.47	2.11
		2	5.68	0.212	7.01	8.53	91.68	0.44	1.75
		3	5.72	0.207	6.92	8.69	94.25	0.44	1.9
	4	1	5.1	0.338	7.19	9.02	97.35	-0.03	2.44
		2	5.35	0.347	7.21	9.3	95.41	0.01	2.46
		3	5.35	0.333	7.09	9.15	95.42	0.01	2.48
	6	1	4.59	0.549	7.25	9.8	97.67	-0.26	3.1
		2	4.56	0.536	7.08	9.59	97.63	-0.25	3.1
		3	4.64	0.522	7.29	9.57	98.2	-0.28	3.3
	8	1	4.45	0.639	7.45	9.8	97.84	-0.37	3.26
		2	4.41	0.626	7.39	9.89	97.74	-0.42	3.07
		3	4.33	0.648	7.47	9.73	98.3	-0.43	3.31
Only Curcumin	0	1	5.83	0.176	6.75	6.97	93.91	-3.1	18.58
		2	5.84	0.180	6.81	6.79	94.35	-3.27	18.55
		3	5.84	0.180	6.84	6.95	93.99	-3.28	18.56
	2	1	5.68	0.203	6.87	8.2	94.62	-5.82	24.89
		2	5.68	0.212	6.99	8.68	90.51	-5.72	23.36
		3	5.7	0.225	6.98	8.34	90.88	-5.8	24.5
	4	1	4.98	0.369	7.14	8.99	94.88	-7.59	27.62
		2	5.3	0.342	7.21	9.18	94.84	-7.7	27.69
		3	5.25	0.374	7.23	9.32	94.85	-7.9	27.7
	6	1	4.56	0.554	7.3	9.55	95.6	-8.33	29.52
		2	4.55	0.540	7.25	9.74	95.26	-8.37	29.29
		3	4.7	0.536	7.23	9.82	96.5	-8.5	30.2
	8	1	4.28	0.621	7.41	9.84	95.51	-8.97	29.74
		2	4.38	0.621	7.3	9.76	95.59	-8.8	30.26
		3	4.4	0.648	7.37	9.9	96.2	-8.6	29.5
Only CGA	0	1	5.66	0.207	6.64	6.88	71.5	3.37	15.13
		2	5.66	0.216	6.73	6.93	70.91	3.31	15.04
		3	5.8	0.212	6.79	6.83	70.6	3.35	15.15
	2	1	5.58	0.239	7.05	8.27	71.18	3.39	15.21
		2	5.61	0.239	7.03	8.55	71.75	3.41	15.43
		3	5.65	0.243	6.96	8.72	71.5	3.35	15.45
	4	1	5.2	0.324	7.05	9.05	71.09	2.77	14.97
		2	5.27	0.347	7.24	9.17	71.05	2.72	14.8
		3	5.26	0.351	7.19	9.35	71.1	2.75	14.95
	6	1	4.6	0.509	7.17	9.68	70.07	2.39	14.83
		2	4.76	0.513	7.19	9.79	71.97	2.69	15.53
		3	4.68	0.522	7.23	9.45	72.1	2.8	15.9
	8	1	4.41	0.617	7.3	10.02	72.44	2.68	15.87
		2	4.5	0.612	7.48	9.78	72.59	2.6	15.86
		3	4.4	0.621	7.51	9.74	72.9	2.71	15.1
Curcumin + CGA	0	1	5.64	0.207	6.6	7.02	69.68	2.42	15.21
		2	5.68	0.203	6.66	6.98	70.92	2.46	18.58

		3	5.65	0.207	6.85	6.79	70.95	2.49	18.36
	2	1	5.6	0.221	6.97	8.42	71.14	1.42	20.82
		2	5.61	0.239	6.99	8.29	70.61	1.39	20.45
		3	5.7	0.243	6.94	8.66	70.9	1.45	20.7
	4	1	5.2	0.347	7.25	9.16	70.43	0.24	21.34
		2	5.3	0.347	7.14	9.18	70.22	0.17	21.15
		3	5.25	0.342	7.17	9.22	70.4	0.18	21.3
	6	1	4.64	0.527	7.3	9.63	68.74	-0.44	21.07
		2	4.7	0.527	7.17	9.89	71.33	-0.47	22.29
		3	4.63	0.504	7.19	9.56	72.41	-0.51	22.8
	8	1	4.51	0.612	7.5	10.05	72.98	-0.26	24.04
		2	4.42	0.630	7.29	9.76	72.51	-0.45	23.71
		3	4.38	0.612	7.34	9.71	72.1	-0.49	23.89

Statistical Analysis
Control sample
pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.019	4	1.255	224.598	.000
Within Groups	.056	10	.006		
Total	5.075	14			

pH

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
8 h	3	4.39667				
6 h	3		4.59667			
4 h	3			5.26667		
2 h	3				5.69000	
0 h	3					5.85333
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.481	4	.120	1475.438	.000
Within Groups	.001	10	.000		
Total	.482	14			

TA

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
0 h	3	.18167				
2 h	3		.21033			
4 h	3			.33933		
6 h	3				.53567	
8 h	3					.63767
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.911	4	.228	32.676	.000
Within Groups	.070	10	.007		
Total	.980	14			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.71333			
2 h	3		6.94000		
4 h	3			7.16333	
6 h	3			7.20667	
8 h	3				7.43667
Sig.		1.000	1.000	.539	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.409	4	4.102	313.145	.000
Within Groups	.131	10	.013		
Total	16.540	14			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.92000			
2 h	3		8.54000		
4 h	3			9.15667	
6 h	3				9.65333
8 h	3				9.80667
Sig.		1.000	1.000	1.000	.132

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	27.648	4	6.912	4.281	.028
Within Groups	16.146	10	1.615		
Total	43.794	14			

L*

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
2 h	3	94.25000	
4 h	3	96.06000	96.06000
0 h	3		96.78333
6 h	3		97.83333
8 h	3		97.96000
Sig.		.112	.118

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.723	4	.431	687.457	.000
Within Groups	.006	10	.001		
Total	1.729	14			

a*

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
8 h	3	-.40667				
6 h	3		-.26333			
4 h	3			-.00333		
0 h	3				.37667	
2 h	3					.45000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.342	4	1.335	106.720	.000
Within Groups	.125	10	.013		
Total	5.467	14			

b*

Duncan[a]

		Subset for alpha = 0.05		
Time	N	1	2	3
0 h	3	1.80000		
2 h	3	1.92000		
4 h	3		2.46000	
6 h	3			3.16667
8 h	3			3.21333
Sig.		.218	1.000	.620

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Only curcumin sample

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.076	4	1.269	154.883	.000
Within Groups	.082	10	.008		
Total	5.158	14			

pH

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
8 h	3	4.35333			
6 h	3		4.60333		
4 h	3			5.17667	
2 h	3				5.68667
0 h	3				5.83667
Sig.		1.000	1.000	1.000	.070

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.473	4	.118	781.803	.000
Within Groups	.002	10	.000		
Total	.475	14			

TA

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
0 h	3	.17867				
2 h	3		.21333			
4 h	3			.36167		
6 h	3				.54333	
8 h	3					.63000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.644	4	.161	61.149	.000
Within Groups	.026	10	.003		
Total	.670	14			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.80000			
2 h	3		6.94667		
4 h	3			7.19333	
6 h	3			7.26000	
8 h	3				7.36000
Sig.		1.000	1.000	.143	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.304	4	4.326	176.903	.000
Within Groups	.245	10	.024		
Total	17.548	14			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.90333			
2 h	3		8.40667		
4 h	3			9.16333	
6 h	3				9.70333
8 h	3				9.83333
Sig.		1.000	1.000	1.000	.333

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.382	4	7.346	6.357	.008
Within Groups	11.556	10	1.156		
Total	40.938	14			

L*

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
2 h	3	92.00333	
0 h	3		94.08333
4 h	3		94.85667
8 h	3		95.76667
6 h	3		95.78667
Sig.		1.000	.100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	63.793	4	15.948	997.595	.000
Within Groups	.160	10	.016		
Total	63.953	14			

a*

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
8 h	3	-8.79000				
6 h	3		-8.40000			
4 h	3			-7.73000		
2 h	3				-5.78000	
0 h	3					-3.21667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	267.956	4	66.989	331.936	.000
Within Groups	2.018	10	.202		
Total	269.974	14			

b*

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	18.56333			
2 h	3		24.25000		
4 h	3			27.67000	
6 h	3				29.67000
8 h	3				29.83333
Sig.		1.000	1.000	1.000	.666

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Only CGA sample

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.786	4	.947	253.987	.000
Within Groups	.037	10	.004		
Total	3.823	14			

pH

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
8 h	3	4.43667			
6 h	3		4.68000		
4 h	3			5.24333	
2 h	3				5.61333
0 h	3				5.70667
Sig.		1.000	1.000	1.000	.091

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.370	4	.093	1529.170	.000
Within Groups	.001	10	.000		
Total	.371	14			

TA

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
0 h	3	.21167				
2 h	3		.24033			
4 h	3			.34067		
6 h	3				.51467	
8 h	3					.61667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.821	4	.205	32.615	.000
Within Groups	.063	10	.006		
Total	.884	14			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.72000			
2 h	3		7.01333		
4 h	3			7.16000	
6 h	3			7.19667	
8 h	3				7.43000
Sig.		1.000	1.000	.584	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.162	4	4.291	165.066	.000
Within Groups	.260	10	.026		
Total	17.422	14			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.88000			
2 h	3		8.51333		
4 h	3			9.19000	
6 h	3				9.64000
8 h	3				9.84667
Sig.		1.000	1.000	1.000	.148

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.232	4	1.308	3.993	.034
Within Groups	3.275	10	.328		
Total	8.507	14			

L*

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
0 h	3	71.00333	
4 h	3	71.08000	
6 h	3	71.38000	
2 h	3	71.47667	
8 h	3		72.64333
Sig.		.367	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.712	4	.428	42.143	.000
Within Groups	.102	10	.010		
Total	1.813	14			

a*

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
6 h	3	2.62667	
8 h	3	2.66333	
4 h	3	2.74667	
0 h	3		3.34333
2 h	3		3.38333
Sig.		.194	.637

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.915	4	.229	2.198	.143
Within Groups	1.040	10	.104		
Total	1.955	14			

b*

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
4 h	3	14.90667	
0 h	3	15.10667	15.10667
2 h	3	15.36333	15.36333
6 h	3	15.42000	15.42000
8 h	3		15.61000
Sig.		.099	.105

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Curcumin + CGA sample

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.760	4	.940	397.144	.000
Within Groups	.024	10	.002		
Total	3.783	14			

pH

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
8 h	3	4.43667			
6 h	3		4.65667		
4 h	3			5.25000	
2 h	3				5.63667
0 h	3				5.65667
Sig.		1.000	1.000	1.000	.626

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.386	4	.097	1109.216	.000
Within Groups	.001	10	.000		
Total	.387	14			

TA

Duncan[a]

		Subset for alpha = 0.05				
Time	N	1	2	3	4	5
0 h	3	.20567				
2 h	3		.23433			
4 h	3			.34533		
6 h	3				.51933	
8 h	3					.61800
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.819	4	.205	27.074	.000
Within Groups	.076	10	.008		
Total	.895	14			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.70333			
2 h	3		6.96667		
4 h	3			7.18667	
6 h	3			7.22000	7.22000
8 h	3				7.37667
Sig.		1.000	1.000	.649	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16.925	4	4.231	183.648	.000
Within Groups	.230	10	.023		
Total	17.155	14			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
0 h	3	6.93000			
2 h	3		8.45667		
4 h	3			9.18667	
6 h	3				9.69333
8 h	3				9.84000
Sig.		1.000	1.000	1.000	.264

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.115	4	2.279	2.613	.099
Within Groups	8.719	10	.872		
Total	17.835	14			

L*

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
4 h	3	70.35000	
0 h	3	70.51667	
6 h	3	70.82667	70.82667
2 h	3	70.88333	70.88333
8 h	3		72.53000
Sig.		.528	.058

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.279	4	4.820	1210.992	.000
Within Groups	.040	10	.004		
Total	19.319	14			

a*

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
6 h	3	-.47333			
8 h	3	-.40000			
4 h	3		.19667		
2 h	3			1.42000	
0 h	3				2.45667
Sig.		.185	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	67.981	4	16.995	19.235	.000
Within Groups	8.836	10	.884		
Total	76.816	14			

b*

Duncan[a]

		Subset for alpha = 0.05		
Time	N	1	2	3
0 h	3	17.38333		
2 h	3		20.65667	
4 h	3		21.26333	
6 h	3		22.05333	
8 h	3			23.88000
Sig.		1.000	.113	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Between samples at same time point

At t=0

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.084	3	.028	13.975	.002
Within Groups	.016	8	.002		
Total	.100	11			

pH

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Curcumin + CGA	3	5.65667	
Only CGA	3	5.70667	
Only curcumin	3		5.83667
Control	3		5.85333
Sig.		.209	.661

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	3	.001	44.323	.000
Within Groups	.000	8	.000		
Total	.003	11			

TA

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Only curcumin	3	.17867	
Control	3	.18167	
Curcumin + CGA	3		.20567
Only CGA	3		.21167
Sig.		.422	.129

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.018	3	.006	.632	.615
Within Groups	.075	8	.009		
Total	.093	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Curcumin + CGA	3	6.70333
Control	3	6.71333
Only CGA	3	6.72000
Only curcumin	3	6.80000
Sig.		.282

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	3	.001	.195	.897
Within Groups	.059	8	.007		
Total	.063	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only CGA	3	6.88000
Only curcumin	3	6.90333
Control	3	6.92000
Curcumin + CGA	3	6.93000
Sig.		.519

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1837.610	3	612.537	2992.242	.000
Within Groups	1.638	8	.205		
Total	1839.248	11			

L*

Duncan[a]

		Subset for alpha = 0.05		
Sample	N	1	2	3
Curcumin + CGA	3	70.51667		
Only CGA	3	71.00333		
Only curcumin	3		94.08333	
Control	3			96.78333
Sig.		.224	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.535	3	25.512	7596.480	.000
Within Groups	.027	8	.003		
Total	76.561	11			

a*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Only curcumin	3	-3.21667			
Control	3		.37667		
Curcumin + CGA	3			2.45667	
Only CGA	3				3.34333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	539.581	3	179.860	202.181	.000
Within Groups	7.117	8	.890		
Total	546.698	11			

b*

Duncan[a]

		Subset for alpha = 0.05		
Sample	N	1	2	3
Control	3	1.80000		
Only CGA	3		15.10667	
Curcumin + CGA	3			17.38333
Only curcumin	3			18.56333
Sig.		1.000	1.000	.164

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

At t=2 h

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.013	3	.004	3.364	.076
Within Groups	.010	8	.001		
Total	.023	11			

pH

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Only CGA	3	5.61333	
Curcumin + CGA	3	5.63667	5.63667
Only curcumin	3		5.68667
Control	3		5.69000
Sig.		.447	.117

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	9.845	.005
Within Groups	.001	8	.000		
Total	.003	11			

TA

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Control	3	.21033	
Only curcumin	3	.21333	
Curcumin + CGA	3		.23433
Only CGA	3		.24033
Sig.		.668	.400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	3	.003	1.175	.378
Within Groups	.022	8	.003		
Total	.032	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Control	3	6.94000
Only curcumin	3	6.94667
Curcumin + CGA	3	6.96667
Only CGA	3	7.01333
Sig.		.149

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.032	3	.011	.252	.858
Within Groups	.338	8	.042		
Total	.370	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only curcumin	3	8.40667
Curcumin + CGA	3	8.45667
Only CGA	3	8.51333
Control	3	8.54000
Sig.		.475

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1453.068	3	484.356	162.448	.000
Within Groups	23.853	8	2.982		
Total	1476.921	11			

L*

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Curcumin + CGA	3	70.88333	
Only CGA	3	71.47667	
Only curcumin	3		92.00333
Control	3		94.25000
Sig.		.685	.150

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	141.015	3	47.005	38112.081	.000
Within Groups	.010	8	.001		
Total	141.025	11			

a*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Only curcumin	3	-5.78000			
Control	3		.45000		
Curcumin + CGA	3			1.42000	
Only CGA	3				3.38333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	862.739	3	287.580	1601.744	.000
Within Groups	1.436	8	.180		
Total	864.176	11			

b*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Control	3	1.92000			
Only CGA	3		15.36333		
Curcumin + CGA	3			20.65667	
Only curcumin	3				24.25000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

At t=4 h

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.014	3	.005	.345	.794
Within Groups	.109	8	.014		
Total	.123	11			

pH

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only curcumin	3	5.17667
Only CGA	3	5.24333
Curcumin + CGA	3	5.25000
Control	3	5.26667
Sig.		.399

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	3	.000	2.232	.162
Within Groups	.001	8	.000		
Total	.002	11			

TA

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Control	3	.33933
Only CGA	3	.34067
Curcumin + CGA	3	.34533
Only curcumin	3	.36167
Sig.		.063

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	.172	.912
Within Groups	.039	8	.005		
Total	.041	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only CGA	3	7.16000
Control	3	7.16333
Curcumin + CGA	3	7.18667
Only curcumin	3	7.19333
Sig.		.594

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S.thermophilus

ANOVA

S.thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	.047	.986
Within Groups	.142	8	.018		
Total	.144	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Control	3	9.15667
Only curcumin	3	9.16333
Curcumin + CGA	3	9.18667
Only CGA	3	9.19000
Sig.		.779

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1839.669	3	613.223	1943.449	.000
Within Groups	2.524	8	.316		
Total	1842.193	11			

L*

Duncan[a]

		Subset for alpha = 0.05		
Sample	N	1	2	3
Curcumin + CGA	3	70.35000		
Only CGA	3	71.08000		
Only curcumin	3		94.85667	
Control	3			96.06000
Sig.		.150	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	184.799	3	61.600	9025.603	.000
Within Groups	.055	8	.007		
Total	184.854	11			

a*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Only curcumin	3	-7.73000			
Control	3		-.00333		
Curcumin + CGA	3			.19667	
Only CGA	3				2.74667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1041.288	3	347.096	66218.643	.000
Within Groups	.042	8	.005		
Total	1041.330	11			

b*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Control	3	2.46000			
Only CGA	3		14.90667		
Curcumin + CGA	3			21.26333	
Only curcumin	3				27.67000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

At t=6 h

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	3	.005	1.203	.369
Within Groups	.033	8	.004		
Total	.048	11			

pH

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Control	3	4.59667
Only curcumin	3	4.60333
Curcumin + CGA	3	4.65667
Only CGA	3	4.68000
Sig.		.173

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	4.440	.041
Within Groups	.001	8	.000		
Total	.003	11			

TA

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Only CGA	3	.51467	
Curcumin + CGA	3	.51933	
Control	3	.53567	.53567
Only curcumin	3		.54333
Sig.		.057	.422

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	3	.002	.474	.709
Within Groups	.039	8	.005		
Total	.046	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only CGA	3	7.19667
Control	3	7.20667
Curcumin + CGA	3	7.22000
Only curcumin	3	7.26000
Sig.		.326

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.008	3	.003	.117	.947
Within Groups	.192	8	.024		
Total	.200	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only CGA	3	9.64000
Control	3	9.65333
Curcumin + CGA	3	9.69333
Only curcumin	3	9.70333
Sig.		.648

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1989.241	3	663.080	494.808	.000
Within Groups	10.721	8	1.340		
Total	1999.961	11			

L*

Duncan[a]

		Subset for alpha = 0.05	
Sample	N	1	2
Curcumin + CGA	3	70.82667	
Only CGA	3	71.38000	
Only curcumin	3		95.78667
Control	3		97.83333
Sig.		.574	.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	201.473	3	67.158	4938.069	.000
Within Groups	.109	8	.014		
Total	201.582	11			

a*

Duncan[a]

		Subset for alpha = 0.05		
Sample	N	1	2	3
Only curcumin	3	-8.40000		
Curcumin + CGA	3		-.47333	
Control	3		-.26333	
Only CGA	3			2.62667
Sig.		1.000	.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1135.766	3	378.589	1144.839	.000
Within Groups	2.646	8	.331		
Total	1138.411	11			

b*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Control	3	3.16667			
Only CGA	3		15.42000		
Curcumin + CGA	3			22.05333	
Only curcumin	3				29.67000
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

At t=8 h

pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.014	3	.005	1.237	.358
Within Groups	.031	8	.004		
Total	.045	11			

pH

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only curcumin	3	4.35333
Control	3	4.39667
Only CGA	3	4.43667
Curcumin + CGA	3	4.43667
Sig.		.160

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	3	.000	2.451	.138
Within Groups	.001	8	.000		
Total	.002	11			

TA

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only CGA	3	.61667
Curcumin + CGA	3	.61800
Only curcumin	3	.63000
Control	3	.63767
Sig.		.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.013	3	.004	.589	.639
Within Groups	.060	8	.007		
Total	.073	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Only curcumin	3	7.36000
Curcumin + CGA	3	7.37667
Only CGA	3	7.43000
Control	3	7.43667
Sig.		.335

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	3	.001	.054	.982
Within Groups	.136	8	.017		
Total	.139	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05
Sample	N	1
Control	3	9.80667
Only curcumin	3	9.83333
Curcumin + CGA	3	9.84000
Only CGA	3	9.84667
Sig.		.732

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1775.305	3	591.768	4925.588	.000
Within Groups	.961	8	.120		
Total	1776.266	11			

L*

Duncan[a]

		Subset for alpha = 0.05		
Sample	N	1	2	3
Curcumin + CGA	3	72.53000		
Only CGA	3	72.64333		
Only curcumin	3		95.76667	
Control	3			97.96000
Sig.		.699	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	217.995	3	72.665	5416.028	.000
Within Groups	.107	8	.013		
Total	218.102	11			

a*

Duncan[a]

		Subset for alpha = 0.05		
Sample	N	1	2	3
Only curcumin	3	-8.79000		
Control	3		-.40667	
Curcumin + CGA	3		-.40000	
Only CGA	3			2.66333
Sig.		1.000	.946	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b *

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1196.663	3	398.888	4097.811	.000
Within Groups	.779	8	.097		
Total	1197.442	11			

b*

Duncan[a]

		Subset for alpha = 0.05			
Sample	N	1	2	3	4
Control	3	3.21333			
Only CGA	3		15.61000		
Curcumin + CGA	3			23.88000	
Only curcumin	3				29.83333
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

II) Physico-chemical and microbial permeates for coconut yogurt during storage for 15 days at 4ºC

Sample	Day	Replicate	pH	TA	LB count (cfu/g)	ST count (cfu/g)	L*	a*	b*	Syneresis (%)	Firmness (g)
Control	1	1	4.42	0.644	7.15	9.52	97.46	-0.29	3.8	0.24	34.9
		2	4.35	0.657	7.25	9.8	97.84	-0.35	4.1	0.38	32.7
		3	4.39	0.648	7.27	9.12	96.05	-0.41	2.81	0.17	33.8
	5	1	4.34	0.675	7.09	9.43	96.38	-0.38	2.2	0.15	34.97
		2	4.32	0.684	6.65	9.65	97.94	-0.52	4.26	0.43	35.4
		3	4.37	0.666	6.78	8.93	97.6	-0.25	4.06	0.25	35.48
	10	1	4.33	0.684	6.78	9.45	97.69	-0.39	3.35	0.43	39.75
		2	4.32	0.689	7.07	9.07	96.94	-0.38	3.96	0.19	39.91
		3	4.32	0.689	6.46	8.75	98.24	-0.47	4.75	0.21	39.85
	15	1	4.31	0.684	6.42	9.2	95.96	-0.4	4.13	0.37	39.23
		2	4.33	0.675	6.95	9.01	98.82	-0.51	5.56	0.19	38.7
		3	4.29	0.689	6.74	8.49	98.4	-0.38	4.17	0.26	38.34
Curcumin + CGA	1	1	4.43	0.621	7.01	9.37	70.96	-0.34	20.24	0.34	29.18
		2	4.38	0.630	7.43	9.33	72.3	-0.45	20.16	0.11	28.85
		3	4.42	0.626	7.07	9.78	72.57	-0.61	23.88	0.23	29.52
	5	1	4.39	0.657	7.09	9.56	73.51	-0.48	24.63	0.27	32.63
		2	4.35	0.648	6.43	9.07	72.18	-0.59	22.83	0.21	32.1
		3	4.4	0.662	6.91	9.02	72.62	-0.36	23.72	0.17	32.37
	10	1	4.38	0.662	6.74	9.05	70.98	-0.44	24.97	0.12	37.11
		2	4.33	0.662	7.15	8.87	75.41	-0.47	26.2	0.31	36.49
		3	4.36	0.666	6.68	8.94	73.2	-0.57	26.64	0.24	36.85
	15	1	4.3	0.657	7.29	9.07	72.31	-0.68	27.43	0.3	35.87
		2	4.36	0.666	6.53	9	72.98	-0.61	25.98	0.14	36.23
		3	4.34	0.657	6.58	8.65	74.4	-0.74	28.48	0.29	36.1

Statistical Analysis
Control sample
pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	3	.003	5.850	.020
Within Groups	.005	8	.001		
Total	.015	11			

pH

Duncan[a]

Time	N	Subset for alpha = 0.05	
		1	2
Day 15	3	4.31000	
Day 10	3	4.32333	
Day 5	3	4.34333	4.34333
Day 1	3		4.38667
Sig.		.141	.058

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	3	.001	18.382	.001
Within Groups	.000	8	.000		
Total	.003	11			

TA

Duncan[a]

Time	N	Subset for alpha = 0.05	
		1	2
Day 1	3	.64967	
Day 5	3		.67500
Day 15	3		.68267
Day 10	3		.68733
Sig.		1.000	.065

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L. bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.488	3	.163	2.964	.097
Within Groups	.439	8	.055		
Total	.927	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
Day 15	3	6.70333	
Day 10	3	6.77000	6.77000
Day 5	3	6.84000	6.84000
Day 1	3		7.22333
Sig.		.512	.053

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.597	3	.199	1.560	.273
Within Groups	1.022	8	.128		
Total	1.619	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05
Time	N	1
Day 15	3	8.90000
Day 10	3	9.09000
Day 5	3	9.33667
Day 1	3	9.48000
Sig.		.099

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L *

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.714	3	.238	.218	.881
Within Groups	8.746	8	1.093		
Total	9.460	11			

L*

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
Day 1	3	97.11667
Day 5	3	97.30667
Day 10	3	97.62333
Day 15	3	97.72667
Sig.		.519

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a *

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.011	3	.004	.510	.686
Within Groups	.058	8	.007		
Total	.069	11			

a*

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
Day 15	3	-.43000
Day 10	3	-.41333
Day 5	3	-.38333
Day 1	3	-.35000
Sig.		.311

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.379	3	.793	1.093	.406
Within Groups	5.804	8	.726		
Total	8.183	11			

b*

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
Day 5	3	3.50667
Day 1	3	3.57000
Day 10	3	4.02000
Day 15	3	4.62000
Sig.		.171

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Syneresis

ANOVA

Syneresis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.008	.999
Within Groups	.115	8	.014		
Total	.115	11			

Syneresis

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
Day 1	3	.26333
Day 15	3	.27333
Day 5	3	.27667
Day 10	3	.27667
Sig.		.901

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Firmness

ANOVA

Firmness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72.880	3	24.293	65.121	.000
Within Groups	2.984	8	.373		
Total	75.864	11			

Firmness

Duncan[a]

		Subset for alpha = 0.05		
Time	N	1	2	3
Day 1	3	33.80000		
Day 5	3		35.28333	
Day 15	3			38.75667
Day 10	3			39.83667
Sig.		1.000	1.000	.062

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Curcumin + CGA sample
pH

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	3	.003	4.345	.043
Within Groups	.006	8	.001		
Total	.016	11			

pH

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
Day 15	3	4.33333	
Day 10	3	4.35667	4.35667
Day 5	3	4.38000	4.38000
Day 1	3		4.41000
Sig.		.079	.050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TA

ANOVA

TA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	3	.001	34.818	.000
Within Groups	.000	8	.000		
Total	.003	11			

TA

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
Day 1	3	.62567	
Day 5	3		.65567
Day 15	3		.66000
Day 10	3		.66333
Sig.		1.000	.114

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L. bulgaricus

ANOVA

L.bulgaricus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.278	3	.093	.894	.485
Within Groups	.828	8	.104		
Total	1.106	11			

L. bulgaricus

Duncan[a]

		Subset for alpha = 0.05
Time	N	1
Day 15	3	6.80000
Day 5	3	6.81000
Day 10	3	6.85667
Day 1	3	7.17000
Sig.		.222

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

S. thermophilus

ANOVA

S. thermophilus

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.660	3	.220	4.192	.047
Within Groups	.420	8	.052		
Total	1.080	11			

S. thermophilus

Duncan[a]

		Subset for alpha = 0.05	
Time	N	1	2
Day 15	3	8.90667	
Day 10	3	8.95333	
Day 5	3	9.21667	9.21667
Day 1	3		9.49333
Sig.		.151	.177

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

L*

ANOVA

L*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.228	3	1.076	.594	.636
Within Groups	14.495	8	1.812		
Total	17.724	11			

L*

Duncan[a]

Time	N	Subset for alpha = 0.05
		1
Day 1	3	71.94333
Day 5	3	72.77000
Day 10	3	73.19667
Day 15	3	73.23000
Sig.		.302

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

a*

ANOVA

a*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.089	3	.030	2.931	.100
Within Groups	.081	8	.010		
Total	.170	11			

a*

Duncan[a]

Time	N	Subset for alpha = 0.05	
		1	2
Day 15	3	-.67667	
Day 10	3	-.49333	-.49333
Day 5	3		-.47667
Day 1	3		-.46667
Sig.		.056	.763

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

b*

ANOVA

b*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.674	3	19.891	10.400	.004
Within Groups	15.302	8	1.913		
Total	74.976	11			

b*

Duncan[a]

		Subset for alpha = 0.05		
Time	N	1	2	3
Day 1	3	21.42667		
Day 5	3	23.72667	23.72667	
Day 10	3		25.93667	25.93667
Day 15	3			27.29667
Sig.		.076	.086	.263

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Syneresis

ANOVA

Syneresis

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	3	.000	.047	.986
Within Groups	.066	8	.008		
Total	.067	11			

Syneresis

Duncan[a]

		Subset for alpha = 0.05
Time	N	1
Day 5	3	.21667
Day 10	3	.22333
Day 1	3	.22667
Day 15	3	.24333
Sig.		.743

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Firmness

ANOVA

Firmness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	112.377	3	37.459	479.273	.000
Within Groups	.625	8	.078		
Total	113.003	11			

Firmness

Duncan[a]

		Subset for alpha = 0.05			
Time	N	1	2	3	4
Day 1	3	29.18333			
Day 5	3		32.36667		
Day 15	3			36.06667	
Day 10	3				36.81667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

III) HPLC analysis for quantification of curcumin and CGA in yogurt samples during storage

Compound	Day	Peak Area	Average retention (%)
Curcumin	1	312904	100
		284076	
		315794	
		294432	
	5	198415	66.607±1.491
		190315	
		179521	
		227007	
	10	172761	68.728±15.258
		146442	
		170311	
		331065	
	15	196378	63.310±3.199
		200669	
		192621	
		166225	
CGA	1	6724861	100
		5942325	
		725970	
		597692	
	5	5845173	90.225±5.467
		6276379	
		495009	
		626897	
	10	5968790	90.589±2.622
		5838409	
		584672	
		579721	
	15	5547896	84.810±3.173
		5597119	
		555834	
		524758	

Statistical Analysis
Curcumin

ANOVA

Curcumin

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1741.978	3	580.659	4.735	.084
Within Groups	490.533	4	122.633		
Total	2232.511	7			

Curcumin

Duncan[a]

		Subset for alpha = 0.05	
Day	N	1	2
Day 15	2	63.3095	
Day 5	2	66.6070	
Day 10	2	68.7280	
Day 1	2		100.0000
Sig.		.654	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

CGA

ANOVA

CGA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	238.849	3	79.616	3.400	.134
Within Groups	93.667	4	23.417		
Total	332.516	7			

CGA

Duncan[a]

		Subset for alpha = 0.05	
Day	N	1	2
Day 15	2	84.8100	
Day 5	2	90.2255	90.2255
Day 10	2	90.5895	90.5895
Day 1	2		100.0000
Sig.		.304	.118

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

IV) Sensory evaluation of yogurt samples during storage

Day	Parameters									
	Appearance		Mouthfeel		Texture		Flavour		Overall acceptance	
	A	B	A	B	A	B	A	B	A	B
1	6.6±1.43	6 ±1	6.6±0.66	6.3±1.01	6.7±0.64	6.4±0.8	6.7±1.62	6.7±1.27	6.9±0.54	6.5±0.81
5	6.4±1.28	6.1±1.38	7±1.48	6.7±0.64	7±1.27	6.5±1.02	6.9±1.22	6.3±0.9	6.7±1.1	6.1±0.83
10	6.1±1.58	6.2±1.47	6.2±1.08	6.7±0.64	6.7±0.78	7±0.45	6.4±1.36	6.6±1.69	6.4±1.11	6.7±1.62
15	6.2±1.47	6.1±1.22	6±1.41	6.5±1.12	6.4±1.36	6.5±0.5	6.6±1.5	6.6±1.11	6.4±1.36	6.2±0.87

Statistical Analysis
Control sample
Appearance

ANOVA

Appearance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.475	3	.492	.212	.887
Within Groups	83.300	96	2.314		
Total	84.775	99			

Appearance

Duncan[a]

		Subset for alpha = 0.05
Day	N	1
Day 10	25	6.1000
Day 15	25	6.2000
Day 5	25	6.4000
Day 1	25	6.6000
Sig.		.510

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Mouthfeel

ANOVA

Mouthfeel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.900	3	1.967	1.221	.316
Within Groups	58.000	96	1.611		
Total	63.900	99			

Mouthfeel

Duncan[a]

		Subset for alpha = 0.05
Day	N	1
Day 15	25	6.0000
Day 10	25	6.2000
Day 1	25	6.6000
Day 5	25	7.0000
Sig.		.116

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Texture

ANOVA

Texture

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.800	3	.600	.484	.695
Within Groups	44.600	96	1.239		
Total	46.400	99			

Texture

Duncan[a]

		Subset for alpha = 0.05
Day	N	1
Day 15	25	6.4000
Day 1	25	6.7000
Day 10	25	6.7000
Day 5	25	7.0000
Sig.		.280

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Flavour

ANOVA

Flavour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.300	3	.433	.191	.902
Within Groups	81.800	96	2.272		
Total	83.100	99			

Flavour

Duncan[a]

Day	N	Subset for alpha = 0.05
		1
Day 10	25	6.4000
Day 15	25	6.6000
Day 1	25	6.7000
Day 5	25	6.9000
Sig.		.506

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Overall acceptance

ANOVA

Overall acceptance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.800	3	.600	.472	.704
Within Groups	45.800	96	1.272		
Total	47.600	99			

Overall acceptance

Duncan[a]

Day	N	Subset for alpha = 0.05
		1
Day 10	25	6.4000
Day 15	25	6.4000
Day 5	25	6.7000
Day 1	25	6.9000
Sig.		.375

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Curcumin + CGA sample
Appearance

ANOVA

Appearance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.200	3	.067	.037	.990
Within Groups	65.400	96	1.817		
Total	65.600	99			

Appearance

Duncan[a]

Day	N	Subset for alpha = 0.05
		1
Day 1	25	6.0000
Day 5	25	6.1000
Day 15	25	6.1000
Day 10	25	6.2000
Sig.		.766

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Mouthfeel

ANOVA

Mouthfeel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.100	3	.367	.429	.734
Within Groups	30.800	96	.856		
Total	31.900	99			

Mouthfeel

Duncan[a]

Day	N	Subset for alpha = 0.05
		1
Day 1	25	6.3000
Day 15	25	6.5000
Day 5	25	6.7000
Day 10	25	6.7000
Sig.		.386

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Texture

ANOVA

Texture

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.200	3	.733	1.234	.312
Within Groups	21.400	96	.594		
Total	23.600	99			

Texture

Duncan[a]

		Subset for alpha = 0.05
Day	N	1
Day 1	25	6.4000
Day 5	25	6.5000
Day 15	25	6.5000
Day 10	25	7.0000
Sig.		.120

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Flavour

ANOVA

Flavour

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.900	3	.300	.166	.918
Within Groups	65.000	96	1.806		
Total	65.900	99			

Flavour

Duncan[a]

		Subset for alpha = 0.05
Day	N	1
Day 5	25	6.3000
Day 10	25	6.6000
Day 15	25	6.6000
Day 1	25	6.7000
Sig.		.551

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.

Overall acceptance

ANOVA

Overall acceptance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.275	3	.758	.580	.632
Within Groups	47.100	96	1.308		
Total	49.375	99			

Overall acceptance

Duncan[a]

		Subset for alpha = 0.05
Day	N	1
Day 5	25	6.1000
Day 15	25	6.2000
Day 1	25	6.5000
Day 10	25	6.7000
Sig.		.294

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 25.000.