FACIAL SKIN CONDITION, HEALTH AND PERCEPTION

IN MALAYSIAN CHINESE

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Abstract

Skin texture and colour play an important role in the judgment of apparent health (Fink, Grammer, & Thornhill, 2001; Jones, Little, Burt, & Perrett, 2004; Matts, Fink, Grammer, & Burquest, 2007; Samson, Fink, & Matts, 2010; Stephen, Coetzee, Law Smith, & Perrett, 2009a; Stephen, Coetzee, & Perrett, 2011), and have been linked to aspects of physiological health, including fitness, immunity and fertility (Armstrong & Welsman, 2001; Jones et al., 2015; Stephen et al., 2011).

The current thesis examines the contribution of skin condition to health perception in Malaysian Chinese. The thesis comprises six chapters. Chapter one offers a general introduction to the topic. It outlines key literature on health perception, and explains the research problem, the objectives and relevance of the studies conducted.

Chapter 2 consists of three studies which examine Malaysian Chinese participants' perception of apparent health. The three studies revealed the significance of both skin texture, and skin colour in forming health perceptions. Examining the sensitivity threshold of human vision to colour changes, Chapter 3 discovered that individuals tend to be more sensitive to changes in redness and yellowness than luminance; and this extra-sensitivity in chrominance is specific to the perception of human faces, and not non-face objects or colour patches.

Following the finding of preference for slightly yellower skin, Chapter 4 reported a supplementation study, whereby an increment in skin yellowness and redness was observed for participants who were prescribed the fruit and vegetable smoothie (and not for the control group). Using the face images obtained in the intervention study, the three perceptual studies in Chapter 5 were designed to examine the amount of carotenoid colouration that is needed to optimize healthy appearance of Malaysian Chinese faces. Too much colour change was not preferred and, in the last study, it seems that the appropriate amount of carotenoid colouration preferred is only one third of the amount observed in the intervention study.

Collectively, these nine studies deepen our knowledge of health perception, especially the importance of skin colour in determining perceived human facial health. Implications and suggestions for future research are presented in Chapter 6.

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CHAPTER 1: INTRODUCTION

This thesis investigates the importance of facial skin as a source of assessment of human health status. As suggested by the findings of recent studies in attractiveness, humans tend to prefer to mate with opposite individuals with attractive bodily and facial features (Buss & Schmitt, 1993; Buss, 1989; Confer, Perilloux, & Buss, 2010; Stein, 2005). These features are thought to serve as cues to the bearer's physical and reproductive health status (Rhodes, Chan, Zebrowitz, & Simmons, 2003; Rhodes, Zebrowitz, et al., 2001; Stephen, Law Smith, Stirrat, & Perrett, 2009; Tinlin et al., 2013). In the first part of the introduction, I will discuss the importance of health, particularly in relation to mate selection and reproduction. The second part of the Introduction will discuss cues to perceived health and their association with the actual physical health of the bearer. Of these cues, one important indicator of both attractiveness and subjective health ratings is skin condition – both its texture and its colour (Coetzee et al., 2012; Fink, Bunse, Matts, & D'Emiliano, 2012; Fink & Matts, 2008; Jones, Little, Burt, & Perrett, 2004; Samson, Fink, & Matts, 2010; Stephen, Coetzee, Law Smith, & Perrett, 2009a; Stephen, Coetzee, & Perrett, 2011). I will introduce the skin cues that have been found to be preferred in various samples from different populations, and the underlying reasons behind these preferences.

Our ability to see in colour has also been proposed to be selected so that we can pay attention to subtle changes in skin condition (Changizi, Zhang, & Shimojo, 2006). Literature regarding to our extra sensitivity in perceiving changes in skin chrominance will be presented in the third part of the Introduction. Increased skin yellowness was found to contribute to human attractiveness and perceived health (Lefevre, Ewbank, Calder, Hagen, & Perrett, 2013; Lefevre & Perrett, 2015; Stephen et al., 2011), and one way to ensure that our skin has its glowing yellowness is by getting sufficient amounts of fruit and vegetables in our diet (Alaluf, Heinrich, Stahl, Tronnier, & Wiseman, 2002; Coetzee & Perrett, 2014; Stahl, Heinrich, Jungmann, & Laar, 1998; Stephen et al., 2011). The last part of the Introduction discusses the importance of having sufficient carotenoids intake, as a mean not only to reduce the risk of future illness, but also improve one's physical appearance.

1.1 Attractiveness as a mechanism for mate selection

To reproduce, individuals of sexually reproducing species need to find a suitable mate which is healthy and fertile. Attractiveness is suggested to be one of the most important mechanisms of mate selection for both non-human animals (Amundsen & Forsgren, 2001) and humans (Kurzban & Weeden, 2005). Individuals which are physically more attractive have a better chance of securing a mate, and have more and better mate options to choose from (Gangestad & Scheyd, 2005). It was suggested that the main reason behind preferring an attractive mate is because physical attractiveness reflect

fitness and health condition, including the fertility status of an individual (Henderson, 2003; Rhodes, Chan, Zebrowitz, & Simmons, 2003; Rhodes, Zebrowitz, et al., 2001).

1.1.1 Benefits of selecting healthy mates

Before we go into the discussion about the significance of having a healthy mate, and further exploring the criteria used in assessing perceived health (and attractiveness), it is important for us to define the concept health, specifically in the context of this dissertation.

Tybur and Gangestad (2011) highlighted in their review three main facets of health, which include (i) free from infectious diseases, (ii) good physical and physiological condition, and (iii) immunocompetence and other capacities to resist disease. The definition proposed not only covered current fitness and health condition of an individual, but also his or her capacities in avoiding and coping with future illness.

Selecting a fit and healthy life companion is important, as it confers both direct (see below 1, 2, and 3) and indirect (example no. 4) benefits for survival and reproduction, including:

1. Reduced risk of contracting infectious pathogens
2. Better physical protection
3. Increased and prolonged investment and involvement in child rearing

4. Inheritance of more adaptive genes by the offspring

Being with a healthy and fit individual reduces the chance of acquiring an infectious disease (Able, 1996; Loehle, 1997). If the mating occurs within a short time course, the current health status of an individual will be a crucial assessment, and if it is for a long term commitment, one would need to look for the cues which indicate the immunocompetence of the potential mate, that is their strength of their immune system in avoiding and fighting with harmful ailments (Tybur & Gangestad, 2011).

Health is not merely an absence of illness, but also implies greater fitness and resourcefulness (Gangestad & Scheyd, 2005). Honekopp and colleagues (2007) proposed that performance-related physical fitness is an important component of ancestral male mate value and that females evolved to regard men whose faces or bodies signal physical fitness as attractive. Research conducted by Dixson and colleagues (2003) indicated that women from different cultures showed preferences for male figures with a medium-built body with a pronounced v-shaped upper body, whereas Fan and his fellow researchers (2005) highlighted women's attention to the chest-waist-hip ratio, whereby males with broad chests and small waists were found to be more attractive as compared to their counterparts with smaller chest-waist ratio. With a strong body build and better fitness, men are not only able to provide better physical protection to their partner and their offspring (Buss & Schmitt,

1993), but are also better able to resist infectious diseases, live healthier, live longer and thus able to prolong the duration of the protection and investment. Among a number of non-human animal species, it has been found that females prefer to mate with males with bigger body size (for example, crickets in Bateman, Gilson, & Ferguson, 2001; and gladiator frogs in Kluge, 1981).

In general, women prefer to have a long term relationship with men who are willing to invest in parental efforts, including providing financial resources, protection and emotional support to both the mother and the offspring (La Cerra, 2003). Selecting a healthy mate not only reduces the chance of passing infectious disease to the individual or offspring, but also reduces the possibility of losing income owing to the contraction of infectious diseases by the mate. Apparent health is associated with better financial prospects as healthy individuals tend to have better physical strength, longer life expectancy, are able to stay longer in working force, and thus are able to accumulate more assets (Krupp, DeBruine, & Jones, 2011). As shown by the Halo Effect (the stereotype of "what beautiful is good"; Shinners, Sponsor, & Morgan, 2009), individuals with a higher level of attractiveness are treated differently as compared to their counterparts with a lower level of attractiveness, which contributes to attractive individuals' wider social network, better occupations and higher income (Langlois & Kalakanis, 2000).

When selecting potential life partners, individuals prefer those perceived to have a high mate value (Honekopp et al., 2007), including the possibility of possessing "better genes", for greater fitness and better immunity. By mating with a healthy and fertile mate, these adaptive genes are inherited and provide benefits to the offspring, to help them survive better and have a better chance to reproduce. Indeed, it was found that attractiveness does pass down from both the parents to the daughter; however, the son only shared the same level of masculinity of the father, with no correlation found between the level of attractiveness of the sons and the parents (Cornwell & Perrett, 2008).

1.2 Attractiveness as a reflection of health

The concept of sexual selection arises from the observation that animals have evolved with features that function to maximize the individual's reproductive success rather than helping them to survive *per se* (Darwin, 1871). Both non-human animals and humans use physical attractiveness as an important criterion in the selection of potential mates (Amundsen & Forsgren, 2001; Bourne, Breden, & Allen, 2003; Lee, Loewenstein, Ariely, Hong, & Young, 2008). Many animals, for example, use bright colouration displayed by various parts of their body to attract mates (Amundsen & Forsgren, 2001; Bourne, Breden, & Allen, 2003). Certain bird species, such as the peacock and the cardinal even risk attracting predators and endure restricted movement as a result of their large, bright ornaments. Sexual selection is often divided into

intersexual choice - by making oneself appear attractive to the opposite sex with certain alluring physical attributions and intrasexual competition - defeating same sex rivals (Darwin, 1871; Trivers, 1972).

Attractiveness has also been found to be one of the most important criteria in human mate selection (Buss, 1989; Lee et al., 2008; Rhodes, 2006). Individuals who look attractive tend to have relatively more mating options, thus contributing to the higher possibility of getting a better partner (Gangestad & Scheyd, 2005).

Scientific research conducted in recent years suggested that beauty is not merely a subjective evaluation which differs from one culture to another. Rather there is certain level of agreement across different cultures whereby individuals from different societies use similar physical cues to define what is attractive (Thornhill & Grammer, 1999; Stephen, Coetzee, Law Smith, & Perrett, 2009, 2011). This means that judgments of attractiveness might be biologically driven. This suggests that cues may appear attractive because we have evolved cognitive mechanisms for identifying and being attracted to cues that reflect health and fertility.

Of course, it has also been shown that individuals from different geographical environments show a certain level of disagreement in their preferences, as different environments create different kinds of challenges. For example, it

has been found in several studies that individuals from lower socioeconomic status rural environments preferred women with higher waist-hip ratio (WHR) and higher body mass index (BMI), as compared to their counterparts from urban populations who preferred a slightly smaller WHR and BMI (Swami & Tovée, 2005a; Swami & Tovée, 2005b; Marlowe, Apicella, & Reed, 2005; Furnham, Moutafi, & Baguma, 2002). More examples of the cultural variations in the preference for health physical characteristic will be presented below, when discussing different physical cues of health.

1.2.1 Mixed evidence of the association between health and attractiveness

Early research on health perception has been conducted mainly to show that certain physical attributes are found to be attractive, with researchers suggesting that they may reflect fertility and health status of potential mates (Rhodes et al., 2003b; Rhodes, Zebrowitz, et al., 2001). Physical health has been repeatedly linked with attractiveness in theory and, to a lesser extent, in empirical studies (Rhodes et al., 2003b; Rhodes, Zebrowitz, et al., 2001a). However, evidence for the relationship between physiological health and attractive traits is not conclusive (Rhodes et al., 2003b), and the line of research which focuses on examining this hypothesis is still in its early stages (Jones, Little, Burt, & Perrett, 2004; Jones et al., 2005).

Some researchers were able to find evidence on the association between attractiveness and actual health. Shackelford & Larsen (1997), for example,

showed that attractive individuals displayed better cardiovascular health, reported less headache and less running nose as compared to unattractive individuals, and this health-attractiveness link appeared to be stronger for men than for women. A similar pattern was observed with relation to immunity whereby it was found that attractiveness in males predicts the ability to produce antibodies in response to hepatitis B vaccine, but not in women (Rantala & Coetzee, 2013). Women who are judged as more attractive reported less severe illnesses as compared to less attractive women (Hume & Montgomerie, 2001). Research done by Rhodes and colleagues also showed that symmetry, averageness and sexual dimorphism (femininity in female faces and masculinity in male faces; all cues that impact on facial attractiveness) predict health and attractiveness (Rhodes et al., 2003b; Rhodes, Zebrowitz, et al., 2001).

Facial attractiveness not only reflects physical health, but also predicts future longevity. In Henderson and Anglin's research (2003), 20 undergraduate students were asked to rate black and white photographs of fifty high school students from the 1920s on attractiveness and perceived health, and the researchers found that more attractive individuals lived longer.

However, not all studies have successfully found relationships between attractiveness, perceived health and actual health. Rhodes, Chan, Zebrowitz, and Simmons (2003) in their research found an association between

perceived masculinity in male adolescent faces with both perceived and actual healthiness, but these faces were not judged as more attractive. Even though femininity in female adolescent faces is perceived as healthy and attractive, it did not reflect actual health. Kalick, Zebrowitz, Langlois and Johnson (1998) found no significant association between facial attractiveness of the adolescents and their actual health.

Previous research has identified several cues which individual utilize when judging the health status of another person. Many of these cues to apparent health also predict attractiveness (Jones, Little, Feinberg, et al., 2004). The following section explores some of the important physical cues that have been found to predict perceived health; and their relationships with attractiveness and physiological health. Cross-cultural variation in relation to the preference for these cues is also discussed.

1.2.2 Facial shape and health: Averageness

Faces with increased averageness are perceived as healthier, while distinctiveness (a converse measure of averageness) is perceived as unhealthy (Rhodes et al., 2001). Rhodes and colleagues further explored the relationship between distinctiveness and actual health assessed from medical assessments and previous medical records of participants and found that facial distinctiveness of 17 years olds was related with poorer current and adolescent health in females, and poorer health condition during childhood

for males. A similar study conducted in Chinese and Japanese adults showed similar preferences for faces with high averageness (Rhodes, Yoshikawa, et al., 2001a). Rhodes and colleagues later found that apparent health contributed to the appeal of averageness, especially for female faces (Rhodes et al., 2007).

Facial averageness is related to both perceived health and actual health as it reflects high genetic heterozygosity which creates a more hostile microenvironment for parasites (Thornhill & Gangestad, 1993). However, there is also another hypothesis that suggests that preference for averageness in the face may be merely due to preference for familiarity (Langlois & Roggman, 1990).

Facial averageness is not always perceived as attractive. If facial beauty is all about averageness, we would predict that an older individual (average in term of age within a population) with an androgynous appearance (average in the spectrum between masculinity and femininity) to be most attractive, which is not the case. DeBruine and her research team (2007) illustrated in a series of experiments that the most average facial composition was not the most attractive, and there are certain non-average characteristics which can make faces look attractive.

1.2.3 Facial shape and health: Symmetry

Symmetrical human facial and body features are rated as more healthy than asymmetrical characteristic in several studies (Fink, Neave, Manning, & Grammer, 2006; Grammer & Thornhill, 1994; Rhodes, Zebrowitz, et al., 2001; Tovée, Tasker, & Benson, 2000). Symmetry was proposed to be a cross-cultural criteria for beauty as it has been found to contribute to attractiveness not only in Western societies (Grammer & Thornhill, 1994; Penton-Voak et al., 2001; Perrett et al., 1999) but also in non-Western industrialised (Rhodes, Yoshikawa, et al., 2001a), non-western traditional societies (Little, Apicella, & Marlowe, 2007), and even in monkeys (Little et al., 2008).

Symmetry has been suggested to be a valid indicator of health and genetic quality (Simmons, 2004). It reflects one's ability to resist environmental disturbance such as illness and malnutrition during development, and still develop evenly (Fink et al., 2006; Grammer & Thornhill, 1994; Swaddle & Cuthill, 1995). Only individuals who are fit and with good genetic quality are capable of developing a symmetrical phenotype despite the developmental perturbations caused by disease, toxins and parasites; while fluctuating asymmetry (small deviations from perfect symmetry in bilateral traits, which is commonly used as an index of developmental stability) occurred when individuals struggled to undergo stable development in the face of environmental stressors (Livshits & Kobyliansky, 1991).

Many studies have been conducted to explore the relationship between fluctuating asymmetry and physical health of both humans and non-human animals (Jones & Little, 2001; Pound et al., 2014; Rhodes, Zebrowitz, et al., 2001; Møller, 1990, 1992). Men with more symmetrical faces were found to have fathered more offspring and had fewer serious diseases (Waynforth, 1998) as compared to those with more asymmetrical faces. Thornhill and Gangestad (2006) found positive associations between facial asymmetry and susceptibility to disease, including frequency of respiratory infections, and antibiotic use, for both men and women.

Body symmetry was also found to be related with one's fertility and health status. Men with high fluctuating asymmetry in body shape were found to have poor semen quality (Manning, Scutt, & Lewis-Jones, 1998; Firman, 2003) and higher frequency of respiratory infection (Thornhill & Gangestad, 2006). Further, it was found that level of fluctuating asymmetry of women's breasts is negatively associated with fertility (Møller, Soler, & Thornhill, 1995; Scutt & Manning, 1996)

Nonetheless, attempts to connect facial symmetry to physical health have not always been successful. Pound and colleagues (2014) found no correlation between symmetry and frequency of childhood illness in a large sample. Even though symmetry was found to appear more healthy and more attractive in a study conducted by Rhodes and colleagues (2001b), the researchers also

failed to establish a significant connection between facial symmetry and the health status of the participants at different time points, including their childhood, adolescence and the health status at the moment of study, which were assessed by medical records.

It was suggested that the relationship between symmetry and actual health might be substantially weaker in modern industrialised societies with access to advanced health care and medication (Jones & Little, 2001). Attractiveness as a cue to health may be more relevant in the ancestral past, when one's immune system is the primary mechanism for dealing with pathogens. Thus, failing to find an association between the two variables (symmetry and actual health) in modern populations does not necessarily mean that they were not linked in ancestral populations.

1.2.4 Facial shape and health: Sexual dimorphism and the Handicap Hypothesis

Our facial shape changes during adolescence due to the influence of sex hormones. Men after puberty tend to have a more angular face with prominent brows and larger jaws; whereas women tend to develop higher cheekbones, larger foreheads, smaller jaws and lighter brow-ridges as compared to men (Penton-Voak et al., 2001; Enlow and Hans 1996). Sexual dimorphism is the extent to which an individual appears typically male or typically female.

Both masculine male faces and feminine female faces are perceived as healthier as compared their counterparts with less exaggerated sexual dimorphic faces (Rhodes et al., 2003a). According to the Handicap Hypothesis, the more choosey sex of a species, which usually is the female tend to choose a male that has survived to the reproductive age with certain costly secondary sexual traits. As a result, the male animals with these attractive features are often able to attract more members of the opposite sex of the same species and have better mate selection (Amundsen & Forsgren, 2001; Bourne, Breden, & Allen, 2003; MacDougall & Montgomerie, 2003).

The development of male's secondary sexual characteristic requires more resources in the form of nutrient and energy (Zahavi, 1975) which can be easily acquired by healthy and fit males. Sex hormones which contribute to the sexual dimorphism of facial features exert a cost onto the bearer. As secretion of testosterone suppresses immune function (Alexander & Stimson, 1988), only those highly fit and healthy individuals, who are able to resist pathogens and parasites can afford to suppress their immune system and augment their secondary sexual traits with an increment in the secretion of testosterone (Hamilton & Zuk, 2009). However, the handicap hypothesis might not be applicable to females, as oestrogen which contribute to femininity in women (Smith et al., 2006) is not immunosuppressive.

Indeed, men with a more masculine faces not only being perceived as healthier as compared to their counterparts with more feminine faces, but also tend to have better actual physical health (Rhodes et al., 2007, 2003b). Masculine looking men reported less respiratory infections, shorter duration of respiratory disease, and lower antibiotic usage (Thornhill & Gangestad, 2006). However, the association between masculinity in male face and both the perceived health and actual health of men is inconclusive. In one study, Boothroyd and colleagues (2005) failed to find an association between masculinity in male faces with apparent health. And in another one (Boothroyd, Scott, & Gray, 2013) they found that facial masculinity was only associated with better previous health but with worse future health.

Besides, the masculine male faces are not judged as attractive (Rhodes et al., 2003b). There are certain variations in female's preference for masculine male faces across different societies. It has been suggested that that women show higher preference for masculine male faces, which reflect better health and immunocompetence (Rhodes et al., 2003a), when they are living in regions with higher pathogen prevalence (DeBruine, Jones, Crawford, Welling, & Little, 2010).

Conversely, sex hormones which contribute to femininity were found to be related with increased production of some antibodies (Alexander & Stimson, 1988). Femininity in women might serve as a cue to resistance to certain

diseases (Gray & Boothroyd, 2012) as Thornhill & Gangestad (2006) found a positive correlation between women's masculinity and number of respiratory infections, and days with respiratory infections. Female faces' rated femininity was found to be negatively correlated with the occurrence of cold in the previous 12 months, and usage of antibiotics in the previous 3- and 12 months (Gray & Boothroyd, 2012).

However, the association between femininity of female faces and the actual health of the respective female was not conclusive. Rhodes and colleagues (2003a) failed to discover any relationship association between the femininity of female faces and the actual physical health of women, as measured by their actual adolescent health scores. Other studies have highlighted the role of oestrogen in the initiation of breast cancer (Russo & Russo, 2006; Russo, Hasan Lareef, Balogh, Guo, & Russo, 2003).

More frequently, the femininity of female faces was found to be associated with women's reproductive health (Moore, Law Smith, Taylor, & Perrett, 2011). Female faces with higher levels of femininity were perceived as attractive due to its relationship with fertility (Moore et al., 2011), as it was found that the preferences for femininity were reduced once the preferences for cues to health are controlled for (Rhodes, 2006).

1.2.5 Facial shape and health: Facial adiposity

Obesity is a precursor to the onset of various physical illnesses. Individuals who are overweight and obese have higher risk of developing a variety of chronic diseases such as cardiovascular disease, diabetes (Balkau, Deanfield, & Després, 2007; Cepeda-Valery, Pressman, Figueredo, & Romero-Corral, 2011) and cancer (Calle, Rodriguez, Walker-Thurmond, & Thun, 2003). Since being obese and overweight is a well known predictor of physical illness, we can predict that individuals with higher waist-hip-ratio (WHR), and higher facial adiposity will be perceived as less healthy, as compared to their counterparts with skinnier body shape and lower facial adiposity.

Indeed, Coetzee, Perrett and Stephen (2009) found that facial images that were judged by two independent groups of participants as overweight tend to be rated by another two different groups of participants as less healthy and less attractive. In this study, the researchers also collected objective measurements of body mass index, and blood pressure, and the subjective report of the participants about their usage of antibiotics and respiratory illnesses. A significant negative relationship was found between facial adiposity and the measures of respiratory health and cardiovascular health (Coetzee et al., 2009).

1.2.6 Facial skin as an indicator of current health

In the recent decade, there have been a growing number of studies which have highlighted the importance of facial skin condition as a prime indicator of health and attractiveness (Lefevre & Perrett, 2014; Stephen et al., 2012; Stephen, Coetzee, & Perrett, 2011).

Among non-human animals, it is common that individuals prefer to mate with the opposite sex of the same species with bright and vibrant ornaments (feather, scale or skin colour). Various birds and fish were found to prefer mating with the opposite sex with bright carotenoid-coloured ornaments; as such colouration predicts the both physical fitness and psychological functioning of that particular individual. For example, carotenoid-based colourful traits were found to reflect better sperm quality in *Parus major* and mallards (Helfenstein, Losdat, Møller, Blount, & Richner, 2010; Peters, Denk, Delhey, & Kempenaers, 2004); higher reproductive success in northern cardinals (Trond Amundsen, 2000), better immune function in Zebra finches (Blount, Metcalfe, Birkhead, & Surai, 2003), more efficient parenting skills in yellow-eyed penguins (Massaro, Davis, & Darby, 2003) and hunting at better sites for male American Kestrels (Bostrom & Ritchison, 2006).

Certain species of fish show a similar tendency whereby they are sensitive towards differences in colour of ornaments. Male two-spotted gobies, for example, tend to spend more time, and perform more courtship displays in

front of female gobies with brighter yellow-orange bellies, regardless of whether the colours were obtained naturally or induced artificially (Amundsen & Forsgren, 2001). Female guppies also prefer to mate with male guppies with brighter carotenoid-based colouration (Sargent, Rush, Wisenden, & Yan, 1998), and males with greater areas of such colouration were found to have significantly larger sperm loads, more active and longer sperm as compared to those with relatively lower levels of the orange coloration (Pitcher, Rodd, & Rowe, 2007).

A variety of non-human primate species have also been observed to use their exposed skin colour, either on their face or genitalia to indicate their fertility and to attract mates (Barelli, Heistermann, Boesch, & Reichard, 2007; Setchell, Wickings, & Knapp, 2006).

Stephen and colleagues conducted a few experiments which highlighted the importance of skin colour as a cue to health in humans (Stephen, Law Smith, et al., 2009; Stephen et al., 2012, 2011). It was found in their facial colour manipulation tests, that Caucasian participants increase facial lightness, facial redness, and facial yellowness to make facial images look healthier (Stephen, Law Smith, et al., 2009). Facial luminance, redness and yellowness are proposed to be related with the bearer's melanin concentration, skin blood perfusion and carotenoids consumption.

Melanin which contributes to the decreased luminance of human skin (Edward & Duntley, 1939) has both health benefits and costs. On one hand, it helps protect individuals from damaging ultra violet radiation, and thus offers protection from sunburn and skin cancer; on the other hand, its function in blocking sunlight reduces the efficiency of vitamin D synthesis (Jablonski & Chaplin, 2000; Kourosh, Harrington, & Adinoff, 2010). Therefore, preference toward melanin colouration may be predicted to rely on the geographical location of the population, with individuals living near to the Equator preferring higher concentrations of melanin, with darker skin tone, and individuals living far from the equator preferring a lower concentration of melanin, with fairer skin tone (Jablonski, 2004). Indeed, this hypothesis has been partially supported in the results of the studies of Stephen et al (2009) where British participants prefer a fairer skin tone over a darker one.

Changes in facial redness can be explained with the changes in skin blood perfusion, which is influenced by various physiological functioning of a person, including the efficiency of the circulatory system, cardiovascular system and hormonal health (Charkoudian, 2001; 2003; González-Alonso, Crandall, & Johnson, 2008). Both the amount of blood and the oxygenation state of that blood in the skin are thought to reflect health status. Stephen and colleagues (2009) found that increment in oxygenated blood colouration especially, as compared to deoxygenated blood colouration, contributed to increased perceived health. Oxygenated blood is bright red in colour, with oxygen

attached to the haemoglobin molecules within the red blood cells. Blood is deoxygenated and turns darker and bluer after it has delivered oxygen to the cells. Increased blood oxygenation increases skin redness, and was found to be related with increased aerobic fitness (Armstrong & Welsman, 2001), while blood deoxygenation was found to be associated with cardiac and respiratory illness (Ponsonby, Dwyer, & Couper, 1997) which makes the blood colouration look darker and bluer.

Skin yellowness in humans is also contributed by carotenoids, which are primarily obtained from the consumption of fruits and vegetables (Alaluf et al., 2002; Stephen et al., 2011). Carotenoids have been found to be involved in various health functions of humans, including the enhancement of the immune system, visual acuity, and skin photoprotection (Maxim E. Darvin, Sterry, Lademann, & Vergou, 2011; DA Hughes, 1999; Rao & Rao, 2007; Samimi, 2005). Reduced levels of carotenoids has been observed in patients with infections such as HIV and malaria (Friis et al., 2001).

Carotenoids are required to scavenge surplus reactive oxygen species (ROS) and stimulate the immune function (Dowling & Simmons, 2009). Excessive production of ROS can damage sperm and reduce fertility (Agarwal, Virk, Ong, & du Plessis, 2014; Agarwal, 2005; Walczak-Jedrzejowska, Wolski, & Slowikowska-Hilczer, 2013). Carotenoids, especially beta-carotene and lycopene, are efficient oxygen quenchers, which help prevent lipids in the cell

membrane and seminal plasma from being peroxidized (Agarwal et al., 2014). Lipid peroxidation causes damage to proteins; and is thought to be related to aging and pathogenesis of many diseases (Desai & Tappel, 1963; Gutteridge, 1995; Niki, Yoshida, Saito, & Noguchi, 2005; Niki, 2009). Supplementation of carotenoids was found to improve male fertility (Gupta & Kumar, 2002).

In relation to human skin, the deposition of carotenoids helps reduce the level of damage caused by UV radiation (Alaluf et al., 2002; Bouilly-Gauthier et al., 2010).

Skin colour has been suggested to be a better predictor for attractiveness of male faces than masculinity when judged by females of own ethnicity, for Caucasian and African samples (Stephen et al., 2012).

Homogeneity of skin colour is also important for health judgments. By using skin patches cropped from the left and right cheek areas of facial images, Jones and colleagues (2004) found that the apparent health of skin is relatively stable across different facial locations, and that this perceived skin health was positively associated with the attractiveness of male faces. By using topography (micro-texture) and shape-standardized female faces, it was found that the distribution of facial colour plays an important role in the judgment of attractiveness, health and youthfulness of the faces, with homogenous colouration being perceived as younger, healthier and more

attractive (Fink, Grammer, & Matts, 2006; Matts, Fink, Grammer, & Burquest, 2007). Facial skin of younger women is perceived as younger, healthier and more attractive than those of older women (Fink et al., 2006).

A further study was conducted to explore the relative contribution of skin colour distribution and topography cues on the perception of age and health status of female faces (Fink & Matts, 2008). It was found that skin surface topography contributes more to the variance in age perception whereas skin colour distribution had stronger impact in influencing the judgment of perceived health for female faces. All these studies (Fink et al., 2006; Jones, Little, Burt, et al., 2004; Jones, Little, Feinberg, et al., 2004) have suggested that the distribution of facial colors, and skin texture stand alone as important cues for the judgment of perceived health and attractiveness, independent of facial shape.

1.3 Colour vision and health perception

The theory of evolution proposes that through natural selection, we have evolved both physical and mental attributions that are adaptive, to help us survive and reproduce effectively (Cosmides & Tooby, 1987, 2013). Both human and non-human species rely on sensation and perception when selecting potential mates. Various type of birds, fish and even non-human primates are visually sensitive towards minor changes in the colour of skin (including feathers and scales), and use that visual information to select a high

quality mate (Gouveia et al., 2003; Hill, 1990; Inouye, Hill, & Stradi, 2001; Saks, Ots, & Hõrak, 2003; Setchell & Dixson, 2001), while other species might pay more attention to either auditory or olfactory information (Ruther, Matschke, Garbe, & Steiner, 2009; Sockman, 2007; Vaccaro, Feldhoff, Feldhoff, & Houck, 2010).

Changes in physical appearance have been found in various studies to be honest indicator of the quality of the bearer. Colour vision has been proposed to be selected in certain species, to attend to differences in skin colour for social signalling (Changizi et al., 2006). This is especially important for highly social animals such as primates, as visually attending to skin colour not only helps one select a mate with good health and good fertility (Barelli et al., 2007; Setchell et al., 2006) but also helps us recognise the emotion, and in some species the social ranking of conspecifics (Setchell & Wickings, 2005)

By analyzing images of 97 species from 35 primate genera, Changizi and colleagues (2006) discovered that the trichromacy is strongly related with the average bareness of the face and other parts of the primates' bodies. Primates with trichromatic colour vision usually have more skin exposed. It was proposed that trichromacy evolved in order to enable individuals to perceive the changes in blood oxygenation and perfusion through subtle changes in skin colour.

Female rhesus macaques were observed to have changed their visual attention when attending to faces of male rhesus macaques with different levels of redness (Waitt et al., 2003). They preferred and stared longer at faces with redder skin tone as it was suggested that the face colouration provides a cue to the owner's quality. A similar pattern was observed in mandrills whereby bright red faces and genital areas are linked to higher status in males (Setchell & Dixson, 2001) and better fertility status in females (Setchell et al., 2006).

The same extra sensitivity and preference for redness has been observed in several studies on humans (Elliot & Niesta, 2008; Elliot et al., 2010; Hagemann, Strauss, & Leissing, 2008; Re, Whitehead, Xiao, & Perrett, 2011). Re and colleagues (2011) for example found that individuals are sensitive towards minor changes in skin redness, and rely on that information for the assessment of health and attractiveness.

Changes in skin redness have been found to be related with females' fertility and fluctuates across the oestrus cycle of female animals (Waitt, Gerald, & Little, 2006) and menstrual cycle of women (Jones et al., 2015). Women tend to dress in red more often when nearing ovulation, and this display of redness is perceived by men as attractive (Elliot & Niesta, 2008). Similarly, women tend to perceive men as physically and sexually more attractive when they

were associated with redness, for example when they were wearing red clothes or stood in front a red background (Elliot et al., 2010).

As skin colour is found to affect perceived health and attractiveness (Coetzee et al., 2012; Lefevre & Perrett, 2014; Scott, Pound, Stephen, Clark, & Penton-Voak, 2010; Stephen, Coetzee, et al., 2009; Stephen et al., 2011), which are important for mate selection, it is possible that our sensory receptors and the associated mental mechanisms may have been selected to enable us to detect changes in colour associated with health, fertility and socially-relevant information.

1.4. Fruit and vegetable consumption induce skin yellowness

Previous studies showed that the level of fruit and vegetable consumption is positively correlated with the skin yellowness of Caucasians (Stephen et al., 2011), as certain fruits and vegetables contain carotenoids, which become deposited in the skin. Carotenoids are natural pigments which give the yellow–orange–red colour in various kind of fruits and vegetables (Mortensen, 2006; Paiva & Russell, 1999).

Capsules containing beta carotene have been found to induce an increment in skin yellowness in both Caucasian (Stahl, Heinrich, Jungmann, Sies, & Tronnier, 2000) and African samples (Coetzee & Perrett, 2014). Nonetheless, there is no research that observed changes in skin yellowness in relation to the

supplementation of natural sources of carotenoids. Further, to our knowledge, no such study has been conducted on an Asian population.

1.4.1 The importance of carotenoid colouration in health perception

Increased skin luminance, redness, and yellowness improve apparent health of human faces (Stephen, Coetzee, et al., 2009b; Stephen et al., 2011). This result is found not only for the Caucasian sample but also the African (Coetzee et al., 2012). The importance of carotenoid colouration has also often been highlighted in the studies of mate selection in various species of bird and fish (Bourne et al., 2003; Grether, 2000; McGraw, Hill, & Parker, 2005; Tapiero, Townsend, & Tew, 2004a).

Even though both melanin and carotenoids contribute to skin yellowness (Alaluf et al., 2002), and both colours have been found to contribute to increased perceived health and attractiveness (Lefevre & Perrett, 2014; Stephen et al., 2011), research conducted by Lefevre and Perrett (2014) found that carotenoid colouration is consistently preferred over melanin colouration, especially for female faces, regardless of the sex of the observer. It is suggested that humans' preference for carotenoid colouration in faces was not due to the fact that carotenoid colouration is an aesthetically pleasing colour, rather because it serves as a social signal of health. Using both facial and non-facial stimuli, Lefevre and colleagues (2013) found in their research

that this carotenoid colouration only showed its charm when applying on human face.

1.5 Objective of the studies

Nine studies were conducted to examine the significance of skin condition on the health perception of Malaysian Chinese, and its implications.

The first two studies reported in Chapter 2 explore the contribution of facial skin, including both its colour and texture, to judgments of perceived health. This will be the first intensive psychophysical study on health perception conducted in Asian setting. As facial colour has been suggested to be the primary cue in determining the immediate health status of an individual (Coetzee et al., 2012; Lefevre, Ewbank, Calder, von dem Hagen, & Perrett, 2013; Stephen, Coetzee, et al., 2009; Stephen et al., 2011), Study 3 in Chapter 2 examined the type and direction of changes in skin colour, which optimize perceived facial health.

In Chapter 3, there will be two studies focused on examining the sensitivity threshold of colour vision. Based on the literature presented earlier (Changizi et al., 2006; Re et al., 2011), it was suggested that individuals should be more sensitive towards changes in chrominance, specifically towards the changes in blood colouration, as compared to changes in luminance. And this extra

sensitivity towards chrominance over luminance should be found primarily when perceiving human faces, and not other non-facial objects or patches. Study 4 in Chapter 3 was designed to compare the sensitivity threshold of detecting changes in facial colours between different colour axes (luminance L*, redness a* and yellowness b*). To further validate our hypothesis that human vision's extra sensitivity in chrominance is only unique for the judgment of face perception, Study 5 was designed, comparing the sensitivity threshold of detecting colour changes in non-face objects and patches.

Study 6 reported in Chapter 4 was designed to examine if the consumption of a high carotenoid fruit and vegetable smoothie is able to induce changes in skin yellowness, in an Asian sample. As changes in skin redness and skin luminance also contribute towards changes in perceived health, we also investigate if the supplementation can induce changes in those two colour axes. In the same chapter, the dietary pattern of Malaysian Chinese, especially their fruit and vegetable consumption, was examined.

Three perceptual studies were conducted to examine if the change in skin colour induced by the supplementation of carotenoid rich smoothie can improve the perceived health of faces. Study 7 compared between the pre-intervention face images and mid-intervention face images (of individuals who consumed smoothie for a month). Controlling for the various physical changes of faces over the course of intervention, Study 8 compared between

the original facial images and those with an increment in carotenoid colouration. Lastly, Study 9 explored the amount of carotenoid colouration that is appropriate to improve apparent health of face images.

1.5.1 Malaysian Chinese as the targeted population

A variety of psychological studies have been conducted to study the perceived health of human faces. Most of these studies were conducted in the West, and we have very limited knowledge about the perception of health among the Asian population. Skin colour has been suggested as a evolved signal, selected to help in mate selection (Changizi et al., 2006). If this is true, we should be able to observe such preference in different populations. Previous research has found evidence for the importance of skin colour in health perception from both Caucasian and African samples (Coetzee et al., 2012; Stephen, Coetzee, et al., 2009b; Stephen et al., 2011). However, it is uncertain whether the same would be observed in an Asian sample.

Besides, there may be a certain level of variation in this health preference. For example, it was hypothesized that communities who are living in different regions should have different preferences for healthy skin colour, as the preferred amount of the melanin which contributed to the darkness of the skin relies on the amount of sunlight available. We know from previous studies (Stephen et al., 2011; Stephen, 2008) that Caucasians prefer a lighter skin tone, as high amount of melanin would screen away the limited sunlight

which is crucial for the synthesis of vitamin D. However, it is not known if communities who are living near the equator would prefer a darker skin tone.

All the nine studies reported in this thesis were conducted on Malaysian Chinese (Malaysia-born, Chinese descent). Asia is a big continent with a huge variety of ethnicities, with various skin tones. To control for the variation in skin tones and the influence of confounding factors (such as of the difference in diet and activity level) on the dependent variables; our research has focused on one specific ethnicity instead of attempting to study Asians as whole. The Chinese were selected here, as it is not only one of the major ethnicities in Asia, but also the second largest ethnic group in Malaysia (Hirschman, 1980).

Since differences have been found between Malaysian Chinese and Caucasian populations in the looking strategies used to recognise faces, (Tan, Stephen, Whitehead, & Sheppard, 2012), it would be interesting to know how the these two populations differ in term of their sensitivity threshold in colour discrimination, and judgment of apparent facial health.

1.5.2 CIELAB Model

All the colour manipulation administered in this thesis was based on the CIELAB 1976 colour space, that was specified by the International Commission on Illumination (*Commission Internationale de L'éclairage*). It describes all the

colors that are visible to the human eye, and was created to serve as a device-independent model.

As shown in Figure 1.1, there are 3 axes in the model. The vertical L* axis represents luminance, ranging from 0-100 (black to white). The (horizontal) a* axis has red at one extremity (+a), and green on the other (-a); while the horizontal b* axis has yellow on one end (+b), and blue (-b) on another. There are no maximum values of a* and b*, but in practice they are usually numbered from -128 to +127 (256 levels).

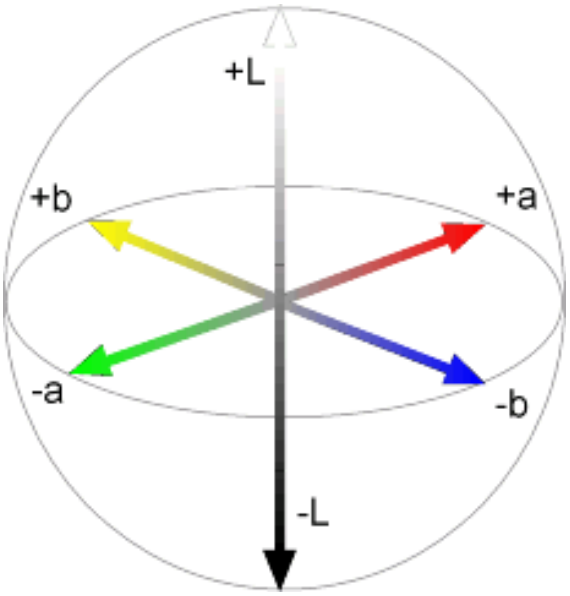


Figure 1.1. A graphical representation of the CIELab colour space.

The CIELAB colour space was selected to be used in this thesis, as the model is well accepted in human perceptual work (Martinkauppi, 2002) and designed

to reflect the human visual system. It is perceptually uniform, which means that a change of one unit along one axis should be perceptually similar in magnitude to a change of one unit in any other colour axis.

1.6 Summary

In summary, this chapter explored the facial cues that are utilized when making judgments about others' health. The chapter began by highlighting the importance of attractiveness and health assessment as criteria for mate selection. While studying physical cues that were perceived as attractive by individuals from different cultures, contemporary psychologists found that all these cues are related to the person's physical and occasionally psychological health. The chapter then discussed the facial cues that are perceived as healthy, and its relation with the bearer's actual health. Among the different facial cues, skin condition stood out as an important cue for both attractiveness and perceived facial health. Various aspect of skin, including its colour, homogeneity of colour distribution and its texture were found to play an important role in defining health perception.

Since health perception is important for survival and reproduction, it is predicted that there should be sensory mechanisms that was adapted to make efficient health judgment possible. We suggested that one of the significant functions of humans' colour vision may be their sensitivity towards facial colours that are important for health judgments.

The final part of this chapter highlighted the importance of carotenoid colouration, and its relation with fruit and vegetable consumption. Objectives of all the ten studies conducted in this thesis were also presented to provide the reader an overview of the whole thesis.

CHAPTER 2: FACIAL SKIN QUALITY AS A PREDICTOR OF PERCEIVED FACIAL HEALTH

2.1 Introduction

The human face provides a variety of useful information about an individual and the surrounding environment, from sex, ethnicity, and age; to more subtle and implicit judgements such as making inferences about one's personality (Carre & McCormick, 2008; Penton-Voak, Pound, Little, & Perrett, 2006; Little & Perret, 2006; Fink, Neave, Manning, & Grammer, 2006), intelligence (Kleisner, Chvátalová, Flegr, 2014; Moore, Filippou, & Perrett, 2011; Zebrowitz, Hall, Murphy, & Rhodes, 2002) and health status (Coetzee, Perrett & Stephen, 2009; Stephen et al., 2011). These judgements are made automatically, with little conscious effort and provide cues which can be crucial for our physical well being and social functioning, including the very important life task of mate selection (Peters, Rhodes & Simmons, 2007; Currie & Little, 2009).

Physical appearance is one of the most important criteria used for mate selection, for both sexes (Rhodes, 2006; Stirrat, Gumert, & Perrett, 2011). Individuals who are physically attractive tend to have more options when choosing a mate and have more opportunity to pursue a good quality mate. Researchers have identified a number of cues commonly employed to

determine facial attractiveness, including averageness (Judith H Langlois & Roggman, 1990; Rhodes, Zebrowitz, et al., 2001a), symmetry (Hume & Montgomerie, 2001; D. I. Perrett et al., 1999; Rhodes, Zebrowitz, et al., 2001a; Thornhill & Gangestad, 1993), sexual dimorphism (Jones et al., 2005; Penton-Voak et al., 2001) and skin condition (Matts et al., 2007; Scott et al., 2010; Stephen et al., 2011; Stephen, Law Smith, et al., 2009).

Over the last two decades, psychologists have suggested that these attractive facial features indicate health (Jones, Little, Feinberg, et al., 2004), such as good genes, developmental health or current health status. For example, facial averageness is thought to be associated with heterozygosity and a better defense against parasites (Thornhill & Gangestad, 1993); while low fluctuating asymmetry has been suggested to reflect developmental stability (Grammer & Thornhill, 1994; Thornhill & Gangestad, 2006). Attempts to empirically link facial symmetry to developmental health, however, have not always be promising (Pound et al., 2014; Rhodes et al., 2001a). Studying detailed health histories of a large sample (n = 4732) from the South West of England, Pound and colleagues (2014) failed to identify any association between facial fluctuating asymmetry and childhood health. Rhodes and colleagues also found no association between facial asymmetry and actual health.

Facial sexual dimorphism has been linked with developmental health and reproductive functioning, whereby men with masculine faces were found to be physically stronger (Fink, Neave, & Seydel, 2007) than those with less masculine faces. Masculinity has also been found to correlate with men's actual health scores during adolescence (Rhodes, Chan, Zebrowitz, & Simmons, 2003). Even though there was no significant correlation found between femininity and actual health, femininity was found to be associated with perceived health and late follicular estrogen levels in females (Rhodes et al., 2003a; Law Smith et al., 2006) which is related with fertility.

2.1.1 Skin condition as a health indicator

Most of the previous studies on attractiveness and healthy appearance have focused mainly on facial shape (Said & Todorov, 2011; Carre & McCormick, 2008; Rhodes et al., 2001; Fink et al., 2006). Nonetheless, recent studies have begun to highlight the importance of skin texture and colour (Fink, Bunse, Matts, &, D'Emiliano, 2012; Fink, Matts, D'Emiliano, Bunse, Weege, & Röder, 2012; Stephen, Coetzee, & Perrett, 2011). In some cases, skin condition has been proposed to hold a more important role in defining perceived facial health and attractiveness (Scott et al., 2010; Stephen et al., 2012) than structural cues, such as morphological masculinity. Using geometric morphometric methods (GMM), researchers (Scott et al., 2010; Stephen et al., 2012) found no contribution of morphological masculinity in predicting attractiveness. Increment in skin yellowness and decrement in skin luminance,

however, predicted rated attractiveness, but only when judged by own ethnicity raters.

Torrance and colleagues (2014) suggested that both shape and surface information of the face contributed to the judgment of others' attractiveness and dominance. However, there is a sex difference in the relative importance of the two information sources in defining the two perceptions. Surface information was particularly important for men's facial attractiveness; while shape information was relatively important for women's facial physical dominance. Jones and colleagues (2004) further validate the importance of skin in the judgment of men's facial attractiveness when they revealed an association between male facial attractiveness and the health rating of their skin patches, after controlling for structural cues.

Fisher and colleagues (Fisher, Hahn, DeBruine, & Jones, 2014) found in their study that both structural cues and colour cues work hand in hand to predict the apparent health of human faces. While faces with low adiposity were perceived as healthier than those with high adiposity, the effect was greater for faces with healthy looking colouration (increased redness and yellowness).

The subjective perception of one's skin condition has a major impact on that person's social and emotional well being and their overall quality of life, especially those diagnosed with dermatological diseases (Ahmed, Leon, Butler,

& Reichenberg, 2013; Barankin & DeKoven, 2002). Women with skin disease may be prone to psychological disturbance such as depression and suicidal ideation (Gupta & Gupta, 1998; Gupta, 2002; Picardi, Lega, & Tarolla, 2013; Picardi, Mazzotti, & Pasquini, 2006; Rehn, Meririnne, Höök-Nikanne, Isometsä, & Henriksson, 2008). The induced psychological stress and stigmatization can further exacerbate the severity of the disease (Ahmed et al., 2013; Gupta, 2002). An improvement in the skin condition, for example with the use of make up, significantly improves the quality of life of patients with various kind of skins conditions (Levy & Emer, 2012; Seité et al., 2012).

2.1.2 Objective of the studies

Many studies have been conducted to investigate the facial cues that are associated with perceived facial health (Fink et al., 2007; Matts et al., 2007; Rhodes et al., 2001, 2007, 2003; Scott, Pound, Stephen, Clark, & Penton-Voak, 2010; Thornhill & Grammer, 1999; Tovée & Maisey, 1999), however, most studies have focused on facial shape (Fink, Neave, Manning, & Grammer, 2006; Rhodes et al., 2001b; Rhodes, Chan, Zebrowitz, & Simmons, 2003). Only in recent years, skin condition, specifically its texture and colour, has started to gain more attention. However, these studies were mainly conducted in the West. To our knowledge, no such study has been conducted in an Asian population. As there is wide variation in skin colours across different geographical locations (Jablonski & Chaplin, 2000; Jablonski, 2011), it is uncertain if there will be agreement in preference for healthy skin condition,

both in terms of texture and colour, across different cultures. Cross-cultural differences have previously been reported in other attractiveness cues, such as body shape (Swami & Tovée, 2005a, 2005b; Tovee, Swami, Furnham, & Mangalparsad, 2006).

The purpose of this chapter is to explore the relationship between perceived facial health and facial skin condition, in an East Asian setting, specifically among the Malaysian Chinese. Three studies are reported in this chapter. Study 1 investigated the relationship between perceived facial health and skin texture. Study 2 examined the association between perceived facial health, perceived skin health, skin colour and texture. Study 3 investigated the amount of skin luminance, redness and yellowness which are considered as healthiest by the Asian sample.

2.2 Study 1: The relationship between facial skin condition and perceived facial health

2.2.1 Introduction

Distribution of colour and texture of facial skin was found to be associated with perception of age, health and attractiveness, in both men (Jones, Little, Burt, et al., 2004) and women (Fink, Grammer, & Matts, 2006; Fink, Grammer, & Thornhill, 2001; Fink et al., 2008; Matts et al., 2007).

Facial skin condition, as a rapidly changing health signal, provides useful information about an individual's current health and physiological status. Both the smoothness and colouration of human skin change with the influence of various internal and external factors. Skin colour is related to skin blood perfusion and oxygenation, melanin and carotenoid concentration (Alaluf et al., 2002; Charkoudian, 2003; Edwards & Duntley, 1939), which fluctuate according to the amount of exposure to sunlight, changes in diet, physical activity and hormonal level (Charkoudian, 2001; González-Alonso, Crandall, & Johnson, 2008; Jablonski, 2004; Stephen et al., 2011).

The smoothness of the facial skin is influenced by both internal biological factors (such as aging and hormonal balance) and external environmental factors (for example diet and humidity). As we age, our skin loses its elasticity, becoming fragile and frail (Al-Nuaimi, Sherratt, & Griffiths, 2014; Naylor, Watson, & Sherratt, 2011), mainly due to the progressive loss of skin tissue

(Robert, Labat-Robert, & Robert, 2009). Research conducted by Nkengne and colleagues (2008) showed that skin condition was one of the most important criteria used when judging the perceived age of individual. Elevated level of hormones has also been highlighted as a factor contributed to the physiological changes in skin. Specifically, women with increased levels of androgen were found to have more severe dermatoses problems (Paulina a Essah, Wickham, Nunley, & Nestler, 2006; Karrer-Voegeli, Rey, & Reymond, 2009).

Kammeyer and Luiten (2015) suggested that aging of skin is more influenced by external factors, such as solar radiation, cigarette smoke, and air pollution, than internal factors. Environmental dryness and individual differences in diet, for example, have also been found to be related with the prevalence of acne (Danby, 2005; Davidovici & Wolf, 2010; Egawa, Oguri, Kuwahara, & Takahashi, 2002; Spencer, Ferdowsian, & Barnard, 2009).

The colour distribution of facial skin was found to influence the perception of age and attractiveness in female faces, independent of facial shape and skin surface topography (Fink et al., 2006). Faces with homogenous skin colour distribution were perceived as younger, healthier and more attractive as compared to those faces with relatively inhomogeneous skin colour distribution. The same pattern was found for male faces, whereby the rating

of cheek skin can be used to predict the age, health status and attractiveness of male faces (Fink, Bunse, Matts, & D'Emiliano, 2012).

By using a computer programme that implemented an algorithm based on the co-occurrence matrix to analyse skin texture, Fink and colleagues (2001) found that skin texture affected male judgments of attractiveness in female faces. It is apparent from previous literature that the physical appearance of human skin, both its texture and the evenness of skin colour play an important role in determining its attractiveness and perceived health.

2.2.1.1 Hypothesis

Based on the results of previous studies conducted in the West, exploring the inter-correlation between skin texture and attractiveness (Fink et al., 2006, 2001; Fink, Matts, Röder, Johnson, & Burquest, 2011; Jones, Little, Burt, et al., 2004; Matts et al., 2007; Samson, Fink, & Matts, 2010), it was predicted that there will be a relationship between perceived facial health and a measure of skin texture (measured using Gabor wavelet analysis).

2.2.2 Methods

This study was approved by the Ethics Committee at the University of Nottingham Malaysia Campus. All participants gave both verbal and written informed consent prior to the experiment. The current study was divided into two main parts. In Part 1, a group of participants (n=143) were recruited to

obtain their body measurements and for their facial photographs to be taken. In Part 2, the standardized photos collected in Part 1 were evaluated by an independent group of participants (n=72) for their perceived facial health.

2.2.2.1 Body measurement and photography session

One hundred and forty three Malaysian Chinese were recruited for the first part of this study by two different experimenters (TKW, VH), with 67 males and 76 females (mean age = 20.41, SD=1.24). All were undergraduate or postgraduate students at the University of Nottingham Malaysia Campus.

Participants recruited for the first part of the study had their facial photograph taken under controlled conditions. Facial photographs of the participants were taken, with a Canon EOS 550D digital camera in a photo booth painted Munsell N5 grey and illuminated with nine Verivide F20 T12/D50 daylight stimulation tubes in high frequency fixtures to reduce the effects of flicker. Shutter speed, ISO, aperture and white balance settings of the camera used were held constant. Participants were requested to sit on a chair inside the photo booth. The hair was held back from the face with a black head band, and participants were asked to pose with a neutral expression while holding a Munsell N5 painted board over their shoulders to obscure clothing.

2.2.2.2 Analysis of skin texture and colours

Psychomorph was used to delineate the all 143 facial images with a total of 179 coordinate landmarks plotted on each face. The size of the facial photos used was then standardized by aligning the eyes of the images to the same locations.

One hundred forty three skin patches were cropped from the left cheek area of 143 facial photographs using computer software Matlab. Examples of skin patches are shown in Figure 2.1.



Figure 2.1. Examples of three skin patches produced by cropping the left cheek area of 143 facial photographs with Matlab.

Gabor wavelet analysis was performed, using Psychomorph (Tiddeman & Perrett, 2001), on the 143 skin patches created, to obtain objective measurements of skin texture. Gabor wavelet analysis divides the data into different frequency components, and studies each component with a resolution matched to its scale (Vinay & Shreyas, 2006). Thirty two outcomes

(indices) were produced, after analyzing the image's imaginary and real components, and loops from finest scale to lowest scale (four different scales, where the filter of the final scale was 8 times bigger than the first scale), over 4 different orientations (0 degrees, 90 degrees, 45 degrees, -45 degrees).

Principle component analysis was then performed with SPSS to reduce these 32 indices into a smaller set of factors. Correlational matrices were examined prior to the administration of the factor analysis, to check the strength of the correlations. Three factors (named as Gabor wavelet factor 1, Gabor wavelet factor 2 and Gabor wavelet factor 3) were selected, with eigenvalues higher than 1.00, which accounted for 97.18% of the variance.

2.2.2.3 Rating of perceived facial health and attractiveness

Facial images were presented in Qualtrics, a web based data collection software, in two randomized blocks. Participants were asked to rate the faces presented on a scale from 1 (very unhealthy) to 7 (very healthy), and key in 9 if they know of any of the faces presented. Seventy two Malaysian Chinese participants (28 male, 44 female; mean age = 23.54, SD=2.19; all with normal colour vision) were allowed to take as much time as they want to rate the images. All the images within the blocks (each block consists of all photos taken by the same researcher) were presented in random order.

2.2.3 Results

2.2.3.1 Preliminary test of normality

Tests of normality were performed. According to the test of Shapiro-Wilk, the perceived facial health (p=.813) rating is normally distributed. However, all the three Gabor wavelet factors were not normally distributed (p<.001) and could not be transformed (and normalized). Therefore, Spearman's correlations were used in the analysis.

2.2.3.2 Spearman correlation between health rate and Gabor wavelet factors

Spearman's correlation was chosen to analyse the correlation between perceived facial health and the Gabor wavelet factors, as all of the independent variables were not normally distributed. Rated health was correlated with all three Gabor wavelet factors, namely Gabor wavelet factor 1, $\rho(143) = .30$, p<001; Gabor wavelet factor 2, $\rho(143) = -.26$, p = .002 and Gabor wavelet factor 3, $\rho(143) = -.21$, p = 014 (Fig 2.2-2.4).

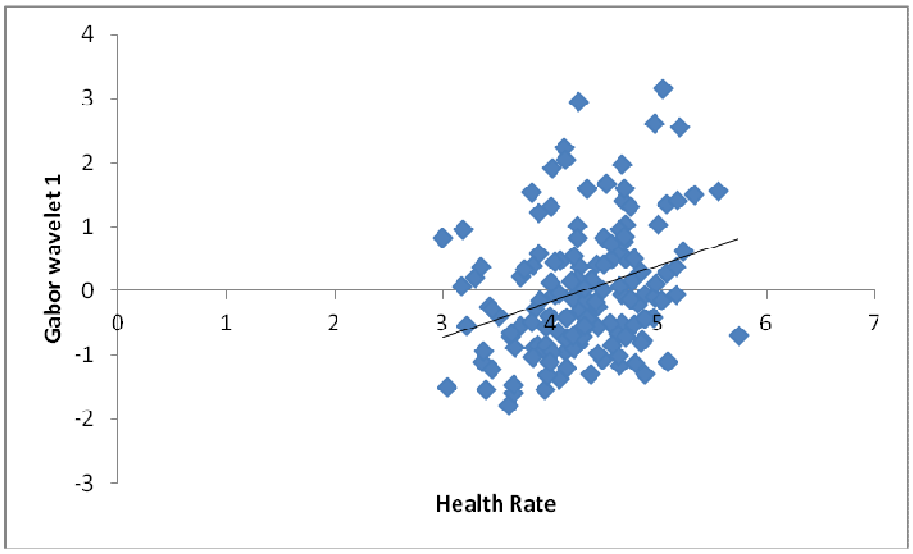


Figure 2.2. Correlation between health rate and Gabor wavelet factor 1

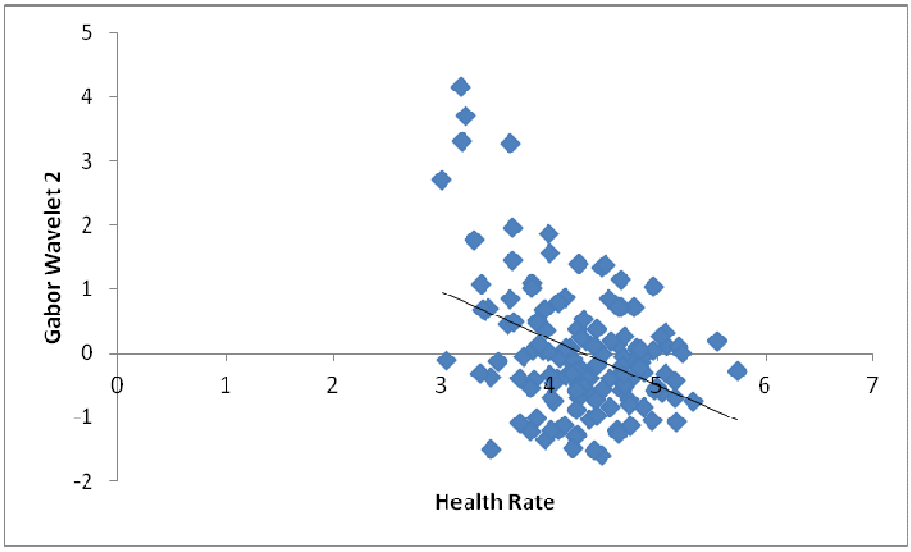


Figure 2.3. Correlation between health rate and Gabor wavelet factor 2

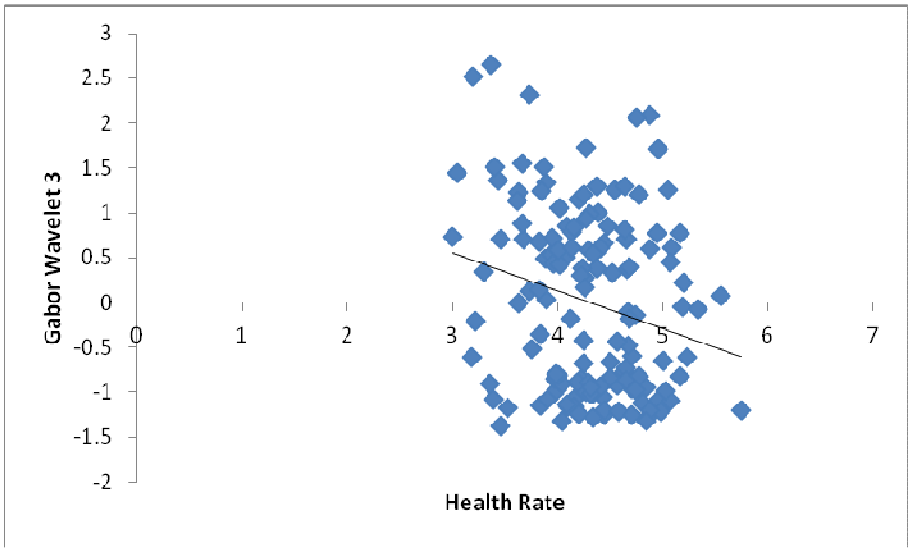


Figure 2.4. Correlation between health rate and Gabor wavelet factor 3

2.2.3.3 Sex differences in Gabor wavelet factors

Non-parametric Mann-Whitney's U tests were performed to examine the sex differences in the three Gabor wavelet factors. The analysis indicated that there was a significant sex difference only for Gabor wavelet factor 2 (GW2), Mann-Whitney U = 1293, $p<.001$, with males scoring higher (median = .19) than the females (median = -.38). The results of the analysis were summarized in the Table 2.1 below.

Table 2.1

Sex differences for the Gabor wavelet factors (medians are presented because of the nonparametric nature of the data)

Variable	Male	Female	U	Z	P
GW1	-.049	-.158	2231	-1.27	.20
GW2	.188	-.381	1293	-5.07	.00**
GW3	.222	-.454	2125	-1.70	.09

**p<.001

2.2.4 Discussion

Consistent with results from the West (Lefevre, Ewbank, Calder, von dem Hagen, & Perrett, 2013; Lefevre & Perrett, 2015; Stephen, Coetzee, et al., 2009; Stephen, Law Smith, et al., 2009; Stephen et al., 2012), Study 1 found significant correlations between skin condition and perceived face health. The three outcomes of the Gabor Wavelet analysis obtained from the facial skin patch were all significantly correlated with the perceived face health. Gabor Wavelet factor 1 was positively correlated with perceived face health, while Gabor Wavelet factor 2 and 3 were negatively correlated with perceived face health.

2.2.4.1 Relationship between perceived facial health and Gabor Wavelet factors

Examples of the three skin patches with highest values of Gabor wavelet factor 1 are presented in Figure 2.5 (which the overall face were perceived as relatively healthier) while those with lowest values were presented in Figure 2.6. Visually, skin patches with highest Gabor wavelet factor 1 (GW1) values look lighter and yellower as compared to those low in GW1. Besides differences in skin colours, from the samples selected from the two extreme ends of the values of GW1, can be seen that those skin patches with lower GW1 values has lots red spots on them, as compared to those high in GW1 values, which are relatively smoother.



Figure 2.5. Skin patch from left check with highest Gabor wavelet factor 1 values



Figure 2.6. Skin patch from left check with lowest Gabor wavelet factor 1 values

Figure 2.7 shows examples of the three skin patches with highest values of Gabor wavelet factor 2 (which the overall face were perceived as relatively less healthier), while Figure 2.8 shows patches with the lowest GW2 values. As found in the analysis, skin patches with higher Gabor wavelet factor 2 (GW2) values are perceived as least healthy. From six samples selected from the two extreme ends of the values, it can be seen that those skin patches with highest values have more scarring and holes, as compared to those scoring the lowest in GW2. It seems that there might be a difference in skin colouration between the skin patches from two extreme of the GW2.



Figure 2.7. Skin patch from left check with highest Gabor wavelet factor 2 values



Figure 2.8. Skin patch from left check with lowest Gabor wavelet factor 2 values

Results showed that skin patches with higher Gabor wavelet factor 3 (GW3) values are perceived as less healthier. Figure 2.9 showing example of the three skin patches with highest values of Gabor wavelet factor 3 (which the overall face were perceived as relatively less healthy), while Figure 2.10 shows patches with the lowest GW3 values. Again, we can see that images with low GW3 values look smoother as compared to those with highest values. There seems to be a slight difference in the colouration between the skin patches from the two extemes of GW3.



Figure 2.9. Skin patch from left check with highest Gabor wavelet factor 3 values



Figure 2.10. Skin patch from left check with lowest Gabor wavelet factor 3 values

By comparing the skin pacthes obtained from the two extreme ends of the three Gabor wavelet factors, it is apparent that skin condition, including its smoothness and colour homogeneity, does play an important role in defining perceived facial health of human faces. Individuals preferred smooth skin with even colouration, suggesting that these traits may act as cues to underlying physiological condition, including the level of denegeration and photodamage due to UV radiation (Kammeyer & Luiten, 2015; Naylor et al., 2011) or hormone levels (Essah, Wickham, Nunley, & Nestler, 2006; Karrer-Voegeli et al., 2009) which can reflect the person's reproductive health.

2.2.4.2 Limitations of the study

There are a few limitation in this study. Firstly, it seems that there were some differences in the colourations of the skin patches from the two extremes of the Gabor wavelet factors. However, as we do not have the data of the actual skin colour of those facial images, it cannot be concluded.

Secondly, the perceived health ratings were made on participants's private computers. Aside from the differences in surroundings, the contrast and colour settings of the computers might be slightly different too. Future research should be conducted in a controlled setting, on a standardized colour-calibrated computer. Therefore, a new study (Study 2) was designed, collecting data on skin pigmentation using spectrophotometry and conducting rating data collection on a standardized colour-calibrated computer.

Nonetheless, the current study provides insight into the health perception of an Asian population, and is the first study of which we are aware conducted in an East Asian setting, exploring the inter-relationship bettwen facial skin condition and perceived facial health of Asian faces.

2.3 Study 2: Predicting apparent health from skin patches

2.3.1 Introduction

Skin carries numerous biological and social messages. In many non-human animal species, the quality and colour of skin, feather and scale are important indicators of health (Peters et al, 2004; Saks et al., 2003). Various animal species use vibrant ornaments to attract mates (Blount et al., 2003; Bourne et al., 2003; Massaro et al., 2003; MacDougall & Montgemerie, 2003). Human skin is thought to have a similar function, whereby skin condition, including both its texture and colour, has been linked to various aspects of physiological health status (Fink et al., 2001; Stephen, Law Smith, et al., 2009).

Jones and colleagues (2004) showed that skin condition is an important cue for attractiveness, independent of facial shape. By using small skin patches extracted from the left and right cheeks of digital images, they found significant correlations between health ratings of skin patches and the attractiveness of male faces.

Fink and colleagues (2001) studied the importance of skin texture to facial attractiveness by using the co-occurrence matrix to examine spatial relationships of pairs of grey values of pixels in digital images, and found a significant correlation between the skin texture and women's facial attractiveness, as judged by men. The homogeneity of the skin colour distribution is one factor that influences apparent health of the faces,

especially for female faces (Fink et al., 2012, 2006; Matts et al., 2007). Faces with evenly distributed skin colouration were perceived as younger, healthier and more attractive as it may signal underlying physiological condition of a person.

Colour of the face too was found to be related with both facial attractiveness and apparent health (Fink et al., 2001; Stephen, Coetzee, Law Smith, & Perrett, 2009b; Stephen et al., 2011). While increments in skin redness and skin yellowness were perceived as healthier in a range of samples (Coetzee et al., 2012; Re et al., 2011; Scott et al., 2010; Stephen, Coetzee, et al., 2009a; Stephen et al., 2011), there was some disagreement about whether lighter or darker skin tones was preferred (Coetzee et al., 2012; Fink et al., 2001; Stephen et al., 2011).

Skin condition has been argued to better predict attractiveness than facial shape. By using an objective measure of masculinity, measurement of skin colour and rated attractiveness of 72 men's faces, Scott et al. (2010) found that skin yellowness predicts attractiveness more accurately than structural masculinity.

Stephen and colleagues (2009, 2011) found that both Caucasian and African samples increased skin yellowness (b*), skin redness (a*) and skin luminance (L*) to optimize the apparent health of facial photographs. It was suggested

that increased facial redness reflects better aerobic fitness (Armstrong & Welsman, 2001; González-Alonso et al., 2008); increased facial yellowness reflects higher fruit and vegetable consumption (Stephen et al., 2011) and thus reflects better immune system (DA Hughes, 1999); while increased skin lightness is preferred by both Caucasian and African samples (Stephen et al., 2011), because with darker skin tone, the increased level of melanin will block more ultra violet sunlight, inhibiting the ability to synthesize vitamin D in the skin (Kourosh et al., 2010). Contrary to what was found in the above study (Stephen et al., 2011; Stephen, Law Smith, et al., 2009), Fink and colleagues (2001) however suggested a preference towards darker suntanned skin tone, as an honest signal of status (time for leisure.)

Study 2 was conducted as an extension to Study 1. There are three major improvements in this study as compared to the previous one. Firstly, Study 2 measured actual skin colour of a sample of individuals with a spectrophotometer. Second, the experiments were conducted on a colour-calibrated computer, to ensure that the participants were exposed to the same standardized settings of luminance, contrast of the presented stimulus. Third, a subjective judgement of facial skin health was obtained from an independent sample of participants, to examine if facial skin (cropped from cheek area) can be a reliable predictor of facial health.

2.3.1.1. Objective of the study

Using both the actual colouration obtained from skin pigmentation, and the subjective rating of cropped skin patches by an independent sample of participants, Study 2 hypothesized that:

1. There will be a positive correlation between perceived skin health and perceived face health.

2. There will be a correlation between the Gabor Wavelet factors, and subjective ratings of skin health.

3. There will be a positive correlation between skin yellowness, skin redness, skin luminance, and subjective ratings of face health and skin health.

2.3.2 Methods

The current study was divided into two main parts. In Part 1, a group of participants (n=53) were recruited and skin colour measurements were made using a spectrophotometer. Standardized photos were taken, colour calibrated and analysed using Gabor wavelet analysis and then evaluated by another group of participants (n=42) for their perceived facial health in Part 2 of the study using Psychopy. A colour calibrated computer was used to standardize the colouration of the image presented to the participants.

2.3.2.1 Photography session and skin pigmentation

Fifty three Malaysian Chinese (24 male, 29 female; mean age = 20.62, SD=1.33) volunteered to participate in this study. They were all students from University of Nottingham Malaysia Campus. Participants had their skin colour measured, and their facial photographs were taken under the same controlled conditions as described in Study 1. All images were taken with a Gretag Macbeth Mini Color Checker colour chart included in the frame, and were colour-calibrated using Psychomorph (Tiddeman, Stirrat, & Perrett, 2005). It was a least squares transform from the colours represented in the image to published values of the patches on the colour card.

The skin colours were measured using a handheld Konica Minolta CM2600D spectrophotometer. This is a painless, non-invasive method utilising the Commission International de l'Eclairage (CIE) L*a*b* colour system measuring the reflectance of luminance (L*), redness (a*) and yellowness (b*) on the targeted object.

Three facial regions of participants were measured: the right cheek, left cheek and forehead. Two sets of measurements were taken for each region, and the averaged figures were used for analysis. The spectrophotometer was calibrated each time it was switched on.

All facial photos were colour-calibrated and their eyes were aligned to the same locations using Psychomorph (Tiddeman & Perrett, 2001). Matlab was used to create a skin patch by cropping the left cheek part of the photos. Fifty two skin patches were created. Gabor wavelet analysis was performed, using Psychomorph (Tiddeman & Perrett, 2001), on the 52 skin patches created, the same way it was performed in Study 1. Principle component analysis was then performed, within the same software, to reduce these 32 indexes into a smaller set of factors. Three factors (named as Gabor wavelet factor 1, Gabor wavelet factor 2 and Gabor wavelet factor 3) were selected, with eigenvalues higher than 1, which accounted for 96.87% of the variance.

2.3.2.2 Rating of perceived facial health

Forty eight Malaysian Chinese were recruited as the raters of perceived facial health, with 21 males and 27 females (mean age = 21.96, SD=2.81). All participants were students pursuing undergraduate or postgraduate degrees at two private institutions of higher education in Malaysia - University Putra Malaysia (UPM) and Universiti College Sedaya International (UCSI). All participants have either normal or corrected vision; and normal colour vision.

Colour calibrated facial images were presented using Psychopy (Peirce, 2009), on a computer attached to a 15" TFT monitor that was colour calibrated with a Data Color Spyder3 Pro. All the facial images were presented once to the participants, one at a time, for one second, during the Preview Phase. No

rating was needed during the Preview Phase. Participants were then presented with the same facial images again, one image at a time, during the Rating Phase where they were asked to rate the faces shown on a scale from 1 (very unhealthy) to 7 (very healthy). Participants were requested to key in 9 if they were acquainted with of any of the faces shown in the experiment, and thus that data will be excluded from the analysis. They were allowed to take as much time as they wanted for each trial. The images were presented to the participants in random order.

2.3.2.3 Rating of skin's health

Another 42 participants (22 male, 20 female; mean age = 22.5, SD=3.022) were recruited to judge the apparent health of the skin patches created by cropping the left cheek region of the facial photos. All participants were Malaysian Chinese university students, with normal colour vision.

Skin patches were presented using Psychopy (Peirce, 2009); the same way facial images were presented to the participants. Participants were asked to rate the skin patches shown on a scale from 1 (very unhealthy) to 7 (very healthy), and to key in 9 if they think that the patch presented was not clear. The skin patches were presented in random order.

2.3.3 Results

2.3.3.1 Preliminary test of normality

Shapiro-Wilk tests of normality were performed on the two ratings (perceived facial health, and perceived skin health), the three facial colours (luminance, redness and yellowness), and the three Gabor wavelet factors. Results showed that both perceived facial health and perceived skin health are normally distributed, together with Gabor wavelet factor 1 and facial skin yellowness (all $p>.05$). All the other factors (facial luminance, facial redness, Gabor wavelet factor 2 and 3) were not normalized, and could not be normalized with transformation.

2.3.3.2 Correlation between subjective rating of face health and skin health

Pearson correlation was conducted to investigate the relationship between perceived facial health and rated skin health. As expected, we found a significant positive correlation (two tailed), $r =.678$, $p<.001$ between perceived face health and rated skin health.

2.3.3.3 Correlation between Gabor Wavelet factors with subjective rating of skin health.

Spearman's rank correlation was chosen to investigate the relationship between objective measurements of skin texture (with Gabor Wavelet analysis) and rated skin health.

As predicted, results indicated significant correlations (two tailed) between rated skin health and Gabor wavelet factor 2, ρ (53) =-.782, p<.001. There was no significant correlation between rated skin health and Gabor wavelet factor 1, ρ (53) =-.122, p=.384; between rated skin health and Gabor wavelet factor 3, ρ (53) =-.052, p=.711. As we suspected that the Gabor Wavelet factors were influenced by the skin colouration (L*, a* and b*), partial correlations were conducted between skin health and the Gabor Wavelet factors, controlling for the three skin colour axes. The relationship between skin health and Gabor Wavelet factor 2 remained significant, ρ (53) =-.746, p<.001, while the correlation between rated skin health and Gabor Wavelet factor 1 ρ (53) =-.07, p=.631, and Gabor Wavelet factor 3 ρ (53) =.093, p=.521, remained non-significant.

2.3.3.4 Correlation between skin colours with subjective rating of perceived facial health and perceived skin health.

The average colours of the three facial regions (left cheek, right cheek and forehead) were compiled. Spearman's rank correlation was chosen to investigate the relationship between the facial skin colours (in luminance, redness and yellowness), perceived face health, and rated skin health.

Result found a significant correlation between perceived face health and facial yellowness, ρ (53) = .29, p = .034, between perceived skin health and facial luminance, ρ (53) = .42, p = .002, between perceived skin health and

facial yellowness, ρ (53) = .30, p=.03. The results of the Spearman correlation were compiled below, in Table 2.4.

Table 2.4

Spearman correlation between perceived facial health, rated skin health and three facial colourations

		Face L*	Face a*	Face b*
Perceived facial health	ρ	.034	.014	.292*
	p	.809	.921	.034
Perceived skin health	ρ	.423**	-.214	.298*
	P	.002	.124	.030

* p<.05, ** p<.01

2.3.4 Discussion

The healthiness of the skin patches (obtained from left cheek area of facial images) was found to be positively correlated with the perceived facial health of the respective faces, as judged by independent groups of raters. Jones and colleagues (2004) had previously showed that healthier skin patches were judged as more attractive. Here, we found an agreement between the perceived health of cheek skin patches and facial images, in an Asian sample. This suggests that the condition of skin patch alone is sufficient enough to be used to predict the apparent health of human face, independent of facial structure.

Previous research (Roberts et al., 2005) has found an association between health in skin patches and heterozygosity at three key loci in the major histocompatibility complex (MHC). Heterozygosity in major histocompatibility complex is suggested to be advantageous in the resistance to infectious disease. Roberts and colleagues (2005) too found agreement between rated skin health and facial attractiveness.

Consistent with the hypothesis, the outcomes of the Gabor Wavelet analysis, which were proposed to be reflecting skin texture, were found to be correlated with subjective ratings of skin health and face health. However, as we suspected that the skin colours contributed to the outcomes of the analysis, partial correlations were conducted to control for the influences of skin colours. The association between Gabor wavelet factor 2 and rated skin health remained significant after controlling for skin colours (L*, a* and b*). Overall, results of the analyses indicated that skin condition (smoothness and colour homogeneity), especially the Gabor wavelet factor 2 impacts on skin health ratings.

Skin yellowness was found to be correlated with both skin health and facial health rate, while skin luminance was correlated with skin health. This result is consistent with what was being found by a few recent researches (Stephen et al., 2011; Stephen, Law Smith, et al., 2009). While there were

inconsistencies on the preference on skin luminance (Fink et al., 2001; Stephen et al., 2011), the result on preference on skin yellowness had been somehow consistent, with individual from various culture perceived a slight yellower skin tones as healthier (Coetzee et al., 2012; Stephen et al., 2011; Stephen, Law Smith, et al., 2009).

Both carotenoids and melanin contributed to skin yellowness, but previous research has shown that carotenoid colouration held a more significant contribution to judgment of apparent health, as compared to melanin colouration (Lefevre & Perrett, 2014). Detailed explanation about the importance of skin texture and skin colours on health perception will be discussed in General Discussion.

2.4 Study 3: Healthy skin colouration

2.4.1 Introduction

Facial skin colour has been shown to influence perceived attractiveness (Jones et al, 2004) and health, with increased lightness (CIELab L*), redness (a*) and yellowness (b*) perceived as healthier (Stephen et al, 2009a), suggesting that skin colour may represent a valid cue to health.

Skin lightness is primarily determined by the concentration of melanin (Edwards & Duntley, 1939). Previous studies have found inconsistent results on the preference for skin luminance with some arguing the significance of lighter skin tones (Coetzee et al., 2012; Stephen et al., 2011), while others suggested darker skin tones were perceived as more attractive (Fink et al., 2001). Perhaps it can be related with the evolution of skin colours according to the unique demands of different locations (Jablonski & Chaplin, 2000). Individuals living far away from the equator, with insufficient sunlight, might prefer reduced melanin concentration, which may be associated with better absorption of sunlight which helps the production of vitamin D (Hochbergh & Templeton, 2010). Individuals who live near the equator on the other hand need higher concentrations of melanin to protect themselves from ultraviolet radiation, so that they won't get sunburn, photodamage and skin disease easily (Jablonski, 2004; Yuen & Jablonski, 2010).

Facial redness is determined by skin blood perfusion, which is controlled by vascularisation (formation of blood vessels) and vasodilation (widdening of blood vessels). Blood flow increases during vasodilation, which makes the skin look redder. A slight increment in facial redness enhances apparent health, as it reflects healthy blood circulation. Skin vasodilation was found to be compromized among smokers (Richardson, 1988) and patients with type 2 diabetes (Charkoudian, 2003). Conversely, exercise was found to improve vasodilation. With the frequent demand for increased blood flow to muscle tissues, physical training enables the circulatory system to meet those demands (J. Johnson, 1998).

Skin redness also depends on the blood oxygenation state. Whilst oxygenated blood is bright red as the hemoglobin molecules are attached with oxygen when travelling away from the heart, the deoxygenated blood looks slightly purplish after blood has delivered oxygen to the cells. Oxygenated blood colour, which is associated with cardiovascular fitness, has been shown to make an individual's face look healthier (Stephen, Coetzee, Smith, & Perret, 2009) and thus more attractive (Jones, Little, Burt, & Perret, 2004).

Increased facial skin yellowness has been associated with higher carotenoid concentration (Alaluf et al., 2002). Individuals with higher levels of fruit and vegetable intake tend to have yellower skin tones as compared to their counterparts with low fruit and vegetable intake (Pezdirc et al., 2014; Stephen

et al., 2011). Carotenoids are associated with better immune system and protection against various diseases in humans (Krinsky & Johnson, 2005; Rao & Agarwal, 2000; Tapiero, Townsend, & Tew, 2004b). Their deposition in skin also contributes to photoprotection against UV radiation (Alaluf et al., 2002; Fraser & Bramley, 2004; Stahl et al., 2000).

2.4.1.1 Objective of the study

Despite the great volume of research on human attractiveness, research on the contribution of facial colour to human perceived facial health and attractiveness were very limited (Fink et al., 2008). It has been found in a previous study that Caucasian and African participants perceived faces with increased redness, yellowness and lightness as healthiest (Stephen et al., 2009a). However, no similar studies have been conducted in an Asian setting.

Individuals' perception of health changes according to the environment that they are living in, as different societies have different health priorities. For example, while being overweight might have certain advantages, and thus is perceived as healthy by individuals living in rural societies (Johnston & Franklin, 1993), it imposes future health risks for individuals who live in urban societies with an abundance of food (Cepeda-Valery et al., 2011; Rampal et al., 2007). Previous studies have discovered cultural variation in individuals' preference for body size and (Swami & Tovée, 2005a, 2005b) waist to hip ratio (Marlowe, Apicella, & Reed, 2005; Marlowe & Wetsman, 2001) whereby

preference for body size and shape changes according to the economical gradient, with individuals living in rural areas prefer a slightly bigger body size, higher WHR as compared to those living in urban cities.

Previous research (Stephen et al., 2011) has found a similar pattern in skin colour preferences for Caucasian and African samples. However, as human skin colour has been suggested to adapt according to the demands of the environment such as the availability of UV light source and bacterial load (Jablonski & Chaplin, 2000), it is uncertain whether the same preferences can be found in East Asian populations. Here in Study 3 , the importance of skin luminance, skin redness, skin yellowness, oxygenated blood colouration and deoxygenated blood colouration were examined. It was hypothesized that:

- Participants will prefer redder skin tone, as skin redness is associated with aerobic fitness and fertility.
- Participants will also prefer yellower skin tone, which reflects fruit and vegetable consumption, and indirectly gives cues about one's immune system.
- Participants will prefer a lighter skin tone, as it indicates a better absortion of sunlight which is useful for the systhesis of Vitamin D.

2.4.2 Methods and Materials

This is a within-participants design whereby the participants went through all five conditions in the study. The study was approved by the Ethics Committee at the University of Nottingham (Malaysia Campus). All participants gave both verbal and written informed consent prior to the experiment.

Twelve facial photographs of three different ethnicities (4 Caucasians, 4 Africans and 4 East Asians) were used in this study. All photos were taken the same way as described in Study 1.

2.4.2.1 Skin Colour Pigmentation

To obtain the oxygenated and deoxygenated blood colouration for East Asian faces, ten Malaysian Chinese with equal number from each gender were recruited from the student population at UNMC. Participants' skin colour on the dorsal interosseous region (the region between the muscles in the back of the hand that act to abduct the index, middle, and ring fingers away from hand's midline, refer Figure 2.11) of their left hand were measured using Konica Minolta CM2600D reflectance spectrophotometer. The reflectance spectrophotometer is a painless, non-invasive device for colour measurement. Participants' skin colour was measured in four different conditions whereby the participants were asked to:

1. raise their left hand for 10 seconds before the measurement, to collect skin pigmentation for low deoxygenated blood condition;

2. stand with both hands placing gently by both sides of the body for 30 seconds, for high deoxygenated blood condition;

3. rest their left hand on the table for 2 minutes while sitting on a chair, for low oxygenated blood condition, and

4. to soak their left hand inside a bowl of 45-50°C warm water for 5 minutes for high oxygenated blood condition.

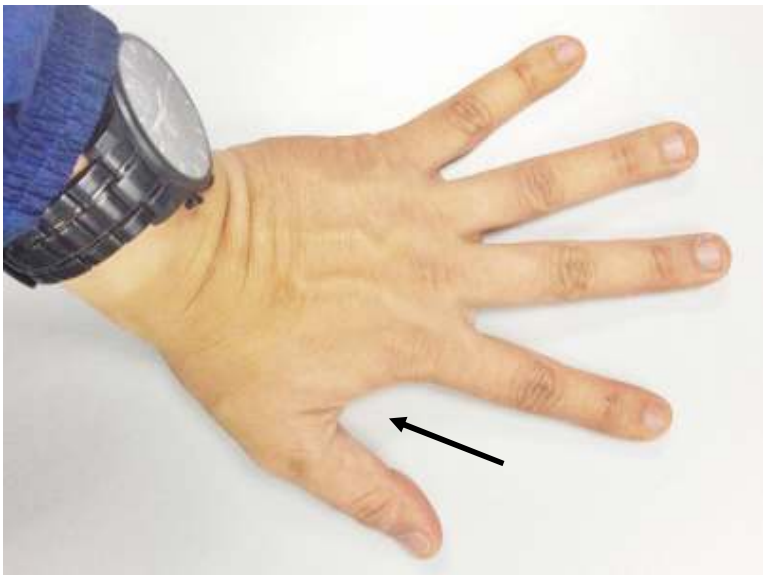


Figure 2.11 Dorsal interosseous region

The purpose of these procedures is to collect the colour of skin with high and low deoxygenated blood, and high and low oxygenated blood (Stephen et al, 2009; Re et al, 2011). The first dorsal interosseous region of the left hand was chosen because of its relatively lack of visible tendons and veins. Furthermore,

the skin colour at the body region is similar to that of the face, and it is more practical to submerge the hand than the face in hot water.

The method that was proposed to collect the skin colour for high oxygenated blood, by soaking the hand in 45-50^{o}C water has been shown to be safe (Stephen et al., 2009; Re et al., 2011; Penneys & Montgomery, 1952). Water with 45-50 degree celcius is warm enough to cause temporary flushing of the skin, and may cause minor discomfort, but is not hot enough to cause any damage. Participants were told to withdraw their hand from the hot water if they feel uncomfortable. The temperature of the hot water used was checked by the experimenter using a thermometer before the participant soaked their hand. Participants who had cuts on their hand were not allowed to participate in the measurement. The same sequence was repeated in the same order for all participants. CIELab measurement in each of the four conditions were later averaged across participants for photo colour manipulation purposes.

The oxygenated and deoxygenated blood colouration for Caucasian and African faces were obtained from Stephen et al. (2009a). The amount of change in skin luminance, redness and yellowness during oxygenation and deoxygenation of the blood are summarized below in Table 2.2

Table 2.2

Changes in skin luminance, redness and yellowness during oxygenation and deoxygenation of the blood, for the faces of three ethnics (African, Caucasian and East Asian).

Ethnic	Colouration	L*	a*	b*
African	Oxygenated	1.31	.81	.09
	Deoxygenated	-2.67	1.26	-1.91
Caucasian	Oxygenated	2.25	5.36	-.17
	Deoxygenated	-4.98	4.29	-1.57
East Asian	Oxygenated	1.34	2.74	-1.58
	Deoxygenated	-3.10	1.48	-1.83

2.4.2.2 Skin Colour Transformation

Matlab was used to produce masks with even colouration representing the skin areas of faces, with a Gaussian blur at the edges. One mask was created to represent average face colour +8 units of a* (increased redness) and another one with average face colour –8 units of a* (decreased redness). The facial redness of all the twelve faces used was transformed by the difference in colour between each of the pairs of masks. This produced a single manipulation composed of a series of 13 frames, numbered from 0 to 12, whereby frame 0 had skin redness reduced by 8 units of a*, increasing incrementally so that frame 6 would be the original image and frame 12 had

skin redness increased by 8 units of a*. For all the transformations, participants' hair, eyes and clothing and the background remained constant.

The same procedures were used to create a series of same-face-photos with different levels of lightness, yellowness, oxygenated and deoxygenated blood colour. Examples of the photos used are shown in Figure 2.12

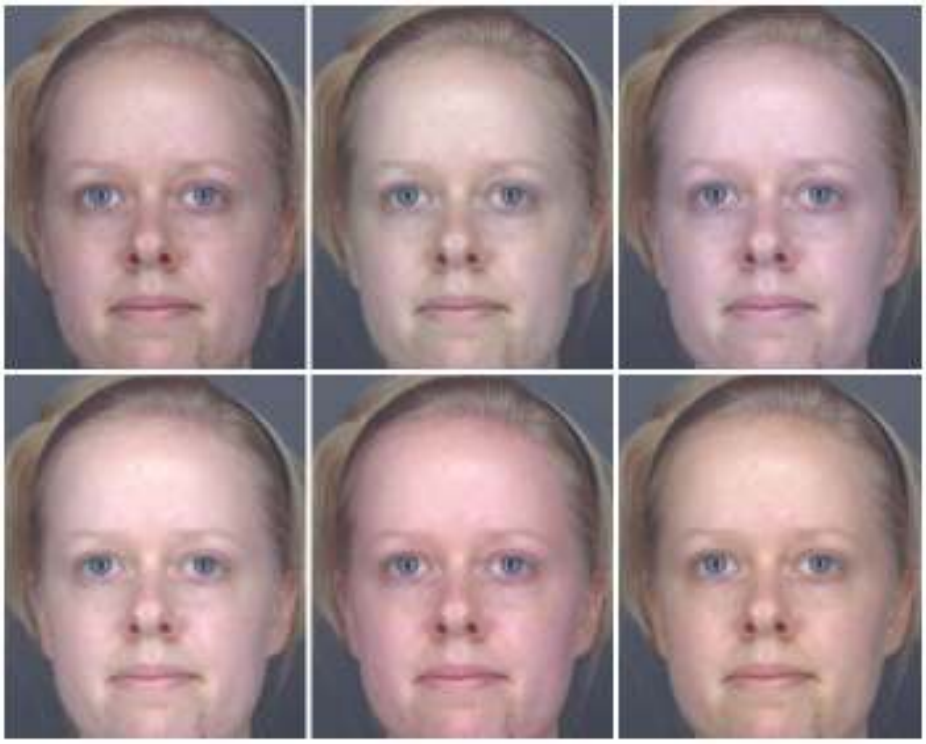


Figure 2.12. Facial images of a Caucasian female after CIELab colour transformation. Facial image with decreased lightness (top left), decreased redness (top middle), decreased yellowness (top right), increased lightness (bottom left), increased redness (bottom middle) and increased yellowness (bottom right).

2.4.2.3 Participants and Procedure

Forty four Malaysian Chinese (18 males, 26 females; mean age = 22.05, SD= 1.23) were recruited for this study. All participants were students at Universiti Tunku Abdul Rahman.

Stimuli were presented using computers attached to 15" TFT monitors that were colour calibrated with a Data Colour Spyder3 Pro. Participants were presented with facial images, one image at a time, and were asked to adjust the colour of skin portions of the facial images presented. By moving the computer mouse horizontally, the participants cycled through the 13 frames of the images (same face, difference level of colour intensity). The participants clicked on the mouse when they felt that the face looks the healthiest.

Each facial image was presented once on each of the five different colour dimensions (lightness, redness, yellowness, oxygenated colouration, and deoxygenated colouration), making a total of 60 trials (12 faces * 5 colourations) for the whole experiment. The location of the midpoint was randomized, and the order of the trials was also randomised.

2.4.3 Results

Paired sample t-tests were performed to investigate if there were sex differences for all the colour changes, and there was no significant difference

detected for lightness, t(43)=1.39, p=.17, redness, t(43)=.9, p=.38, yellowness t(43)=.17, p=.87, oxygenated blood t(43)=.56, p=.58 or deoxygenated blood t(43)=.27, p=.79 chosen to optimise healthy appearance. Data from both sexes were therefore combined.

The frames selected were then converted into Euclidean distances, and it was found that participants increased facial yellowness by 1.31 units (SD = 1.27), facial redness by 0.85 units (SD = 1.08), oxygenated blood colour by 0.67 units (SD = 1.15), and deoxygenated blood colour by 0.83 units (SD = 1.73), and decreased facial lightness by 0.37 units (SD = 1.05), as showed in Figure 2.13.

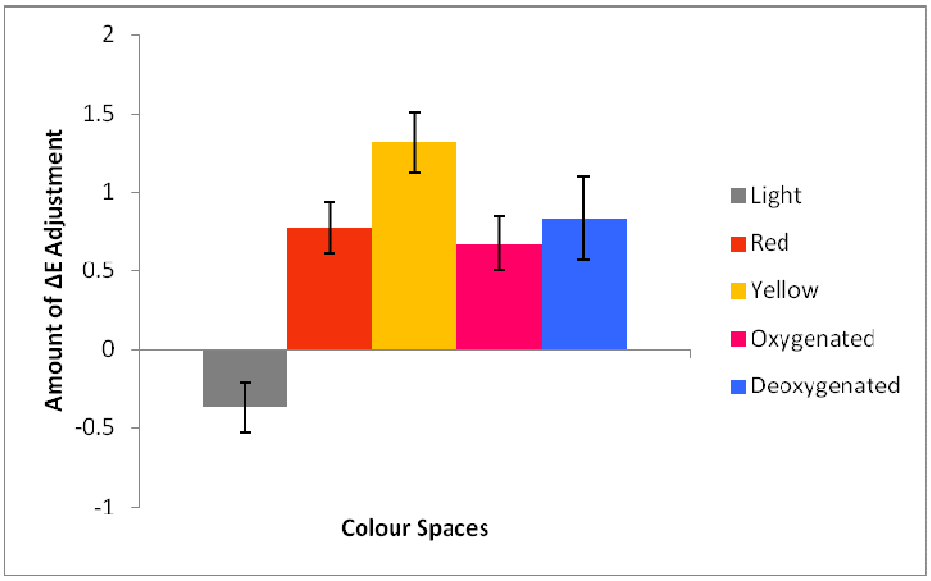


Figure 2.13. Amount of colours being adjusted by the participants.

Mean colour changes that were applied to the twelve faces within the same colour space were calculated. A one-sample t-test was conducted to check if

the colour of the faces was changed to optimise the bearer's perceived facial health. It was found that participants significantly increased facial yellowness, redness, oxygenation and deoxygenation of blood colour, and decreased facial lightness, to make the stimuli look the healthiest (Table 2.3).

Table 2.3

Summarised statistics from the one sample t-test.

Facial Colour	t	p
Lightness	-2.291	p=.027
Redness	4.722	p<.001
Yellowness	6.846	p<.001
Oxygenated blood	3.864	p<.001
Deoxygenated blood	3.185	p=.003

2.4.3.1 Difference in adjustment between sexes, ethnicities and main colour axes.

A 3 colour axes * 3 ethnicities * 2 sexes repeated-measures ANOVA was performed to investigate the differences in the amount of colour change applied to the faces of different ethnicites for all the three colour axes and between sexes. There was a significant main effect for colour axes, $F(1.71, 73.52) = 21.374$, $p < 0.001$, $\eta_p^2 = .332$; and for ethnicity $F(2, 86) = 38.803$, $p < 0.001$, $\eta_p^2 = .474$. Pairwise comparisons showed that there is a significant

difference between the amount of increment in facial yellowness and increment in facial redness (mean difference = .068, p = .012) and also decrement in facial luminance (mean difference = .210, p<.001). Adjustment of skin redness also significantly higher than the adjustment in skin luminance (mean difference = .143, p<.001).

As for the ethnicities, Caucasian faces received significantly more colour adjustment as compared to African faces (mean difference = .127, p<.001) and Asian faces (mean difference = .046, p = .001). Asian faces also received more adjustment in facial colouration than African faces (mean difference = .081, p<.001).

No significant main effect was found for sex, F (1, 43) = .177, p = .676, η_p^2 = .004; and no interaction between colour*ethnicity, F (3.27, 140.71) = .543, p = .704, η_p^2 = .012; colour*sex, F (2, 86) = .605, p = .548, η_p^2 = .014; ethnicity*sex F, (2, 86) = .339, p = .714, η_p^2 = .008 and colour*ethnicity*sex, F (2.67, 115.04) = 1.421, p = .243, η_p^2 = .032.

2.4.4 Discussion

The current study examined Malaysian Chinese participants' perception of healthy facial colour. Participants chose to increase facial yellowness and redness, while decreasing facial lightness to make faces appear healthiest.

Increased skin yellowness is associated with higher levels of dermal carotenoids, which can be achieved with high consumption of fruits and vegetables (Alaluf et al., 2002; Stahl et al., 1998; Stephen et al., 2011). Carotenoid colouration is not only perceived as attractive and healthy in both animals (Bourne, Breden, & Allen, 2003; MacDougall & Montgomerie, 2003) and humans (Coetzee et al., 2012; Lefevre et al., 2013; Lefevre & Perrett, 2015; Stephen et al., 2011; Whitehead, Ozakinci, & Perrett, 2012), but also bring upon various health benefits such as improvement in visual acuity, photoprotection of skin and boosting of the immune system (Maxim E. Darvin et al., 2011; Krinsky & Johnson, 2005; Stahl et al., 2000; Tapiero et al., 2004b).

Skin redness is mainly determined by blood perfusion. A slight increment in facial redness improves perceived facial health, as it reflects healthy blood circulation (Stephen, Coetzee, et al., 2009a). As women's skin redness changes across their menstrual cycle (Burriss et al., 2015; Jones et al., 2015), it might also provide cue about women's fertility.

We also found in this study that Caucasian faces received the highest amount of colour adjustment (in all three facial colouration), followed by Asian faces, and finally African faces. This might be related to the initial skin colour of the face images, whereby faces with lighter, less red, and less yellow skin tones received more adjustment than those with darker, redder and yellower skin tones. Since averageness contributes to attractiveness, (Apicella, Little, &

Marlowe, 2007; Rhodes, Zebrowitz, et al., 2001b) whereby characteristics with extreme values tend not to be preferred, it appears that this aversion to extreme characteristics extends to extreme colouration (Houde, 1987).

The relationship between skin colours and human's health will be discussed in more details in General Discussion.

2.5 General Discussion

2.5.1 The importance of skin texture as a predictor of perceived facial health.

In line with what was observed in the West, the current studies (Study 1 and 2) found an association between skin condition and perceived face health. By using only skin patches extracted left cheek part of the face images, Study 2 found agreement between perceived skin health rated from skin patches and perceived facial health, showing that one can make inferences about another person's apparent health merely by assessing the skin condition of the cheek. A similar finding had been observed in the West when Jones and his colleagues (2004) found that skin patches which were rated as healthiest by the Caucasian faces belonged to faces that were being rated as most attractive.

Both Study 1 and Study 2 found associations between perceived facial health, perceived skin health and the Gabor Wavelet factors. Gabor Wavelet analysis

examines the texture of the face images by scanning the images from different angles and with different frequencies. The correlations between the Gabor Wavelet factors remain significant even after controlling for skin colour, suggesting that the texture itself should account for some variance in the rating of perceived facial health.

Examination of the examples of skin patches on the extreme ends of the Gabor wavelet factors, showed that skin patches on the lowest end of Gabor Wavelet factor 1, and highest end of Gabor Wavelet factor 2 and 3 have rough texture, with more red spots, and less homogenous colouration as compared to those skin patches on the other extremes.

The result of these studies (Study 1 and 2) are consistent with what were discovered in the West. Research by Fink and colleagues (Fink et al., 2012; Fink et al., 2008; Matts et al., 2007) have also showed that faces with even colouration were judged as healthier and more attractive by the Caucasian sample. Jones and colleagues (2004) showed that apparent health of facial skin patch alone is good enough to predict attractiveness of male faces.

2.5.2 Skin yellowness as an indicator of carotenoid intake

Skin yellowness was found to be correlated with perceived facial health and skin health in Study 2. Meanwhile, in Study 3, participants increased yellowness and redness of the skin the most, and slightly decreased the skin

lightness to make faces of three ethnicities look healthiest. The similar pattern (for preference in skin redness and skin yellowness) was observed in previous studies (Stephen et al., 2009a, 2009b, and 2011) whereby Caucasian participants make a significant increment in facial yellowness and skin redness to optimize perceived facial health of the faces shown. However, a different preference for skin lightness was detected, as compared to the preference of Caucasian and South African samples (Coetzee et al., 2012; Stephen et al., 2011) whereby these two groups of participants increased skin lightness for the optimization of facial health while Malaysian Chinese participants decreased skin lightness.

Previous studies have established the relationship between carotenoid consumption and skin yellowness via both correlational and experimental research. Stephen et al. (2011) showed that individuals with higher dietary carotenoid intake tend to have slightly yellower skin tone than those with low dietary carotenoid intake. Stephen (2009) highlighted in his study that skin yellowness is directly affected by natural fruit and vegetable intake, as he found in his study that, even though melanin also contributes to skin yellowness, controlling exercise level of participants did not remove the relationship between these two variables.

By a utilizing an experimental approach, Stahl and colleagues (1998) gave participants capsules containing approximately 25mg of beta carotene on a

daily basis for 12 weeks, and found a significant increment in skin carotenoids after four weeks of supplementation. Alaluf and colleagues (2002) showed that skin carotenoid levels were consistently and closely related with one's skin yellowness.

Lefevre and Perrett highlighted the importance of carotenoids colouration in attractiveness as found in their study (2015) that even though both carotenoid and melanin colouration improves attractiveness of the faces, carotenoids colouration was much preferred than melanin colouration when levels of colouration are matched. This effect was especially strong for female faces, regardless of the sex of the perceivers.

Carotenoids do more than making human faces look healthy and attractive. The associations between carotenoids and actual human health has long being established. Various compounds of carotenoids have been found to be useful in boosting one's immune system (Gracia et al., 2003, Hughes, 1999), helping to prevent chronic disease such as cancers (Finley, 2005; Tang et al., 2005; Mullick & Gasser, 2004) and cardiovascular diseases (Palace et al., 1999; Granado et al., 2003). Illness such as malaria and HIV infection was found to cause low carotenoid levels (Friis et al., 2001).

Carotenoids were also found to be related with women's fertility. Carotenoid concentrations of premenopausal women were at their lowest during their

menses and increased thereafter, except for alpha-carotene (Forman et al., 1996). Beta-carotene especially, peaked in the late follicular phase when women are most fertile.

Scientists have observed similar colour preferences in non-human animals. Several species of birds and fish prefer mating with opposite sex individuals that sport bright carotenoid-coloured ornaments (Blount et al., 2003; Hill, 1990; Bourne et al., 2003; Amundsen & Forsgren, 2001; Massaro et al., 2003; MacDougall & Montgemerie, 2003). This can be explained with the immunocompetence handicap hypothesis (ICHH), with females preferring the exaggerated secondary sexual characteristics of males. These secondary sexual characteristics are thought to be honest cues to heritable immunocompetence as the costliness of these traits prevent cheating (Verhulst, Dieleman, & Parmentier, 1999). Males with weak immunity are unable to afford the full expression of these characteristics as it will compromise their immunity, making them more vulnerable to diseases.

Carotenoids are limited in supply, cannot be synthesized de novo in one's body (Alaluf et al., 2002), and are needed for various bodily functions (Olson & Owens, 1998; Stahl & Sies, 2005; Toomey, Butler, & McGraw, 2010). To develop colourful and flamboyant ornaments in animals, individuals need to retract this valuable resource from other bodily functions, for example their immune systems (Hughes, 2001). Thus, only individuals with good genes and

good health can afford to suppress their immune systems to develop such ornaments (Saks et al., 2003). An association between ornament size and colouration and immunity or antioxidant activity has been observed in several species (Blount et al., 2003; McGraw & Ardia, 2003; Peters et al, 2004; Saks et al., 2003; Pike et al. 2007) whereby animals with bright and flamboyant ornaments were found to have a stronger immunity and higher levels of antioxidant activity.

Furthermore, carotenoid levels and coloration have been found to be predictive of mate quality in several species, including higher reproductive success among the male northern cardinal (Wolfenbarger, 1999), more food collected by the male house finches during nesting season (Hill, 1991), sperm quality in male mallards (Peters et al., 2004) and even parental ability among the yellow-eyed penguin, and house finches (Massaro et al., 2003; Hill, 1991).

2.5.3 Skin redness as a cue to fitness and fertility

Participants in the current study also increased skin redness to enhance healthy appearance. Increased facial skin redness was related with optimum health possibly due to its association with skin blood perfusion and oxygenation (Stephen et al., 2009b, Re et al., 2011). Skin redness, associated with skin blood perfusion, can be enhanced with aerobic training (Armstrong & Welsman, 2001) as our blood flow increases when we are engaged in exercise (Gonzalez-Alonso et al., 2008). Exercise improves vasodilation, or the

dilatation of blood vessel, which further smoothen the blood circulation. Regular exercise might be helpful for our long term blood circulation, as it set into action adaptive response, enables our circulatory system to meet the constant demands for improving blood flow to muscle tissues (J. Johnson, 1998).

The importance of blood circulation to human health can be observed in various examples. For example, skin colour has been used as an indicator of health status in the APGAR scale to assess infants' probability of survival (Dimich et al., 1991). Illness such as diabetes and hypertension can reduce skin blood flow (Charkoudian, 2003; Panza et al., 1900), making the unhealthy face look pale.

Redness may also be a subtle cue to fertility, as found in several non-human primate species (Barelli et al., 2007) and humans. The facial skin of male rhesus macaques reddens during the mating season as testosterone levels increase (Rhodes et al., 1997). Facial redness in female mandrills has also been associated with their reproductive quality and fertility across the oestrus cycle (Setchell et al., 2006). Reproductive hormone levels were found to have influences on skin blood flow in humans (Charkoudian, 2003).

In the current study, participants increased both oxygenated and deoxygenated blood colouration levels to about the same level. One plausible

explantion was because, perhaps the difference between these two colourations were not that obvious; for example, the difference in skin redness (a*) in only .45 unit for African faces, 1.07 unit for Caucasian faces and 1.26 unit for East Asian faces.

3.5.4 Geographical differences in the preference for skin luminance

In contrast with what has been discovered by Stephen et al. (2009), that Caucasian samples increased skin lightness when they were requested to make faces look healthiest, participants in the current study decreased facial lightness, making the facial skin look darker to achieve optimum health. There is a mixed preference for skin lightness-darkness, subject to sex and geographical differences. It was found that men with slightly darker skin tone were perceived as healthier and more attractive (Broadstock, Borland & Gason, 1992). This preference for darker skin tone has also been reflected in males' own self evaluation whereby men with darker skin tone judged themselves as sexually more attractive as compared to their counterparts with lighter skin tone (Wade, 1996). Women, on the other hand preferred lighter skin tone. South Asian-Canadian females, for example, preferred a much lighter skin as compared to the lightness of the skin they possessed (Sahay & Piran, 1997). Chinese community perceived females with lighter skin tone as more attractive as it's reflected in the common Chinese saying that "fair skin can hide three facial flaws" (Mak, 2007).

There was also a mixed result on skin lightness preference among individuals from different geographical populations. Kishen et al. (2010) found in their study that Asians prefer lighter skin tone while Caucasians prefer a much darker skin tone, while Stephen and colleagues reported that both Caucasian and South African samples perceived lighter skin tones as healthier. It is important to notice the difference between attractiveness and perceived facial health. Although these two factors were found to be inter-related (Jones et al., 2004), the inter-relationship between health, attractiveness and skin lightness may be more dynamic and it can't be conceptualized as equal.

Melanin was found to have both health benefits and costs, depending on one's exposure to the ultraviolet (UV) radiation. Melanin filters a large amount of UV radiation, making limited amounts of UV reach the dermis and be available for the synthesis of vitamin D (Hochbergh & Templeton, 2010). Although it helps protect us from ultra-violet radiation, this photo protective property reduces synthesis of vitamin D (Arianne et al., 2010) as synthesis of vitamin D requires ultra violet light. Vitamin D deficiency was found to not only negatively impact on our physical wellbeing, such as the increased risk of having musculoskeletal pain (Heath & Elovic, 2006) but also on one's emotional status and cognitive ability (Wilkin et al., 2006).

Ultra violet radiation can produce reactive oxygen species and free radicals which can damage molecules such as DNA and protein (Herrling, Jung & Fuchs,

2007). Individuals with darker skin tones are less susceptible to sunburn and skin cancer as compared to individual with lighter skin tone (Gloster & Neal, 2006). Perhaps that is the reason why darker skin tone is perceived as healthier in a region where UV radiation is relatively high, such as the equator (Jablonski & Chaplin, 2000; Jablonski, 2004). Even though dark-skinned individuals require six times longer exposure to sunlight to acquire the same level of serum vitamin D as compared to fair-skin individuals (Clemens et al., 1982), with the sufficient sunlight in Malaysia, individuals are able to synthesize vitamin D even with darker skin tone. The ability to protect one from harmful UV radiation might be more important here, thus being perceived as healthier by the participant in this study.

Besides, disease-causing organisms of humans, such as parasites are more prevalent in equatorial regions and decrease as the latitude increases (Nunn et al., 2005; Guernier et al., 2004). Since melanin has both the function of photo protection and immunity, it might relatively serve a much more important role for people living in region which has higher parasite loads and higher UV radiation, such as Malaysia which is near to the equator.

2.5.5. Conclusion

In summary, both skin texture and skin colour were found to contribute to the judgment of perceived face health and rated skin health.

One can spontenously make inferences about another person's health status by attending to the subtle clues obtained from face. Previous studies (Baudouin & Tiberghien, 2004; Coetzee, Perrett, & Stephen, 2009; Jones & Little, 2001; Little, Apicella, & Marlowe, 2007; Penton-Voak et al., 2001; Rhodes et al., 2003b; Swaddle & Cuthill, 1995) have highlighted the significance of facial structure in the perception of apparent health and attractiveness. Whilst some recent research (Coetzee et al., 2012; Fink et al., 2012, 2001; Jones, Little, Burt, et al., 2004; Lefevre & Perrett, 2015; Samson, Fink, & Matts, 2010; Stephen, Coetzee, et al., 2009a; Stephen et al., 2011) has started giving more emphasis to the importance of skin condition in the judgement of health and attractiveness, no such study has been conducted in an Asian setting. In line with what was observed in the West, the current study showed the Malaysian Chinese too rely on skin texture and skin colours when making assessment on face's apparent health. Specifically, skin with homogenous colouration, and slightly yellow was being perceived as healthier.

Further, the individuals increased skin yellowness, redness and decreased skin lightness to make faces look healthiest. Skin blood colouration serves as a cue of the bearer's cardio fitness and absence of certain illness, while skin yellowness reflects the amount of vegetables and fruit that one consumed which serve as an indicator of that person's immunity. Finally, darker skin tone can be a result of sufficient exposure to sunlight which can be associated with one's outdoor activity level. It serves photo protective values, reducing

the risk of getting sun burn and skin cancer, especially for residents who reside in regions which has high exposure of UV radiation such as the countries along the equator.

This information provides us clues on how we can improve our appearance, and should be useful as a way to encourage the society to engage more in healthy behaviours.

CHAPTER 3: SENSITIVITY THRESHOLD TO COLOUR CHANGES IN FACES, NON-FACE OBJECTS AND COLOUR PATCHES

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3.1 Introduction

Facial skin colour has been shown to influence perceived attractiveness (Jones et al, 2004) and health, with increased lightness (CIELab L*), redness (a*) and yellowness (b*) being perceived as more healthy (Stephen, Coetzee, et al., 2009b; Stephen, Law Smith, et al., 2009). Further, skin colour has been linked to aspects of physiological health status, such as aerobic fitness, fertility and immunity, suggesting that skin colour may represent a valid cue to health (Stephen, Coetzee, et al., 2009b; Stephen et al., 2011; Stephen, Law Smith, et al., 2009).

Skin colour is primarily influenced by three pigments: melanin, haemoglobin (oxygenated and deoxygenated) and dietary carotenoids (Edwards & Duntley, 1939)). Skin lightness is mainly determined by melanin - the dark brown

pigment - and melanoid, a derivative of melanin (Edwards & Duntley, 1939); skin yellowness is contributed by melanin and also individuals' dermal carotenoid levels (Alaluf et al., 2002; Stephen et al., 2011); while skin redness is primarily caused by blood underneath the epidermis (Piérard, 1998).

3.1.1 Humans' preference for facial redness

Changes in skin redness are influenced by the changes in skin haemoglobin concentration and oxygen saturation (Changizi et al., 2006).
Skin redness is also associated with increased oestrogen levels in women (Charkoudian, 2003). Oestrogen is important to the reproductive health of women and the maintenance of human skin, specifically with increment in skin thickness and enhancement of vascular blood flow under the skin surface (Thornton, 2002).

Primate colour vision has been suggested to have evolved through selection for the ability to detect skin colour signalling (Changizi et al., 2006). Our inherited trichromat phenotypes possess significant sensitivity to oxygenation saturation, enabling us to perceive the subtle changes in blood oxygenation level through changes in skin colour (Changizi et al., 2006).

Re at al. (2011) further examined the oxygenated blood colour change threshold required to affect perception of facial colour, health and

attractiveness, and found that the perceptual thresholds for colour discrimination were slightly lower than those for health and attractiveness.

The importance of skin redness in providing social cues to emotional and health state suggests that selection may have favoured mechanisms for detecting redness in facial skin (Changizi et al., 2006; Re et al., 2011), due to the high information content of skin redness in social contexts, signalling emotional state, health condition and reproductive status in many primate species, including humans (Changizi et al., 2006; Drummond et al., 2007; Drummond & Lazaroo, 2012; Drummond, 1997; Stephen & McKeegan, 2010).

Tarr and colleagues (2001) suggested that measuring the overall red-green ratio of the face is sufficient for accurate sex discrimination, because the reflectance properties of facial hair and skin produce different degrees of red and green in each sex. Nestor & Tarr (2008) found that, mouth and eyes aside, male faces are on average slightly redder than female faces.

Nonhuman primate species display redness on various part of their body, including the face and the genital region, during the mating season, to attract mates (Setchell, Wickings, & Knapp, 2006; Setchell & Wickings, 2005; Waitt et al., 2003; Waitt, Gerald, & Little, 2006).

Changes in skin redness have been found to reflect fertility in female non-human primates and to signal dominance among the males (Setchell et al., 2006; Setchell & Wickings, 2005). Research has shown that women tend to perceive men as physically and sexually more attractive when they are associated with redness. Male participants were perceived as sexually more attractive when they were wearing red clothes, stood in front of a red background (Elliot et al., 2010), or when they had a redder complexion (Stephen, Law Smith, et al., 2009). Similarly, women wearing red clothes or standing in front of a red background were perceived as more attractive by men, even with a brief glimpse (Elliot & Niesta, 2008).

3.1.2 Sensitivity threshold and CIELAB colour space

This study used a psychophysical task to compare the sensitivity threshold for detecting changes in skin colour. A sensitivity threshold is the level of perception whereby individuals are able to perform certain tasks in sensation and decision making, above chance level. These sensations and decision making tasks usually involve detection, discrimination, recognition or identification of stimuli.

In the current study, colour changes were described using CIE L*a*b* colour space, which is modelled after the human visual system, reflecting the way we perceive and process chromatic information. The three dimensions of this colour space correspond to the three colour channels of the human visual

system: luminance (L*), red-green (a*), and yellow-blue (b*). CIELab is designed to be perceptually uniform whereby a change of one unit on one dimension appears to be of approximately the same magnitude as changes of one unit in another dimension (Martinkauppi, 2002). The Euclidean distance (ΔE) between two points in CIE L*a*b* space mirrors the colour differences perceived by human vision (Wyszecki & Stiles, 1982). This suggests that the sensitivity threshold to simple colour change in a stimulus should be similar in each of the three dimensions of the CIELab colour space.

3.1.3 Cross Race Effect

The Cross Race Effect suggests that that individuals are better at recognizing faces of own- than other-ethnicity (Horry, Wright, & Tredoux, 2010), possibly due to greater experience with and exposure to own-race faces. The Cross Race Effect has accumulated a wealth of evidence from psychological research in face recognition, particularly in the West (Furl, Phillips, & O'Toole, 2002; Horry, Wright, & Tredoux, 2010). A recent study conducted locally (Tan et al., 2012) also found that Malaysian Chinese did equally well when trying to recognise both East Asian and Caucasian faces (which they are familiar with) but less well when recognising less familiar African faces.

By using infants as young as 3 months old, Bar-Haim and his colleagues (2006) showed the importance of environmental factors and social learning in facial perception. Infants in their study were found to be able to discriminate

between own ethnic and other ethnic adult faces, in which African infants raised in a primarily African environment showed own ethnic preference by fixating longer at African faces; Caucasian Israeli infants fixated longer at Caucasian rather than at African faces, while African infants born in Israel showed no preference for either African or Caucasian faces.

Facial skin colour preference also may be restricted to own-race faces, possibly due to the unfamiliarity with other-race skin colour (Stephen et al., 2012). Therefore, it was predicted that participants would perform better (higher accuracy) when discriminating changes in facial colours for faces of their own ethnicity (East Asian, in this context).

3.1.4 Objective of the studies

Although it has been found that human vision is sensitive to facial colours, particularly blood colouration (Re et al., 2011), it is unclear if individuals are more sensitive towards changes in certain facial colours over others. Two studies were conducted to examine the sensitivity threshold of human vision in the face (Study 4), colour patches and non-face objects (Study 5), manipulated along the three colour axes of the CIELab colour space.

In Study 4, participants were asked to discriminate between two same-face-photos with different levels of colour intensity to determine which of the stimuli was lighter, redder or yellower in three separate blocks. Due to the

importance of facial skin colour in social interaction, it was hypothesized that changes in chrominance in general, and changes in skin redness specifically will be easier to detect as compared to changes in skin luminance. Besides, due to the Cross Race Effect, colour changes in East Asian and Caucasian faces should be more easily detected by Malaysian Chinese participants than colour changes in African faces.

In Study 5, participants were asked to discriminate colour differences between pairs of colour-manipulated patches and non-face object images. It was hypothesized that no difference would be observed in one's ability to detect changes in luminance, redness and yellowness in non-face objects. Data from Study 5 was compared with data from Study 4 to determine if colour has special salience in the context of human faces.

3.2 STUDY 4: Sensitivity threshold in CIEL*a*b for human faces

3.2.1 Introduction

Re and colleagues' (2011) study found that individuals are sensitive to subtle changes in skin blood coloration. In their study, facial images with high and low oxygenated blood coloration were presented in pairs, and the participants were asked to determine which of the paired facial images was redder, healthier and more attractive, in three different blocks. Result of the study revealed that the threshold for colour discrimination was lower than those for health and attractiveness. As the stimulus in the three trials differ only in term of their redness, the researchers suggested that changes in health and attractiveness perception also should be attributed only to changes of facial redness, specifically the oxygenated blood coloration. Re and coleagues suggested that even though changes in redness contributed towards changes in judgment of health and attractiveness, a minor but detectable change in facial redness does not necessarily alter perceived facial health and attractiveness

Red coloration in the skin was found to play an important role in the socialization of several primate species. Intensity of redness on exposed skin, specifically on the face and genital regions, indicates social rank among male mandrills (Setchell & Dixson, 2001; Setchell & Wickings, 2005) and higher level of testosterone among male macaques (Rhodes et al., 2007) which was further found to be preferred by female macaques (Waitt et al., 2003). Skin

redness is suggested to be an important cue for mate selection among non-human primates as it reflects reproductive status in females. While skin colour was found not to be related to female mandrills' rank and physical quality, the skin redness increases significantly with age, and when female mandrills are most fertile -- more specifically the skin gets brighter during the follicular phase of the oestrus cycle (Setchell et al., 2006). Male macaques looked for a longer duration when shown female hindquarters with increased redness (Waitt et al., 2006).

Skin redness plays an important role in human life. The human face flushes when experiencing social anxiety, embarrassment (Drummond et al., 2007; Drummond & Lazaroo, 2012; Drummond, 2001) and anger (Drummond, 1999; Drummond, 1997); and becomes pale when feeling fearful (Drummond, 1997). Body painting in blood-colouration was used in ancient civilizations among the women as a symbol of sexual fertility (Knight, Power, & Watts, 2008), while women in modern societies continue to use red ornamentation, such as lipstick, to increase their facial attractiveness (Schaffer, 2007). Although previous research has shown that individuals tend to perceive faces with higher levels of redness, yellowness and lightness as healthier (Stephen, Coetzee, et al., 2009b; Stephen, Law Smith, et al., 2009), the sensitivity threshold – the minimum colour difference that can be perceived by human vision at above chance levels is unclear.

In the current study, the sensitivity threshold of human vision to facial skin colour change was examined. Based on previous research (Re et al, 2011; Changizi et al, 2006) that highlighted the significance of using blood colouration as a cue to physical health and emotional status of others, it was hypothesized that changes in red will be more easily detected than changes in facial yellowness and facial lightness.

Participants' accuracy in detecting colour changes in African, Asian and Caucasian faces were also tested. Since Malaysian Chinese participants have been found to perform better at recognising East Asian and Caucasian than less familiar African faces (Tan et al., 2012), it was predicted that participants will perform better when detecting colour changes in faces with which they are familiar (East Asian and Caucasian) as compared to those with which they are less familiar (African).

3.2.2 Methods

This is a within-participants design whereby the participants went through all three conditions (changes in luminance, redness and yellowness) in the study. This study was approved by the Faculty of Science Ethics Committee at the University of Nottingham Malaysia Campus. All participants gave written informed consent prior to the experiment.

3.2.2.1 Stimuli Preparation

Twelve face photographs – four of each (two male, two female) of three different ethnicities: Caucasian, African and East Asian - were used in this study. Photography took place in a photo booth painted with Munsell N5 grey and illuminated with three Verivide F20 T12/D65 daylight simulation bulbs in high-frequency fixtures to reduce the effects of flicker. Participants were asked to pose with a neutral expression while holding a Munsell N5 painted board over their shoulders to obscure clothing. A Gretag Macbeth Mini ColorChecker colour chart was included in the frame. All photos were colour-calibrated after Stephen et al (2009a).

Matlab was used to produce masks with even coloration representing the skin areas of faces, with a Gaussian blur at the edges. One mask was created to represent average face colour +8 units of a* (increased redness) and another one with average face colour –8 units of a* (decreased redness). The colours of the twelve faces were manipulated by the difference in colour between each of the pairs of masks. This produced a single manipulation composed of a series of 13 frames, numbered from 0 to 12, whereby frame 0 had skin redness reduced by 4.8 units of a*, increasing incrementally so that frame 6 would be the original image and frame 12 had skin redness increased by 4.8 units of a*. For all the transformations, hair, eyes, clothing, and background remained constant. Twelve images were then paired in a way to create six combinations with colour distances of 9.6, 4.8, 2.4, 1.2, 0.6 and 0.3 for each

colour axis. However, due to quantisation effects (errors introduced by rounding numbers to the nearest integer when converting to RGB colour space for presentation on computer monitors), only the highest four levels of the transformation (ΔE 9.6, 4.8, 2.4 and 1.2) were used for the data analysis.

3.2.2.2 Participants and procedures

Fifty one Malaysian Chinese (26 males, 25 females) participants with average age 21.43 (SD= 2.46) were recruited. All participants were students pursuing undergraduate or postgraduate degrees at two private institutions of higher education in Malaysia - University of Nottingham Malaysia Campus and Universiti Tunku Abdul Rahman. Initially, 54 participants were recruited and all completed the three discrimination tasks. However, three participants were excluded, as outliers, because their results on at least two tasks were more than 3 standard deviations above the mean performance. All participants reported having normal colour vision.

Participants were asked to discriminate the lightness, redness, or yellowness of the sequentially-presented, paired facial images in three separate blocks. Twelve facial images with four combinations of colour difference (delta-E 9.6, 4.8, 2.4, 1.2) created 48 trials for each block. The three different colour blocks and the 48 trials within each block were presented to the participants in random order.

The experiment was presented using Psychopy (Peirce, 2008). Participants were given two-alternative forced-choice psychophysics tasks, presented on a computer screen colour-calibrated with a DataColor Spyder3 Pro. Specifically, the participants were shown one face image for 750ms, followed by a visual mask of black dots on a white background for 100ms, followed by the other image in the pair for 750ms. These face images were presented using portrait orientation with 4:5 aspect ratios. Participants were asked to decide which of the paired-images (either the first or the second image) look redder in the redness discrimination task, by using the computer keyboard. The same procedures were administered for yellowness and lightness blocks, except the stimuli used differed in their yellowness or lightness. Two trials (one of the African male faces with ΔE 2.4 of lightness and yellowness) were accidentally omitted from the experiment, and thus are not included in the analysis. To ensure that this does not introduce bias into the analysis (as well as to control for quantisation errors in stimulus production), measured colour distance between the pairs was included as a covariate in the analyses. Presentation of the stimulus in the actual experiment is illustrated in Figure 3.1 below.

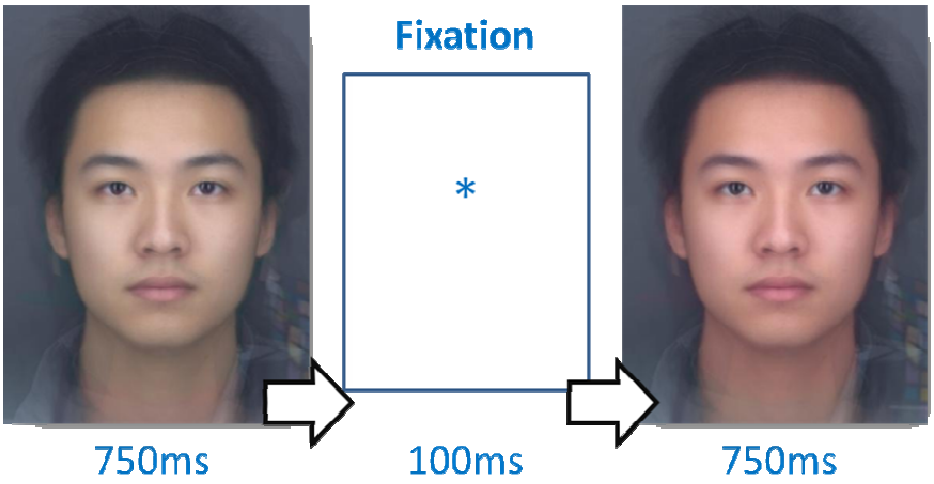


Figure 3.1. Stimulus arrangement in the actual experiment, presented using Psychopy. Each image was presented for 750ms, with a 100ms fixation in between.

3.2.3 Results

An analysis of covariance (ANCOVA) was administered to examine the difference of accuracy in detecting three different colour changes in the images, across three ethnicities. One point was given to each correct response, and the average scores for each colour axis and each ethnicity were calculated, labelled as the accuracy and serves as the dependent variable.

An independent samples t-test was performed to determine if there was any sex difference in participants' performance. No effect of sex was observed, $t(49) = 1.234$, $p = .223$, so results were collapsed across sex for subsequent analyses.

3.2.3.1 Sensitivity threshold in faces

An analysis of covariance (ANCOVA) was used to examine:

1. Difference of accuracy in detecting colour changes in the three different colour axes,
2. Difference of accuracy between faces of different ethnicities
3. Interaction between these two variables.

Participants' accuracy in detecting changes was entered as the dependent variable while colour axis (L*, a* or b*) and ethnicity of the facial images (African vs. Caucasian vs. East Asian) were entered as independent variables. The actual distances of colour changes as validated by Matlab were used as a covariate to control for minor differences in colour changes of different images as a result of quantisation errors. The data were not normally distributed. However, we used ANCOVA as it is robust to violations of normality.

Results of the analysis indicated a significant main effect of the colour dimension, $F(2,132) = 22.346$, $p < .001$. Pairwise comparisons (Bonferroni) showed that participants can see smaller differences in facial redness than yellowness (mean difference=.081, p<.001); facial redness than lightness (mean difference=.130, p<.001) and facial yellowness than lightness (mean difference=.048, p=.045). Participants correctly identified the lighter face in 73.7% of L* trials (estimated marginal mean [EMM] =.737, SE=.014), the

redder face in 86.6% of a* trials (EMM=.866, SE=.014) and the yellower face in 78.5% of b* trials (EMM=.785, SE=.014).

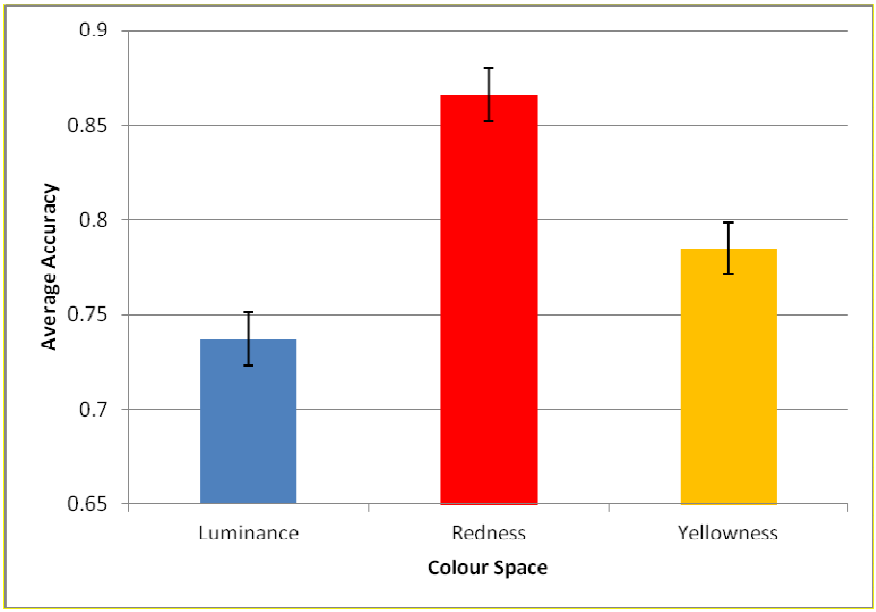


Figure 3.2. Participants' accuracy in identifying colour changes in faces

3.2.3.2 Differences between ethnicities

There was also a significant main effect of ethnicity of face F (2,132) = 9.071, p <.001. Pairwise comparisons revealed that there is a difference in the accuracy of participants' responses when discriminating facial colour of African and Caucasian (mean difference=-.055, p=.018), and between African and East Asian (mean difference=.082, p<.001). However, there was no significant difference in participants' judgment between Caucasian and East Asian faces (mean difference=.028, p=.479). Participants performed equally well when discriminating facial colour of East Asian and Caucasian faces, but

significantly worse when discriminating facial colour of African faces. Participants correctly identified colour differences in 75% (EMM=.75, SE=.014) of African face trials, 80.5% of Caucasian trials (EMM=.805, SE=.014) and 83.3% of East Asian trials (EMM=.833, SE=.014). No interaction between colour axis and ethnicity was found, $F(4,132) = 1.107$, $p = .356$.

Taken together, these results suggest that individuals are perceptually more sensitive towards changes in facial redness, followed by facial yellowness, and lastly facial lightness. Participants were more sensitive to these facial colour differences in faces of the same ethnicity or faces of a familiar ethnicity than those to whom they are less exposed.

3.2.3.3 Correlation between colour distances and accuracies

Since the data was not normally distributed, one tailed Spearman's rho was used to examine the correlation between the accuracy of response and the validated colour distance. Consistent with our prediction, an association was found between the accuracy in discriminating the facial colour difference and the intensity of the colour $r(142) = .689$, $p<.001$ which means, the smaller the difference between the two images being shown, the less accurate the participant's judgment will be. The correlation between colour distance and accuracy were significant for facial lightness $r(47) = .618$, $p<.001$, facial redness, $r(48) = .820$, $p<.001$ and facial yellowness, $r(47) = .814$, $p<.001$. The

associations between the intensity of the colour changes and accuracies of performance are presented in Figure 3.3.

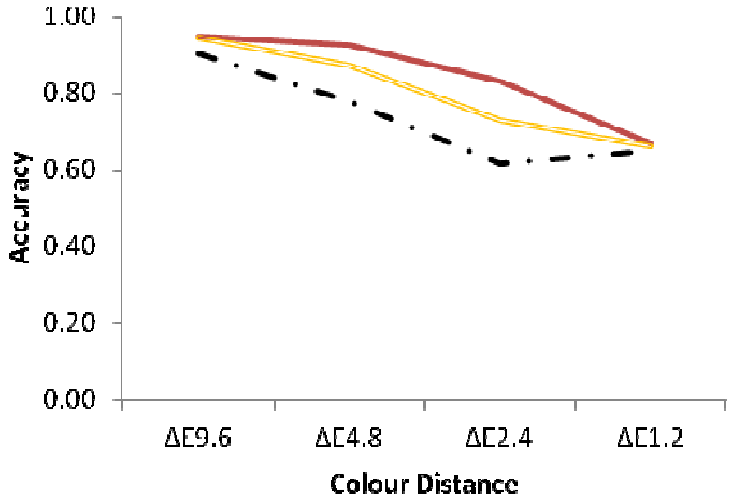


*Figure 3.3. Association between accuracies of performance and colour distance of CIEL*a*b*. The dashed black line shows L*, the solid red line shows a*and the hollow yellow line shows b* distances.*

Non-parametric Wilcoxon Signed Ranks Tests were conducted to check if the performance of each of the four different colour distances were identified above chance, with a standard value of .5. Participants were able to identify colour differences in the facial images above chance level (all $p<0.001$; $-4.36<z<-6.351$), for all four levels of colour distance for all three colour axes (Table 3.1).

Table 3.1

Summarised statistics from the Wilcoxon Signed Ranks Test

Color and Euclidean Distance	z	p
L* ΔE 9.6	-6.286	p<.001
L* ΔE 4.8	-6.116	p<.001
L* ΔE 2.4	-4.362	p<.001
L* ΔE 1.2	-5.347	p<.001
a* ΔE 9.6	-6.458,	p<.001
a* ΔE 4.8	-6.349	p<.001
a* ΔE 2.4	-6.130	p<.001
a* ΔE 1.2	-5.360	p<.001
b* ΔE 9.6	-6.351	p<.001
b* ΔE 4.8	-6.203	p<.001
b* ΔE 2.4	-6.164	p<.001
b* ΔE 1.2	-5.099	p<.001

3.2.4 Discussion

Re and collegues (2011) suggested that individuals are sensitive towards subtle changes in facial redness, and these changes in redness were found to contribute to the perception of apparent health and attractiveness of human faces. The current study compared the sensitivity threshold of human vision in detecting changes in facial colours in respect to three different colour axes (luminance, redness and yellowness) and revealed that individuals were

indeed visually more sensitive towards changes in facial redness, and to a lesser extent skin yellowness.

This may be due to the significance of these two facial colours in providing information about the bearer's health, fertility and emotional status. However, one could argue that this sensitivity towards chrominance, specifically towards redness, might not be unique only to human faces. Thus, this hypothesis warrants further validation, by comparing the sensitivity theshold of human vision towards the changes of colour in non-human-face objects.

There was also a main effect of ethnicity in the colour discrimination task. Participants made more accurate judgments when discriminating facial colour of own and familiar ethnicities (East Asian and Caucasian) than less familiar (African) faces. Further discussion about Cross Race Effect will be presented in General Discussion part of this chapter.

3.3 STUDY 5: Sensitivity threshold in CIEL*a*b for objects and patches

3.3.1 Introduction

In general, our eyes are more sensitive towards changes in luminance (lightness) than changes in chrominance (Ramanath, Snyder, Bilbro, & Sander, 2002) . However, it was found in the previous study (Study 4) that individuals have a lower sensitivity threshold in detecting changes in chrominance (redness and yellowness) than luminance when viewing human faces. Since CIELab colour space used in the study is proposed to be perceptually uniform, this increased sensitivity towards redness, and to a certain extent yellowness, is suggested to be unique only to the perception of human faces. This hypothesis warrants further investigation.

The ability to detect changes in colours should be contextual. It is suggested that individuals might be sensitive towards changes of chrominance as compared to luminance only for human faces, because colours provide richer information about the health and even the emotional status of the face, than lightness.

Study 5 was designed to investigate whether the greater sensitivity towards chrominance which was found in Study 4 is unique to faces, or representative of a more general phenomenon. To this end, facial images used in Study 4 were replaced with objects and colour patches made using the average facial

colours of the three ethnicities used in Study 4. A comparison is also made between the data collected from this study and those from Study 4.

It was predicted that participants' accuracy at detecting changes in chrominance (redness and yellowness) will not be any better than detecting changes in luminance for non-facial stimuli. If our perceptual sensitivity towards changes in chrominance is only greater than luminance for faces and not patches and object, this implies that there may be a special salience of chrominance in facial perception. Also, colour changes in faces were predicted to be easier to detect as compared those in colour patches and the non-face objects.

3.3.2 Methods

This is a 2x3 repeated measures design (type of stimuli vs. colour space). All participants were presented with six non-facial conditions -- three using colour patches and another three using object images. Within the same stimulus type, three blocks were presented; in each block, stimuli differed only in one particular colour space, i.e. luminance, redness or yellowness.

3.3.2.1 Stimulus Creation

Eleven colour patches and 11 objects were used in this study. For object images, pictures of 37 objects were taken in the above mentioned standardised photo booth. A Gretag Macbeth Mini ColorChecker colour chart

was included in the frame. Eleven objects were selected by considering the size of the object and the diversity of the colour of the objects – which include a red pepper, a blue plaster, a green cup, a black pouch, a white cup, a grey sponge, an orange pepper, a brown container and three other objects which have a similar coloration as human skin, i.e. an egg, a dog-shaped cuddly toy and a brown plaster.

Eleven colour patches were created using Matlab. These eleven colour patches included eight highly diverse colours (red, blue, yellow, black, white, grey, violet and pink) and three skin-colour patches that were made based on the average L*a*b (luminance, redness and yellowness) of African and Caucasian faces collected by Stephen et al (2009a) and East Asian faces that were collected previously (refer to Chapter 2, Study 3, and Table 3.2).

Table 3.2

CIELab values used to create skin colour patches.

Skin Colours	L*	a*	b*
African	42	12	17
Caucasian	64	14	15
East Asian	63	9.3	18.2

For each of the 11 patches and 11 objects, images were transformed as in Study 4 to create pairs of images that differed in colour at five different intervals (delta-E of 19.2, 9.6, 4.8, 2.4 and 1.2) along each of the three CIELab colour spaces (luminance, redness, yellowness) respectively. Colour changes within these stimuli were then validated using Matlab. All the colour changes were transformed based on the CIELab colour space. Examples of the stimuli are presented in Figure 3.4.



Figure 3.4. Examples of the stimuli created. The top row depicts skin patches with different levels of lightness (left -4.8 unit L, middle original patch, and right +4.8 unit L*); the bottom row depicts objects with different levels of redness (left -4.8 unit a*, middle original image, and right +4.8 unit a*)*

3.3.2.2 Participants and procedures

Forty eight Malaysian Chinese (15 males, 33 females) participants with an average age of 22.65 (SD= 4.86) were recruited to discriminate colour changes in non-face patches and objects. All participants were students pursing undergraduate or postgraduate degrees at the University of Nottingham Malaysia Campus. All participants reported having normal colour vision.

All stimuli were presented using computers attached to 15 inch TFT monitors and colour calibrated with a Data Colour Spyder3 Pro. The experiment was presented using PsychoPy (Peirce, 2007), following the same procedure as Study 4. Participants were asked to decide which of the paired-images looked lighter in the luminance discriminance task, redder in the redness discrimination task, and yellower in the yellowness discriminance task.

Eleven colour patches and 11 object images, with five combinations of colour difference (delta-E 19.2, 9.6, 4.8, 2.4 and 1.2) for each image, made up 55 sets of trials for each of the six blocks. The six blocks (patch-luminance, patch-redness, patch-yellowness, object-luminance, object-redness and object-yellowness) and the 55 trials within each block were presented to the participants in random order.

3.3.3 Results

There will be two parts of the data analysis in this section – Section 1 presents only the result involving data from both objects and colour patches, while Section 2 will compare the results collected from objects, colour patches and also face stimuli (from Study 4).

3.3.3.1 Colour patch vs. object

ANCOVA was used to examine the difference in accuracy in detecting colour differences in the three different colour axes, and between the two types of stimuli. One point was given to each correct response, and the average scores for each stimulus type on different colour spaces and different type of stimuli were calculated, labelled as the accuracy and served as the dependent variable. Type of tests (object and patch) and colour spaces (luminance, redness and yellowness) were entered as the independent variables. The actual distances of colour changes as validated by Matlab were used as covariates to control for minor differences in colour changes of different patches due to quantisation errors.

Participants correctly identified the colour changes in 70.9% of the patches (EMM=.709, SE=.012) and in 77.6% of the object images (EMM=776, SE=.012). Analysis of covariance indicated a significant main effect for the test type, $F(1, 257) = 13.764$, $p<.001$. Utilising Bonferroni adjustment, participants were

found to have higher accuracies when judging colour changes in object images, as compared to patches (mean difference=0.067, p<0.001).

A significant main effect was found for the colour dimension, F (2,257) = 15.377, p<.001. After applying Bonferroni adjustment, pairwise comparisons showed that participant were less accurate when judging changes in yellowness, as compared to lightness (mean difference =.11, p<.001) and redness (mean difference =-.074, p=.001). There was no significant difference between lightness and redness (mean difference =.036, p=.239). Participants correctly identified the lighter patch in 79.1% of L* trials (EMM=.791, SE=.014), the redder patch in 75.5% of a* trials (EMM=.755, SE=.014), and the yellower patch in 68.1% of b* trials (EMM=.681, SE=.014). No interaction was found between type of stimulus and colour F (2,257) = 2.501, p =.084. The result of the above comparisons is presented in Fig 3.5 below.

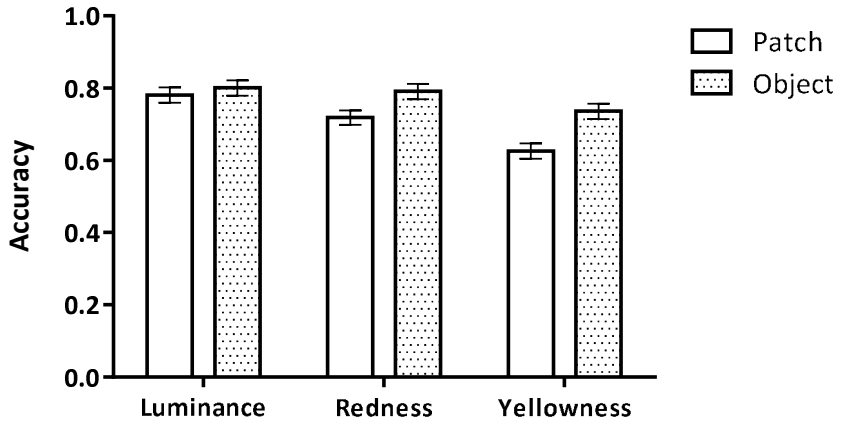


Figure 3.5. Differences in accuracies of performance in two types of stimulus in three different colour spaces. Error bars represent standard error of the mean.

3.3.3.2 Face vs. colour patch vs. object

The data from the face trials were then combined with the data collected from the non-face trials, after excluding all data obtained for patches and objects with a colour distance of 19.2 (as these are not present for face trials). Thus, we are comparing the participants' sensitivity of changes in colour for faces, patches and object on 4 different Euclidean distances (delta-E 1.2, 2.4, 4.8, and 9.6). Response accuracy was the dependent variable, type of test (face vs. patch vs. object) and colour (L*a*b) were independent variables, and actual colour distant of the stimulus was keyed in as covariate.

Participants correctly identified the colour changes in 82.4% of the faces (EMM =.824, SE=.011), in 68.8% of the patches (EMM=.688, SE=.012) and in 76.8% of the object images (EMM=768, SE=.011). Analysis of covariance

indicated a significant main effect for the test, $F(2, 396) = 34.406$, $p<.001$. Utilising Bonferroni adjustment, participants were found to have higher accuracies when judging changes in facial images, as compared to patches (mean difference=0.137, $p<0.001$) and objects (mean difference=0.057, $p=0.001$), and made better judgements for objects than for patches (mean difference=0.08, $p<0.001$).

There is also a main effect of colour, $F(2, 396) = 12.273$, $p<.001$. Participants correctly identified the lighter stimuli in 77.2% of L* trials (EMM =.772, SE=.011), the redder stimuli in 79% of a* trials (EMM=.79, SE=.011) and the yellower stimuli in 71.7% of b* trials (EMM=717, SE=.011). Pairwise comparison revealed a better performance in luminance over yellowness (mean difference=0.055, $p=0.001$), and redness over yellowness (mean difference=0.073, $p<0.001$). No difference was detected between luminance and redness (mean difference=-0.018, $p=0.740$).

There is an interaction between type of test and colour axis, $F(4, 396) = 9.696$, $p<.001$. As shown in Figure 3, while the judgement of luminance remains the same across different tests ($p>0.05$), judgement in chrominance, specifically in redness and yellowness, depends on the type of test that was given. Participants were found to make better judgements in redness and yellowness only for stimuli involving human faces but not patches or objects.

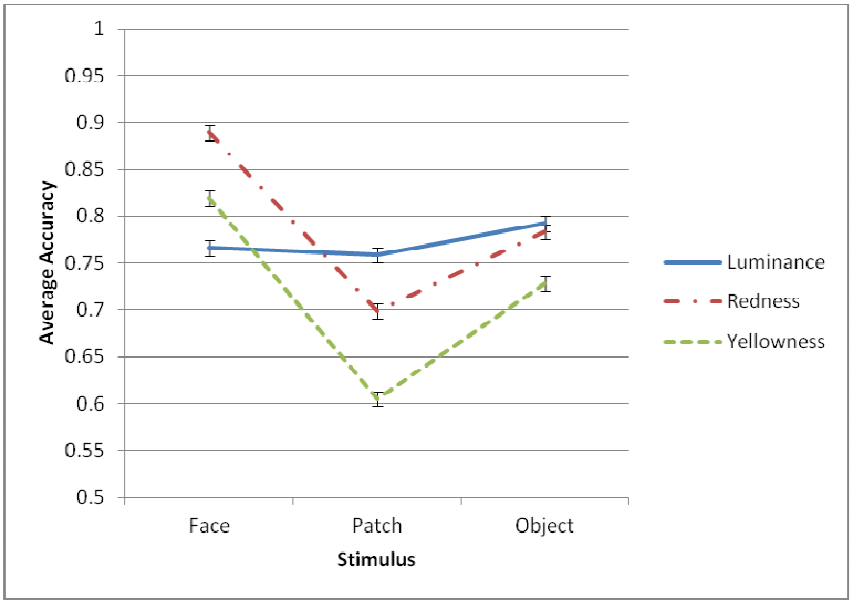


Figure 3.6. Differences in accuracies of performance in three types of stimulus and three different colour spaces. Error bars represent standard error of the mean.

3.3.4 Discussion

Study 5 was created to investigate if the extra sensitivity in detecting changes in redness (found in Study 4) is only unique for face perception, in comparison to stimuli such as colour patches and objects.

Results showed that there were main effects for both the type of stimulus used and colour axis in the accuracy of the participants' visual judgment. Participants made better colour discrimination when perceiving facial images, followed by object images and finally colour patches. This is consistent with the hypothesis that human vision is more sensitive towards colour differences in faces than in non-face stimuli (Farah, Wilson, Drain, & Tanaka, 1998).

Participants did not perform significantly better in detecting changes in redness as compared to changes in luminance. However, the accuracies in these two trials (redness and luminance) are higher than the judgment of changes in yellowness. Based on the result (comparison between faces, colour patches and object images), we suggest that our higher visual sensitivity in detecting changes in redness than luminance is only unique to certain stimuli, specifically human faces, and not a general preference in perception. This further suggests a special salience for chrominance in faces.

3.4 General Discussion

The current study examined Malaysian Chinese participants' ability to discriminate colour changes in human faces, colour patches and objects. Analyses showed that, while participants perform significantly better in detecting changes in chrominance (redness and yellowness) in faces than in patches and objects, their performance on luminance remains the same across three different tests. We suggest that this extra sensitivity in redness and yellowness may indicate special salience for colour in face stimuli, due to the significance of chrominance changes in face perception, particularly in providing information about the bearer's physical health and emotional status.

3.4.1 Sensitivity in Faces

Despite the complexity of faces as compared to colour patches, it was found in the study that individuals make better judgements of colour changes in faces than in colour patches. Faces are important for human socialisation and survival as they provide useful information about the bearer. It is proposed that individuals use different mechanisms when perceiving faces and non-facial objects. The perception of faces tends to be more holistic, whereby the overall structure of a face is used for analysis and recognition, which is different from object recognition which involves part decomposition (Farah, Wilson, Drain, & Tanaka, 1998).

Humans, in general, have a preference towards seeing faces over other patterns. A preference towards faces over objects and other patterns can be observed in newborn infants, where they fixated longer on facial configurations than non-face stimuli (Fantz, 1963; Ichikawa, Tsuruhara, Kanazawa, & Yamaguchi, 2013; Johnson, Dziurawiec, Ellis, & Morton, 1991). Some researchers even suggest that facial perception is an experience-independent, inborn ability, as it has been found that preference towards face-like patterns can be observed in newborns as young as nine-minutes old (Goren, Sarty, & Wu, 1975). Based on a case study on a one-day-old boy with brain damage, Farah and colleagues (2000) also suggest that explicit information about faces is specified within the human genome, and does not require much learning. Perhaps due to our preference toward faces, and our

higher exposure to human faces than other objects, our sensitivity towards changes in faces is also relatively higher.

3.4.2 Chrominance over luminance

In general, our vision is more sensitive towards changes in luminance (lightness) than changes in chrominance (Ramanath et al., 2002). A similar pattern was observed in Study 5 whereby participants performed better in detecting changes of luminance over yellowness for objects and colour patches. However, individuals performed significantly better in detecting changes of both redness and yellowness, over luminance, in trials using human faces. It seems like changes in redness, and to a certain extent in yellowness, are easier to detect when they occur in human faces (Changizi et al., 2006; Re et al., 2011). The ability to discriminate minor changes in facial colour might have evolutionary advantages whereby it allows one to identify healthy individuals as potential mates (Perrett, 2010, Re et al., 2011).

Both facial redness and yellowness provide useful information about an individual's current health status, fertility (Re et al., 2011; Stephen, Coetzee, et al., 2009b; Stephen et al., 2011) and their emotional status (Drummond, 1999; Drummond et al., 2007; Drummond & Lazaroo, 2012) which are important to human interaction.

Facial redness gives clues about one's current physical health, fertility (Re et al., 2011; Stephen, Coetzee, et al., 2009b) and emotional status (Drummond, 1999; Drummond et al., 2007; Drummond & Lazaroo, 2012). Increased facial redness was perceived as healthy as it is associated with skin blood perfusion and oxygenation (Re et al., 2011; Stephen, Coetzee, et al., 2009b). Skin redness also serves as a cue to fertility for both primates and human beings (Barelli, Heistermann, Boesch, & Reichard, 2007; Charkoudian, 2003; Setchell et al., 2006). Changes in facial redness can be useful for the identification of negative emotions such as fear and anger (Drummond & Lazaroo, 2012; Drummond, 1997; Lin & Cheng, 2012), and the ability to detect these emotional changes can be very beneficial for our socialisation and to a certain extent, our survival and reproduction efforts.

Facial yellowness is also used as an important indicator of perceived health. Research conducted by Stephen and colleagues (2011) showed that increased yellowness in one's facial skin was perceived as healthier in both white Caucasian and black South African faces. Similar preferences had been observed among Malaysian Chinese, as reported in Study 3, Chapter 2 of this dissertation. It has been found that individuals who consumed more vegetables and fruits, particularly those high in carotenoids, tend to have slightly yellower skin than those with a low dietary carotenoid intake (Stephen et al., 2011). Carotenoids are thought to be beneficial for immune (Hughes, 1999; 2001) and reproductive systems (Dowling & Simmons, 2009b).

Even though skin luminance is important for ethnicity recognition, it says little about one's health status, especially within the same ethnicity. Borrell and colleagues (2006) found no significant relationship between skin darkness and reported physical health among African Americans. There have been mixed results in determining whether a lighter or a darker skin tone is perceived as healthy. While Caucasian participants in Stephen et al (2009) and Asian participants in Krishen et al. (2014) preferred a much lighter skin tone, It was found in Study 3 (Chapter 2) that Malaysian Chinese decreased the luminance of the faces to make them look healthier.

It was argued that the preferred skin luminance can be influenced by geographical location. People who live in regions where ultraviolet radiation is high tend to perceive a darker skin tone as healthier, because melanin (which contributes to skin darkness) helps protect one from UV radiation, sunburn and skin cancer (Gloster & Neal, 2006). Meanwhile, an individual who lives further away from the equator would prefer a lighter skin colour. High concentration of melanin, which makes skin darker, filters large amounts of UV radiation, which are needed for the synthesis of vitamin D (Hochberg & Templeton, 2010). Deficiency in vitamin D does not only negatively affect one's physical well being, e.g. an increased risk of having musculoskeletal pain (Heath & Elovic, 2006), but also one's emotion and cognitive ability (Wilkins, Birge, Sheline, & Morris, 2009).

3.4.3 Extra sensitivity to facial redness

Individuals were found to have a lower sensitivity threshold in detecting facial redness as compared to facial lightness and facial yellowness. Anatomically, human ought to be visually more sensitive towards redness because they have red-green sensitive cones clustered around the centre of the retina where visual acuity is the sharpest (Cepko, 2000). Furthermore, there are more long-wavelength cones which are sensitive to orange and red in the *fovea centralis*, as compared to medium-wavelength cones which are sensitive to green (Cicerone & Nerger, 1989). However, the CIELab colour space which was used in the current research is designed to be perceptually uniform across colour axes, thus sensitivity towards a* should be similar to the other two colour dimensions.

Increased facial redness is related to perceived health, possibly due to its association with skin blood perfusion and oxygenation (Re et al., 2011; Stephen, Coetzee, et al., 2009b). Skin redness, due to blood perfusion and oxygenation, is associated with enhanced aerobic fitness (Armstrong & Welsman, 2001; González-Alonso et al., 2008), the levels of reproductive hormones (Charkoudian, 2003) and is sexually dimorphic (Nestor & Tarr, 2008). Such colour signals of fertility are found in females of other primate species (Barelli et al., 2007; Setchell et al., 2006)

Facial redness is also important in human social interaction as it serves as an important cue to negative emotions such as fear (blanching) and anger (reddening). The blood perfusion of our skin fluctuates as our emotions change (Drummond, 1999; Drummond et al., 2007; Drummond & Lazaroo, 2012). Humans have a negativity bias or the tendency to allocate more attention and to learn more from negative events and stimuli (Vaish, Grossmann, & Woodward, 2008). This negativity bias serves as a critical evolutionary adaptive function. Attending to negative emotions was proposed to be more important as compared to positive emotion as we need to be able to respond faster to events that provoke negative emotions, which can be dangerous and create a threat for our well being or survival (Vaish et al., 2008).

Öhman, and colleagues (2001) found that angry faces were detected more accurately and quickly as compared to other negative emotions. The negativity bias of processing has gained support from a functional magnetic resonance imaging (fMRI) study (Cunningham, Raye, & Johnson, 2004) whereby the right inferior frontal or the insular cortex, which was associated with the valence-based evaluations of stimuli, showing greater activity to negative events as compared to those being rated as more positive.

As facial redness changes drastically when one is angry (Lin & Cheng, 2012) and is perceived as being aggressive and dominant (Stephen et al., 2012), the

ability to detect facial redness may mean that increased sensitivity to skin colour – especially redness – fluctuations may be advantageous in both social and survival contexts.

Red coloration seems to play a significant role in human competition and achievement. Hill and Barton (2005) found that, wearing red, is associated with a high chance of winning in various Olympic combat sports (boxing, tae kwan do, and wrestling), even though the outfit and body protectors were assigned randomly. Hagemann, Strauss and Leissing (2008) argued that this was probably due to the perceptual bias in the referees. They showed in two studies that professional tae kwon do referees awarded more points to the fighters wearing red even when their performance is identical to their opponents who were wearing blue. Attrill and colleagues (2008) found that football teams wearing red showed significantly better performance than non-red teams over a 55-year period in eight English cities.

3.4.4 Cross Race Effect

The Cross Race Effect was observed whereby Malaysian Chinese participants performed equally well when identifying colour changes in Asian and Caucasian faces, but did less well when judging colour changes in less familiar African faces.

Human experience and familiarity with faces are important for facial recognition (Furl et al., 2002; O'Toole & Jiang, 2006). The differential experience hypothesis suggests that, the greater our experience and exposure with other ethnic groups, the better one's performance in cross-ethnic facial recognition (Malpass & Kravitz, 1969). Malaysian Chinese are exposed to East Asian faces on a daily basis and have, on average, much more exposure to Caucasian faces as compared to African faces. While the United Kingdom was one of the top five sending countries of expatriates in Malaysia (Kanapathy, 2008), none of the African countries was listed as a major source of foreign labour in Malaysia, either as expatriate or low skilled labour (Kanapathy, 2008; Nair & Jantan, 2006). Further, Malaysia is a multicultural country which has strong influence of Western culture. About 82% of the movies shown in cinemas in Malaysia were contributed by the United States of America and the European Union, while local productions contributed only 14% of the movies shown (Epstein, 2011). This relatively high exposure to faces of different ethnicities might explain why our participants were more sensitive towards colour changes in Caucasian faces than in African faces.

The differential ability of our participants to recognize colour changes in faces of different ethnicity suggests that colour, as well as shape, may contribute to the other race effect, whereby people find it easier to recognize the faces of own- and familiar- than other-ethnicity faces.

An alternative explanation of why the initial colour of the face might influence how well one is able to notice such changes. It may be more difficult for one to notice the colour changes in dark skinned faces (Coetzee & Perrett, 2014b). Future research is needed to clarify this confusion.

3.3.5 Conclusion

In summary, we found in our study that individuals are more sensitive towards changes in chrominance (CIELab a* and b*), but not luminance (L*) in face photographs than in colour patches and non-face object images. We suggest that this extra sensitivity in chrominance in face perception, might be attributed to the increased salience for colour in face stimuli, which provides information about health, fertility and emotional status.

CHAPTER 4: SUPPLEMENTATION STUDY

A version of this chapter was published as Tan KW, Graf BA, Mitra SR, Stephen ID. (2015). Daily Consumption of a Fruit and Vegetable Smoothie Alters Facial Skin Color. *PLoS ONE* 10(7): e0133445. doi:10.1371/journal.pone.0133445

4.1 Introduction

Colour of skin, feather and scale is important for mate selection as various animal species use bright and colourful carotenoid-based ornaments to attract mates (Amundsen & Forsgren, 2001; Blount et al., 2003; Bourne et al., 2003; MacDougall & Montgomerie, 2003; Massaro et al., 2003). Carotenoids are natural pigments produced by plant, fungi and microorganism which are responsible for the bright colors of various vegetables and fruits (Paiva & Russell, 1999). Carotenoids which give the yellow–orange–red colour in many fruits and vegetables (Mortensen, 2006) is required in the diet of most animals for photoprotection and protection from oxidative stress (Moran & Jarvik, 2010)

Preference to mate with members of the opposite sex with bright and flamboyant ornaments has been found in a range of species. Carotenoid colouration is an important criterion in a number of species' mate selection

processes as it reflects the physical wellbeing of that animal (Blount et al., 2003; Bourne et al., 2003; MacDougall & Montgomerie, 2003; Massaro et al., 2003; Pitcher et al., 2007; Williamson, Surai, & Graves, 2006). Increased carotenoid concentration was found to be associated with lower occurrence of blood infection and lower parasite load in birds (Hamilton & Zuk, 2009), and higher resistant to bacterial and fungal diseases in fish (Lara-flores, 2013).

Despite an abundance of research investigating the significance of carotenoids in animals' physical quality and mate selection (Blount et al., 2003; Bourne et al., 2003; MacDougall & Montgomerie, 2003; Massaro et al., 2003; Amundsen & Forsgren, 2001; Peters, Denk, Delhey, & Kempenaers, 2004), few studies have investigated their impact on human skin and health functioning.

Human skin has a similar function of providing cues to health status whereby increased skin luminance (CIELab L*), redness (a*) and yellowness (b*) are perceived as healthier by observers (Stephen et al., 2011; Stephen, Law Smith, et al., 2009). When requested to optimize the perceived health of colour calibrated facial images, individuals increased skin yellowness the most as compared to skin redness or skin luminance (Stephen, Law Smith, et al., 2009). Even though skin yellowness is contributed by both melanin colouration (for example, from tanning) and carotenoid colouration (from fruit and vegetable

intake), Lefevre and Perret (2014) found that individuals consistently preferred carotenoid colouration over melanin colouration.

4.1.1 Skin yellowness and carotenoids intake

Skin yellowness is mainly determined by one's dermal carotenoids and melanin (Alaluf et al., 2002; Stephen et al., 2011). Stephen et al. (2009, 2011) highlighted that skin yellowness is directly affected by natural fruit and vegetable intake - individuals with a higher dietary carotenoid intake tend to have slightly yellower skin tone than those with a lower dietary carotenoid intake. Examining the skin colour of 35 undergraduate students at the University of St. Andrews, Whitehead and colleagues (2012) found a marginal effect of change in facial yellowness after increased consumption of fruits and vegetables over a 6-week period.

Similar results have been observed in supplementation trials. Stahl and colleagues (1998) found an increment in carotenoid levels in different parts of human skin after the participants consumed Betatene capsules containing approximately 25mg of beta-carotene daily for 12 weeks. The increments were especially obvious around the forehead, palm and dorsal skin region.

A similar effect was obtained with a mixture of different kinds of carotenoids in a study conducted by Heinrich et al., (2003), whereby 24mg of the three main dietary carotenoids (i.e. carotene, lutein and lycopene) were prescribed

to participants for 12 weeks, and an increment in skin yellowness among participants was observed after 6 weeks.

While previous research has explored changes in skin colour using dietary supplements in the form of capsules, little is known if the same effect can be detected using a natural form of carotenoids, specifically by using fruit juice. Using juice potentially has higher practicality, as not everyone can easily access supplementation pills. Besides, it is also less controversial than using supplementation pills (McCormick, 2010). Dietary supplements are an ineffective substitute for natural sources of nutrients, as it has been shown in several studies and reviews that they are inefficient and might even bring harm to the consumers (Gold, Slone, & Ames, 2001; Pryor, Stahl, & Rock, 2000; Schunemann, Freudenheim, & Grant, 2001; Timbo, Ross, McCarthy, & Lin, 2006). For example, some of the commonly used supplements like ginkgo biloba, ginseng or black cohosh have been reported to have caused side effects such as spontaneous bleeding, hypertension and mild gastrointestinal symptoms (refer to Ashar & Rowland-Seymour, 2008, for more details). A one year multicentre observational study of dietary supplement exposures, involving 21 of the 65 poison control centres in the United State of America (Palmer et al., 2003), reported that approximately one third of the reported cases were greater than mild severity, as reviewed by an independent panel of reviewers. Various symptoms have been reported including myocardial infarction, liver failure, bleeding, seizures and death (Palmer et al., 2003).

Paiva and Russell (1999) highlighted the importance of investigating the appropriate doses of carotenoids needed to bring upon health advantages. Previous studies prescribed supplementation of carotenoids in capsule form (Coetzee & Perrett, 2014; Stahl et al., 1998), to our knowledge, there is no research exploring the changes that might occur from supplementation with real fruit and vegetables. Further, the previous studies (Coetzee & Perrett, 2014; Stahl et al., 1998) did not include a control group in their study, making it difficult to attribute the change observed to the supplementation. All the previous studies were conducted on Caucasian samples (Stahl et al., 1998; Heinrich et al., 2003) and one African sample (Coetzee & Perrett, 2014). It is unknown if the same kind of skin colour changes can be observed in an Asian population.

4.1.2 Fruit and Vegetable Consumption among Malaysian Chinese

Prevalence of chronic diseases has increased in recent decades with changes in dietary habit and with the adoption of sedentary lifestyles (Noor, 2002). Individuals who live in modern cities are shifting away from strenuous, labour intensive activities to more sedentary ones, and consume more refined grains, more food higher in fat, more animal products, more sugar and more processed food, as compared to their rural counterparts (Popkin, 1998) .

Insufficient consumption of fruit and vegetables has been identified as among the most significant reasons for the loss of quality adjusted life years (Ezzati, Lopez, Rodgers, Vander Hoorn, & Murray, 2002).

In the year 2001, approximately 2.7 million deaths and 26.7 million disabilities globally have been associated with low fruit and vegetable consumption (Amal & Paramesarvathy, 2011). Many developing countries, including Malaysia, have been found to consume insufficient amounts of fruit and vegetables (Hall, Moore, Harper, & Lynch, 2009). A research conducted in Selangor, Malaysia (where this study was conducted) revealed that the participants consumed approximately 173 grams of fruits and vegetables per day (Izzah & Fatimah, 2012) - way below the 400 grams per day, or five servings, that is recommended by the World Health Organization and used by many countries (Agudo, 2005).

4.1.3 Dietary Compensation

Unhealthy lifestyle, such as low fruit and vegetable consumption, which causes morbidity in adulthood and early mortality, is often preventable (Grunbaum & Kann, 2002). There is a call to plan, develop and promote effective strategies to promote health-enhancing behaviors, such as promoting fruit and vegetable consumption (Coyle et al., 2009; Rao & Rao, 2007). Although educational-based intervention is relatively cheap and easy to disseminate, it has little impact on lifestyle changes towards improved

dietary habits (Sluijs, Beulens, Grobbee, & Schouw, 2009). A practical-based intervention might be more useful. The Mississippi Department of Education at the United States of America launched a program in 2004-2005 to distribute free fruit and vegetables to students from kindergarten to 12th grade (Coyle et al., 2009). The program not only improved the familiarity of all graders towards fruit and vegetables but also increased preference and willingness of the eighth- and tenth-grade students' to consume more fruit. It seems that one's food preference can be modified with repeated exposures (Coyle et al., 2009).

However, some scientists suggested that sometimes behavioral intervention might result in behavioral compensation; that is the adjustment of habitual energy intake in response to the extra energy consumed in an intervention. Institutionalized seniors with probable Alzheimer's Disease, specifically those with low body weight compensated for the extra energy consumed in a food supplementation study by reducing their lunch intake (Young, Greenwood, van Reekum, & Binns, 2004).

Changes in energy balance induced by exercise may also initiate compensatory behavior adjustments, and alter one's dietary intake, as found by some of the studies focusing on exercise related intervention (King, Hopkins, Caudwell, Stubbs, & Blundell, 2008; Staten, 1991). The variability of the outcome of a weight control related intervention is suggested to be at

least partially related to individual differences in their compensatory adjustment either by changing their energy intake or energy expenditure, which is beyond the control of the intervention. King et al. (2008) found in their study that overweight individuals who participated in a 12-week exercise intervention who experienced a lower than predicted weight loss demonstrated a compensatory increase in energy intake over the course of the intervention. As there was no significant change in participants' resting metabolic rate before and after the intervention, King and colleagues proposed that the adaptations were more behavioral than metabolic. Manthou and Gill (2010) also revealed that the lower than predicted weight and body fat loss can be attributed, at least in part, to a reduction in physical activity outside exercise sessions. Gipson (2010) however found that individuals maintained the same level of energy intake and energy expenditure across different time points along with their participation in structured exercise. Based on the above literature, it seems that individuals may have a tendency to reduce their calories intake, in response to the extra supplementation introduced by the intervention.

4.1.4 Objective of the study

Previous studies have examined the relationship between carotenoid intake and skin yellowness in both Caucasian and African samples, by using beta-carotene pills (Coetzee & Perrett, 2014; Stahl et al., 1998). Stephen and colleagues (2011) have also found an association between self-reported fruit

and vegetable intake and skin yellowness. Yet, no study has investigated the impact of supplemented natural sources of carotenoids on changes in skin colour, and no similar study has been conducted in an Asian population. Study 6 in this chapter aimed:

1. To investigate if supplementation with a smoothie high in carotenoids will induce a change in Malaysian Chinese participants' skin colour, specifically in skin yellowness (b*), skin redness (a*) and skin luminance (L*).

It was hypothesized that only participants in the intervention group will increase in their skin yellowness after the supplementation with smoothie.

By using data collected from the food frequency questionnaire from the supplementation study, we aimed:

2. To investigate if participants achieved the minimum requirement of 400 grams of fruits and vegetables per day.
3. To identify the main sources of carotenoids in the Malaysian Chinese diet.

As previous studies (King et al., 2008; Staten, 1991; Young et al., 2004) showed that individuals may vary their diet and exercise level when they participate in diet and exercise related intervention to compensate for the supplementation, this study also aimed:

4. To examine if participants, particularly those assigned to the intervention group will reduce their energy intake and fruit and vegetable consumption after receiving supplementation in the form of a smoothie on daily basis.

It was hypothesized that only participants in the intervention group will reduce their energy intake and consumption of fruit and vegetable as a result of the intervention. No change in diet would be expected for the control group.

4.2 Method

This study was approved by the Ethics Committee at the University of Nottingham (Malaysia Campus). All participants gave both verbal and written informed consent prior to the experiment.

This is a 2x4 mixed-model design (2 groups x 4 measurements). Participants were pseudo-randomly assigned to either the intervention group or the control group. Participants in the intervention group were given a bottle of fresh fruit smoothie (500ml) to drink on a daily basis, whereas participants from the control group were given mineral water described as “purified detoxifying water” as a placebo, to drink on a daily basis. The entire supplementation lasted for six weeks. Participants were requested to fill in an online food frequency questionnaire twice, once before the supplementation started, and another one right after they took their final prescribed drink

(after six weeks of supplementation). Participants were required to attend four measurement sessions, where their body composition and skin colour were measured. The first measument was taken before the supplementation, two measurements were taken during the supplementation, and a follow up measurement was taken two weeks after the supplementation had ended. Comparison of skin colour changes were made between the two groups and between the four measurements.

4.2.1 Participants and Sampling

Eighty-one heathy Malaysian Chinese participants (34 males, 47 females; mean age 20.48, SD= 1.22) were recruited from the University of Nottingham Malaysia Campus. Pseudo random assignment was utilised by which the participants recruited were randomly assigned to either the intervention group (n=41) or the control group (n=40) based on their baseline total carotenoid intake using the following procedure. Participants were ranked by baseline carotenoid intake (assessed using a food frequency questionnaire) and randomly assigned in pairs, such that one of the two participants with the highest daily carotenoid intakes was randomly assigned to the intervention group and one to the control group, and so on, to ensure a balanced distribution of high and low carotenoid consumers in each group. Block randomisation based on baseline carotenoid intake was used to eliminate bias that may be observed due to skewed diet content in one of the groups. Volunteers who consumed more than 3 cigarettes per day were excluded, as

it has been found that consumption of a high volume of carotenoids may lead to potential adverse effects in smokers (Paiva & Russell, 1999).

Participants were not allowed to use any skin colour alteration products, specifically those with bleaching and tanning purposes. We also ensured that none of our participants had any chronic or acute illness, especially any mal-absorption disease, liver disease, disease of lipid metabolism or photosensitivity disorder, as all these ailments could either affect the absorption of carotenoids or affect the changes in skin colour in a way that was not due to the supplementation.

Eighty-one participants were initially recruited and 71 attended the second measurement. However, three of these participants were excluded from the analysis as their data were incomplete. Sixty-seven participants took supplementation until week-6 and attended the third measurement; however, only 50 participants came for the final follow-up measurement. The numbers of participants in both groups across different sessions are presented in Table 4.1.

Table 4.1

Amount of participants per group throughout the study

Measurement	Intervention Group	Control Group	Total
First recruitment	41	40	81
Completed the 2nd measurement	33	36	69
Completed the 3rd measurement	33	34	67
Completed the follow up	27	23	50

For the analysis, we have complete data from 69 participants (28 males, 41 females; mean age 20.51, SD= 1.21) with 33 in the intervention group and 36 in the control group, all of whom attended at least two measurements.

4.2.2. Procedure: Dietary intervention

Participants in the intervention group were given a bottle of fresh fruit smoothie (500 mL, including approximately 50 mL of lipids), while the participants in control group received a bottle of filtered water (500 mL; described as “purified, detoxifying water”) every weekday for six weeks. Both groups were told that both prescribed health drinks (the smoothie and the water) have potentially beneficial effects on skin health.

Researchers from the School of Nutrition at the University of Nottingham Malaysia Campus (BAG) designed six carotenoid-rich smoothies, made by mixing 250ml carrot juice with other juices from local under-utilised fruits. The local under-utilized fruits used included pitaya (dragon fruit), star fruit, hog plum (or ambarella, also known as *kedondong* by the locals), sapodilla (*chiku*) and tropical fruits such as watermelon, pineapple and banana. Two or more fruit juices were blended together in order to improve the overall taste and aroma of the beverages (Bhardwaj & Pandey, 2011). Each of the smoothies contained approximately 20-25 mg of beta carotene.

Carotenoids are lipid soluble, thus 3% (w/w) of lipid, in the form of palm oil (carotino), milk or cream, was added into each smoothie, to ensure optimal bioavailability (Brown et al., 2004). More than 40 different smoothie recipes were designed one month before the supplementation was launched. Ten smoothies were selected to be used in the supplementation, based on palatability ratings given by an independent sample of participants.

Participants assigned to the intervention group were given one smoothie on each weekday for 6-weeks. The smoothies were prepared fresh every morning, and participants were requested to come and collect the smoothie and to finish the smoothie within 3 hours of preparation. After blending the mix for 5 minutes, the smoothie was distributed to participants in reusable opaque plastic drinking bottles, to reduce possible photo-degradation of

carotenoids. Participants from the control group were asked to collect the filtered water on a weekly basis. They were given five bottles of filtered water and were asked to consume one bottle per day, on every weekday.

The samples of smoothie prescribed were later sent to a collaborator (Brig) for HPLC analysis. A summary of the methods is in Appendix 1. The recipes and carotenoid content determined by HPLC are in Appendix 2.

4.2.3 Procedure: Skin colour and body composition measurement

Participants attended four measurement sessions throughout the whole study. In each measurement session, the body composition and skin colour of the participants were measured. The four measurement sessions were conducted 1) before supplementation (on week 0), 2) during the supplementation (on week 5), 3) at the end of the supplementation (on week 7) and 4) two weeks after the intervention had ceased (on week-9).

Participants' body composition was measured using a Tanita SC-330 body composition analyzer -- a non-intrusive leg-to-leg bioelectrical impedance scale that provides detailed information about one's body composition, which includes their body weight, fat mass, fat-free mass, muscle mass, bone mass, total body water, body mass index, visceral fat rating, basal metabolic rate and metabolic age.

Participants' skin colour was measured using a handheld Konika Minolta CM2600D spectrophotometer. This is a painless, non-invasive method utilising the Commission International de l'Eclairage (CIE) L*a*b* colour system measuring the reflectance of luminance (L*; on gray scale from 0 to 100 where 0 is black and 100 is while), redness (a*; scale from +127 to -128, from red to green) and yellowness (b*; scale from +127 to -128, from yellow to blue) on the targeted object.

Seven body regions of participants were measured including the left inner upper arm, right cheek, left cheek, fore head, left outer upper arm, left shoulder and left palm (Figure 4.1). These regions were selected because different patterns of colouration of the skin have been observed after 12 weeks consumption of beta carotene in a previous study (Stahl et al., 1998).

Carotenoids are not equally distributed in the skin (Darvin, Gersonde, Albrecht, Sterry, & Lademann, 2006; Darvin et al., 2009). High levels of carotenoids are found in the forehead, and palm of the hand as compared to other regions (Stahl et al., 1998; Darvin, Gersonde, Albrecht, Sterry, & Lademann, 2006). A few different factors have been found to contribute to the difference in the concentration of carotenoids within different body regions, including exposure to ultra violet radiation. Ultra violet radiation causes production of reactive oxygen species in human skin, which would reduce the amount of antioxidants when present in high concentration.

Previous research (Darvin et al., 2006) detected a decrement in the amount of beta carotene and lycopene in the skin within 90 minutes after an exposure to sunlight. Further, it depends on the thickness of *stratum corneum* which composes the outermost layer of the epidermis. Higher levels of carotenoids are found in the upper layers of the *stratum corneum*, and the concentration is reduced in deeper levels of the skin (Darvin et al., 2009). Darvin and colleagues (2009) discovered that body regions with high densities of sweat glands and sebaceous glands (forehead) have the highest concentration of carotenoids.

Two sets of measurements were taken for each region, and the averaged figures were used for analysis. The spectrophotometer was calibrated each time it was switched on.

Spectral reflectance measurements of skin colour were recorded, sampling every 10nm from 360-740nm (a range that encompasses the entire visual spectrum and extends into ultraviolet at the short wavelength end and infra red at the long wavelength end).



Figure 4.1. From top left, clockwise: the scanned regions, i.e. the left inner upper arm, cheeks, forehead, left outer upper arm, left shoulder and left palm.

4.2.4 Dietary Assessment

Of the 81 participants recruited for Study 6, only 48 participants completed six weeks of supplementation and food frequency questionnaires before and after supplementation. Two out of these 48 were identified as outliers with their fruit and vegetable consumption (outside 3 standard deviations from the mean). Forty six participants' data were used for the dietary assessment with 23 in the intervention group and 23 in the control group (19 males, 27 females).

Participants recruited for the supplementation study were requested to complete a food frequency questionnaire one week before the study began and one week after they received their last supplementation. They were asked to exclude the smoothie that was given as part of the supplementation from their reported diet.

The online food frequency questionnaire was built online at Qualtrics (https://asia.qualtrics.com) based on the food frequency questionnaire created by Loy and colleagues (2011), and modified after consultation between a nutrition scientist (SM) and a local Malaysian Chinese researcher. A series of carotenoid rich local fruit and vegetables that are commonly consumed in Malaysia were considered and added into the questionnaire, including apricot, loquat, peach and persimmon (fruit); spinach, lettuce and water cress (green leafy vegetables); French beans and green peas (legumes); carrots and beetroot (root vegetables); peppers, broccoli and tomatoes (other vegetables).

There were 151 food items in total. For each of the food items listed, a corresponding photograph of the item was included alongside each question to aid item identification and to act as a reference for standard portion size used (https://www.dropbox.com/s/bf9pdk2lpxii3j9/Thesis-Appendice-FFQ.doc?dl=0). The portion sizes shown were based on the 'Atlas of Food Exchanges and Portion Sizes' (Shahar et al., 2009). Participants indicated how

frequently they consumed each of the items listed (never, once a week, 2-4 times a week, 5-6 times a week, once per day, 2-3 times per day, 4-5 times per day), and the portion size consumed per sitting during the previous 7 days.

The quantity of carotenoids in each food item was estimated based on the "Nutrient Composition of Malaysian Foods" by Tee at al. (1997). Values for items that were not found in Tee et al. (1997) were retrieved from the online Canadian Nutrient File (http://webprod3.hc-sc.gc.ca/cnf-fce/index-eng.jsp: apricot, broad beans and loquat) or Fleshman et al. (2011: honey dew). Total daily carotenoid intake was calculated by multiplying the carotenoid content of one portion of each food item by the number of portions consumed per sitting, by the number of times the participant consumed that food item per week (where the response in the FFQ was a range of values, the mid-point was used), divided by 7 to give per day consumption, and finally summed across all food items to give an estimate of total daily carotenoid intake at baseline. A similar process was used to calculate daily energy intake.

4.2.5 Statistical Methods

Mixed ANOVA was chosen to examine the changes in three skin colour dimensions over the course of the study. Skin colour (L*, a* or b*) was the dependent variable, while measurement (baseline, second, third and fourth measurement) and group (intervention vs. control group) were within-subject and between subject factors respectively.

4.3 Results

4.3.1 Preliminary analysis for changes in skin colours

A series of independent samples t-tests were administered to ensure that participants in both intervention group and control group have similar diet and body composition. No significant difference in calories, $t(67) = 1.126$, $p = 0.264$, or carotenoids consumed, $t(67) = .197$, $p = 0.844$) was found between the intervention and control groups. Independent t-tests showed that participants in both groups were similar in their body composition. No significant difference between two groups was found in body height ($t(67) = 1.586$, $p = .117$), weight ($t(67) = .234$, $p = .816$), fat mass ($t(67) = -1.656$, $p = .104$), fat-free mass ($t(67) = 1.45$, $p = .151$), muscle mass ($t(67) = 1.46$, $p = .15$), bone mass ($t(67) = 1.342$, $p = .184$), total body water ($t(67) = 1.05$, $p = .297$), body mass index ($t(67) = -.992$, $p = .326$), or basal metabolic rate ($t(67) = 1.119$, $p = .267$), indicating that if any difference was found between the two groups, it was unlikely to be due to their body composition.

Since facial colour is the main focus, the average of skin luminance, redness and yellowness at the participants' left cheek, right cheek and forehead were calculated. The colour of these three regions were averaged and coded as the facial skin colours. Independent t-tests showed that the average luminance (mean=61.65, SD=4.79), redness (mean=13.62, SD=1.6) and yellowness (mean=18.21, SD=1.86) of facial skin between the two groups were not significantly different ($p>.05$) during the baseline measurement. The

descriptive data of the body composition and skin colour for the two groups are presented in Table 4.1.

Table 4.1

Descriptive data of the body composition and skin colour for the two groups

	Supplementation Group		Control Group	
Measurement	Mean	SD	Mean	SD
Height	167.27	8.75	164.18	8.80
Weight	59.17	10.29	58.43	15.53
Fat Mass	11.52	4.40	14.31	8.95
FFM	47.65	9.77	44.11	10.44
Muscle	45.06	9.36	41.67	9.93
TBW	32.21	6.85	30.39	7.54
Bone	2.59	.42	2.44	.54
BMR	5928.68	1057.48	5620.03	1225.36
Visceral	3.03	2.75	3.71	3.21
BMI	20.84	2.76	21.74	4.57
L	61.1	2.76	62.2	6.16
a*	13.6	1.43	13.64	1.77
b*	18.33	1.66	18.09	2.05

4.3.1.1 Changes in skin yellowness

To examine the changes in skin yellowness over the course of the study, a mixed ANOVA was conducted with skin yellowness (b*) as the dependent variable, measurement (baseline, second, third and forth measurement) as a within subject factor, and group (intervention vs. control group) as a between subjects factor.

Results showed a significant main effect of group $F(1, 48) = 44.127$, $p<.001$, $\eta_p^2 = .479$, with participants in the intervention group exhibiting a yellower skin tone (EMM=20.972, SE=.287) than those in the control group (EMM=18.165, SE=.311). Greenhouse-Geisser correction showed a significant difference in facial yellowness between measurements, $F(3,121) = 43.916$, $p<.001$, $\eta_p^2 = .478$. Bonferroni corrected pairwise comparisons revealed significant changes in skin yellowness between the baseline measurement and second (mean difference=-1.511, $p<.001$), third (mean difference=-1.712, $p<.001$) and fourth measurements (mean difference=-1.556, $p<.001$). No significant change was found between the second, third and fourth measurements (all $p>.05$).

An interaction between group and measurement was detected, $F(3,121) = 62.38$, $p<.001$, $\eta_p^2 = .565$. To investigate this result further, we conducted two separate repeated measures ANOVAs: one for the treatment group and one for the control group. A significant difference in skin yellowness between

measurements was detected for the intervention group F(3, 78) =106.773, p<.001, η_p^2 = .804. Pairwise comparisons showed that participants in the intervention group increased in skin yellowness on the second (mean difference=3.382, SE=.239, p<.001), third (mean=3.793, SE=.306, p<.001) and last measurement (mean=3.257, SE=.239, p<.001) as compared to the baseline measurement (mean=18.36, SD=1.68). No significant changes in skin yellowness were detected between the second, third and fourth measurements (p>.05). Also, no difference between measurements for the control group was detected F(3, 66) =1.103, p=.354, η_p^2 = .048. The interaction between group and measurements is presented in Figure 4.2.

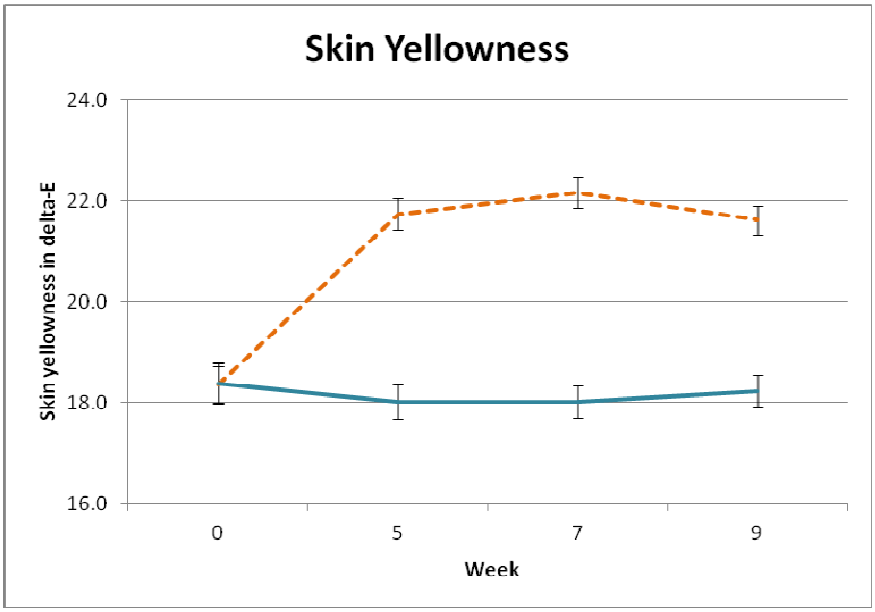


Figure 4.2. Skin yellowness across the intervention. Solid line indicates skin yellowness for participants in the control group whereas the dashed line indicates skin yellowness for participants in the intervention group.

4.3.1.2 Changes in skin redness

As for skin redness, a mixed ANOVA showed a significant difference in facial redness between the two groups F(1, 48)=10.402, p=.002, η_p^2 = .178, taking the study duration as a whole. Participants in the intervention group had overall redder skin tones (EMM=14.431, SE=.274) as compared to those in the control group (EMM=13.127, SE=.297). Greenhouse-Geisser correction showed a significant difference in facial redness between measurements, F(2,112)=3.115, p=.04, η_p^2 = .061. However, Bonferonni pairwise comparisons failed to detect the differences between the four individual measurements. We thus conducted an LSD test which is thought to be more sensitive in detecting changes within groups as compared to Bonferonni (Field, 2009). A significant difference was found between the baseline and second measurements (mean difference=-.443, p=.018) whereby participants' skin redness slightly increased during the second measurement (EMM=13.961, SE=.223) as compared to the baseline measurement (EMM=13.518, SE=.243). An interaction between group and measurement was detected, F (2,112)=9.937, p<.001, η_p^2 = .172. A repeated-measures ANOVA showed significant difference in skin redness between measurements for the intervention group F(3, 78)=11.745, p<.001, η_p^2 = .311. Pairwise comparison showed that participants in the intervention group increased in their skin redness on the second (mean difference=1.077, SE=.239, p<.001), third (mean=1.183, SE=.259, p<.001) and fourth measurements (mean=.953, SE=.234, p<.001) as compared to the baseline measurement (EMM=13.628,

SE=.284). No significant change was detected between the second, third and fourth measurements (p>.05). Greenhouse-Geisser correction showed no significant difference in skin redness between measurements for the control group F(2, 40)=2.006, p=.151, η_p^2 = .084.

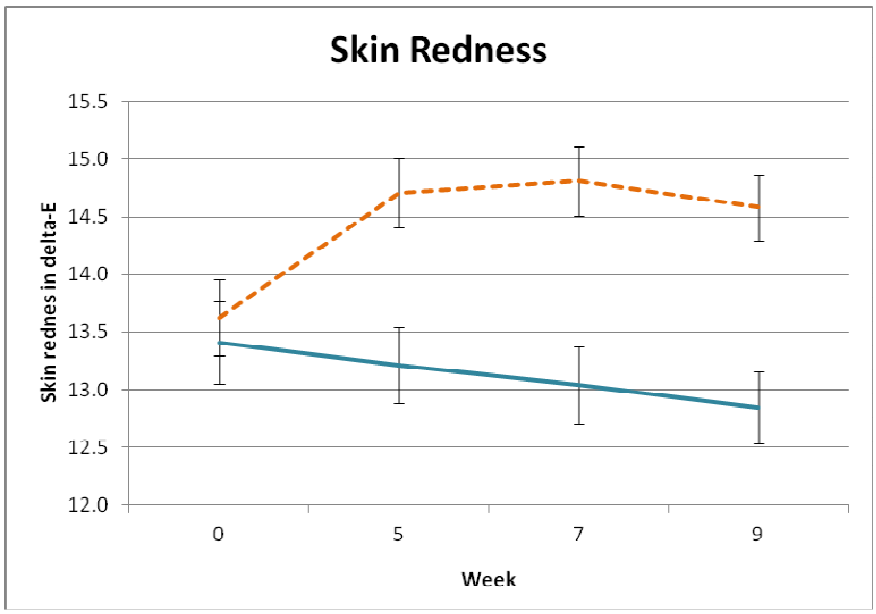


Figure 4.3. Skin redness across the whole intervention. Solid line indicates skin redness for participants in the control group whereas the dashed line indicates skin redness for participants in the intervention group.

4.3.1.3 Changes in skin luminance

A mixed ANOVA was conducted, with skin luminance (L*) as the dependent variable, and measurements (baseline, second, third and forth measurement) and group (intervention vs. control group) as independent variables. Results showed no overall difference in skin lightness between the two groups, F (1,

48)=.862, p=.358, η_p^2 = .018. Using Greenhouse-Geisser correction, a significant change in participants' skin lightness between measurements was detected, F (2,79)=5.031, p=.013. η_p^2 = .095. Using Bonferonni pairwise comparison, participants' skin were found to be darker in their third measurement (EMM=59.98, SE=.455) as compared to the baseline measurement (EMM=61.746, SE=.759) with mean difference= -1.766, p=.037. No interaction between group and measurement was found for skin lightness, F (2,79)=.328, p=.678, η_p^2 = .007 (Figure 4.4).

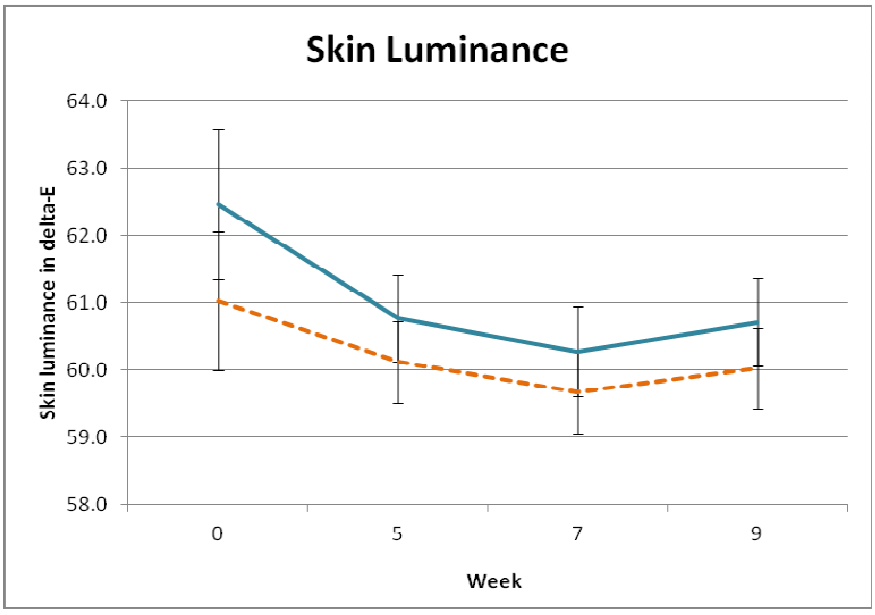


Figure 4.4. Skin luminance across the whole intervention. Solid line indicates skin luminance for participants in the control group whereas the dashed line indicates skin luminance for participants in the intervention group.

4.3.1.4 Initial skin yellowness and changes observed

The amount of change in skin yellowness observed within the participants in the intervention group was normally distributed. Pearson's correlation was conducted to examine the association between increment in skin yellowness at week 5 and baseline skin yellowness. There was a significant negative correlations between the increment observed and the baseline skin yellowness, r(33)=-.462, p=.007. Participants with lower skin yelllowness at baseline experienced higher levels of increment in skin yellowness. The correlation between the increment and baseline skin yellowness are illustrated in the figure below (Fig 4.5)

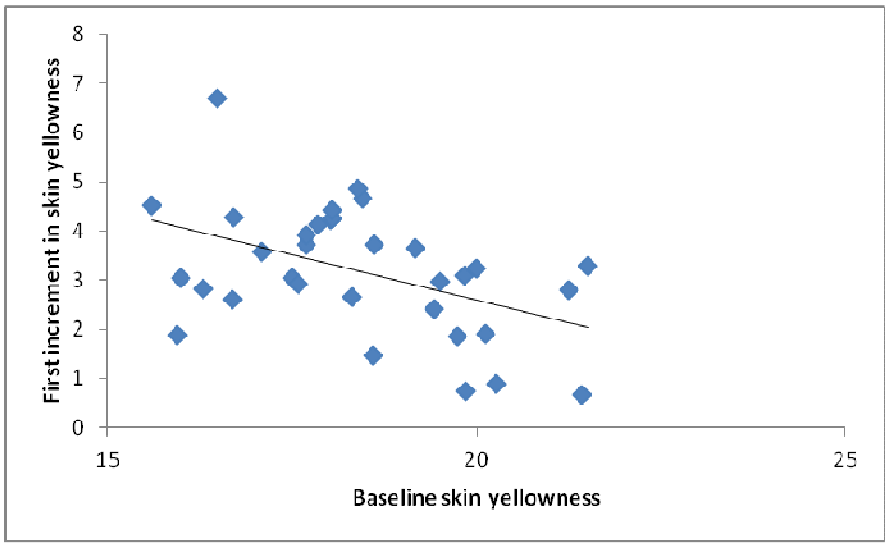


Figure 4.5. Scatter plot showing the correlation between the first increment in skin yellowness and baseline skin yellowness, for supplementation group

4.3.1.5 Spectral analysis

The level of carotenoids within the skin can be measured by using a non-invasive method such as reflectance spectrophotometry. Different pigments in the skin absorb light at certain wavelengths and reflect light at others, and these unique spectra gives the pigments their unique colours (Stephen et al., 2011). Carotenoid absorption is best seen in the range 400-540nm (Stahl et al., 1998). The absorption in the short wavelength part of the spectrum and reflectance at longer wavelengths (yellow, red) give carotenoids their yellow-red colouration.

This section aims to further confirm that the increment in skin yellowness observed from baseline (week 0) to mid-intervention (week 5) was caused by dietary carotenoids deposition within the skin. Standard absorption coefficient values for three common carotenoids (α-carotene, β-carotene and lycopene) were derived from Miller (1937); while the melanin absorption coefficient values were derived from Sarna and Swartz (1988). Since some variables were non-parametrically distributed, Spearman's rank correlations were used to test the relationship between observed changes (between measurements one and two) in reflectance values of skin at the different wavelengths (from 400-540nm) of the two groups (intervention vs. control group) and those from different kind of skin pigments.

Significant correlations were found between the changes in reflectance of skin and the absorption spectra of common carotenoids, specifically β-carotene and lycopene, only for the participants in intervention group ($p<.01$) and not control group ($p>.5$, with exception on right cheek) as shown in Table 4.3. There were significant correlations between the changes in reflectance of skin and the absorption spectra of melanin, only for the participants in control group ($p<.01$) and not the intervention group ($p>.5$).

Table 4.3

Correlation between changes in reflectance of skin and absorption spectra of carotenoids and melanin.

Group	Region		Melanin	β	α	Lycopene
Intervention	Left Cheek	r	043	.674**	.487	.911**
		p	.879	.006	.066	.000
	Right Cheek	r	-.054	.653**	.458	.904**
		p	.850	.008	.086	.000
	Forehead	r	-.054	.653**	.458	.904**
		p	.850	.008	.086	.000
Control	Left Cheek	r	-.914**	-.402	-.541*	.011
		p	.000	.138	.037	.970
	Right Cheek	r	-.679**	.011	-.193	.607*
		p	.005	.970	.491	.016

Forehead	r	-.875**	-.341	-.517*	.357
	p	.000	.214	.048	.191

Changes of the skin reflectance of skin as observed on the participants in intervention group have a similar shape (Fig. 4.6) as the absorption spectra of β-carotene and lycopene. Such a pattern was not found for the changes of the skin reflectance of skin in the control group (Fig. 4.7).

Beta carotene (a yellow carotenoid) shows maximal absorbance in the 450-490nm range of the spectrum, while lycopene (a common red carotenoid) shows maximal absorbance in the 470-510nm range of the spectrum (Miller, 1937). The intervention group exhibited reduced skin reflectance (equivalent to an increase in absorbance) in the 450-510nm range of the spectrum, suggesting deposition of both yellow and red carotenoids, rather than melanin (which absorption peaks at ultraviolet wavelengths). This evidence provides further support for the hypothesis that changes in skin colour of the participants in the intervention group was due to the absorption of carotenoids in the fruit and vegetable smoothies distributed to the participants in the intervention group.

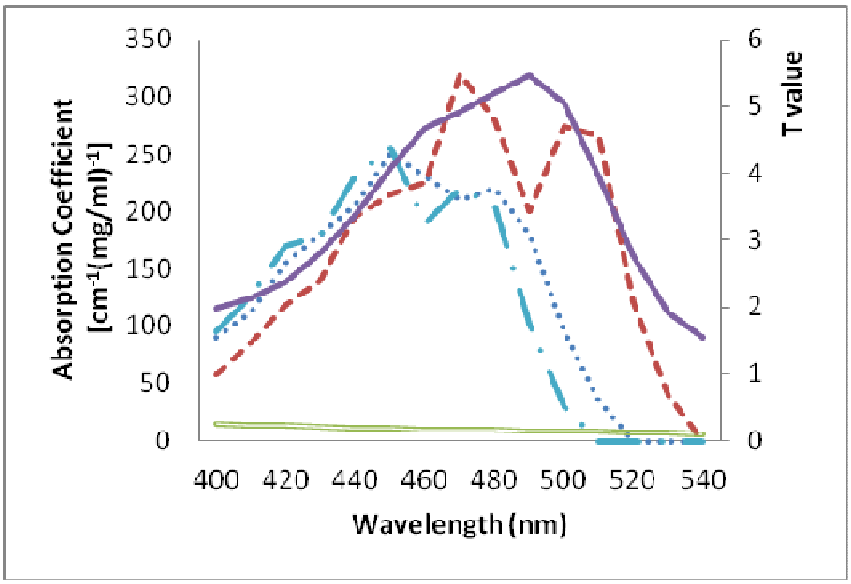


Figure 4.6. Absorption spectra of different skin pigments; and the changes in skin colour of forehead (in t values) for participants in the intervention group. Solid line indicates changes in absorption spectra of the forehead for the participants in intervention group; dotted line indicates absorption spectra of beta carotene; dashed line indicates absorption spectra of lycopene, hollow line indicates absorption spectra of melanin, and dashed line with dots indicates absorption spectra of alpha carotene.

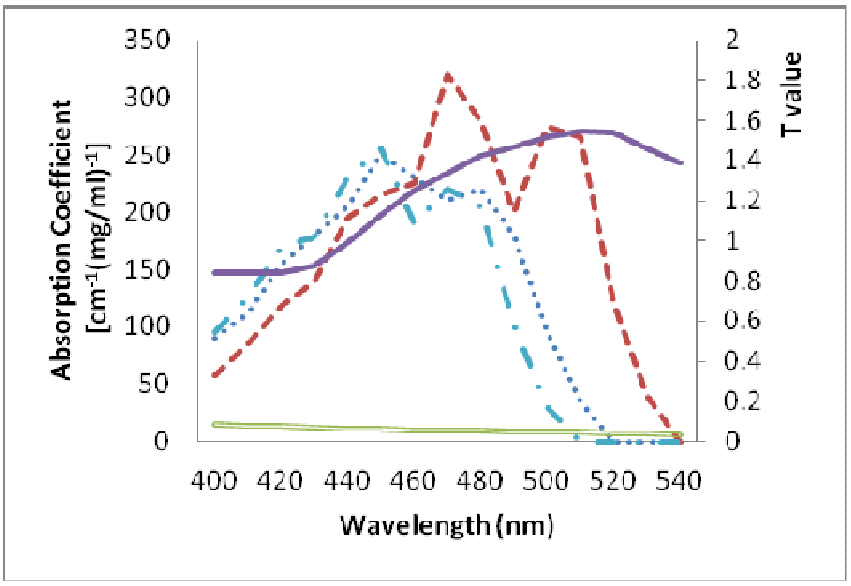


Figure 4.7. Absorption spectra of different skin pigments and the change in skin colours of forehead for participant in control group. Solid line indicates changes in absorption spectra of the forehead for the participants in control group (in t value); dotted line indicates absorption spectra of beta carotene; dashed line indicates absorption spectra of lycopene, hollow line indicates absorption spectra of melanin, and dashed line with dots indicates absorption spectra of alpha carotene.

4.3.1.6 Changes in skin colours in four other regions

A series of mixed ANOVAs were conducted to examine the changes of skin colour in the other four body regions (left inner upper arm, left outer upper arm, left shoulder and left palm) over the course of study between the two groups of participants. Skin colour (CIEL*a*b) was the dependent variable, measurements (baseline, second, third and forth measurement) was coded as

a within subject variable, and group (intervention vs. control group) as a between subject independent variable. The results of all the other four body regions were similar to the results in the face.

4.3.1.6 1 Changes in skin yellowness in four other regions

Results showed significant change in skin yellowness (b*) between the four measurements, for the left inner upper arm, $F(3, 144) = 35.61$, $p<.001$, $\eta_p^2 = .426$; left outer upper arm, $F(2.57, 123.34) = 8.06$, $p<.001$, $\eta_p^2 = .144$; left shoulder $F(3, 144) = 11.23$, $p<.001$, $\eta_p^2 = .190$; and left palm, $F(3, 144) = 60.10$, $p<.001$, $\eta_p^2 = .556$. Pairwise comparisons showed significant differences (or trends) between the first measurement and the three subsequent measurements, for the above four body regions, with skin yellowness increased significantly after the first measurement. No significant difference was detected for between the second, third and forth measurements, except for the palm, whereby a difference was detected between the second and the third measurement. Greenhouse-Geisser corrections were used to correct against all those with violations of sphericity. The pairwise comparisons of skin yellowness across 4 measurements, for the 4 body regions are summarized in Table 4.4, Table 4.5, Table 4.6 and Table 4.7.

Table 4.4

Mean difference across four measurements in b on left inner upper arm*

Measurement		2	3	4
	1	-1.226**	-1.271**	-1.430
	2		-.044	-.204
	3			-.160

**p<.001

Table 4.5

Mean difference across four measurements in b on left outer upper arm*

Measurement		2	3	4
	1	-.471	-.797**	-.526*
	2		-.326	-.055
	3			-.271

*p<.05 **p<.001

Table 4.6

Mean difference across four measurements in b on left shoulder*

Measurement		2	3	4
	1	-.643*	-1.006**	-.980
	2		-.363	-.337
	3			-.026

*p<.05 **p<.001

Table 4.7

Mean difference across four measurements in b on left palm*

Measurement		2	3	4
	1	-2.307**	-3.001**	-2.585**
	2		-.695*	-.278
	3			.416

*p<.05 **p<.001

A significant difference in skin yellowness was detected between the two groups for left inner upper arm, $F(1, 48) = 11.13$, $p = .002$, $\eta_p^2 = .188$; left outer upper arm, $F(1, 48) = 6.23$, $p = .016$, $\eta_p^2 = .115$; left shoulder, $F(1, 48) = 15.89$, $p<.001$, $\eta_p^2 = .249$; and left palm, $F(1, 48) = 65.75$, $p<.001$, $\eta_p^2 = .578$. Pairwise comparisons revealed that participants in the intervention group overall showed yellower skin tones as compared to the participants in the control group, on left inner upper arm (mean difference = 1.778, SE = .533, p

= .002), left outer upper arm (mean difference = 1.173, SE = .470, p = .016), left shoulder (mean difference = 2.316, SE = .581, p<.001), and left palm (mean difference = 3.533, SE = .436, p<.001).

A significant interaction was detected between measurement and group for skin yellowness at left inner upper arm, $F(3, 144) = 31.74$, $p<.001$, $\eta_p^2 = .398$; left outer upper arm, $F(2.57, 123.34) = 16.43$, $p<.001$, $\eta_p^2 = .255$; left shoulder, $F(3, 144) = 15.33$, $p<.001$, $\eta_p^2 = .242$; and left palm, $F(3, 144) = 84.11$, $p<001$, $\eta_p^2 = .637$. Greenhouse-Geisser corrections were used to correct against all violations of *sphericity.*

Eight repeated measures ANOVAs (with skin yellowness as independent variable, measurement as within subject factor), for four different body regions, were conducted to examine the above interactions. Overall, the increment in skin yellowness across measurements was detected on participants in the intervention group but not the control group.

There was a significant main effect in measurement for intervention group (3, 78) = 78.14, $p<.001$, $\eta_p^2 = .750$; and not for control group (3, 66) = .765, p = .52, $\eta_p^2 = .034$, for the changes in skin yellowness on left inner upper arm. The pairwise comparisons of skin yellowness on left inner upper arm across 4 measurements, for the two groups were summarized in Table 4.8.

Table 4.8

Mean difference across four measurements in b on left inner upper arm, for intervention group*

Group	Measurement	2	3	4
Intervention	1	-2.305**	-2.650**	-2.639*
	2		-.345	-.334
	3			.012

**p<.001

There was also a significant main effect in measurement for intervention group (2.24, 58.2) = 27.44, p<.001, η_p^2 = .513; and not for control group (2.25, 49.55) = 1.028, p = .372, η_p^2 = .045, for the changes in skin yellowness on left outer upper arm. The pairwise comparisons of skin yellowness on left outer upper arm across 4 measurements, for the two groups were summarized in Table 4.9.

Table 4.9

Mean difference across four measurements in b on left outer upper arm, for intervention group*

Group	Measurement	2	3	4
Intervention	1	-1.187**	-1.827**	-1.496*
	2		-.640	-.309
	3			.331

**p<.001

There was a significant main effect in measurement for intervention group (3, 78) = 24.10, p<.001, η_p^2 = .481; and not for control group (3, 66) = 1.936, p = .13, η_p^2 = .081, for the changes in skin yellowness on left shoulder. The pairwise comparisons of skin yellowness on left shoulder across 4 measurements, for the two groups were summarized in Table 4.10.

Table 4.10

Mean difference across four measurements in b on left shoulder, for intervention group*

Group	Measurement	2	3	4
Intervention	1	-1.759**	-2.204**	-1.819*
	2		-.445	-.060
	3			.385

**p<.001

There was a significant main effect in measurement for intervention group (2.07, 53.73) = 133.54, p<.001, η_p^2 = .837; and not for control group (3, 66) = 1.969, p = .13, η_p^2 = .082, for the changes in skin yellowness on left palm. The pairwise comparisons of skin yellowness on left palm across 4 measurements, for the two groups were summarized in Table 4.11.

Table 4.11

Mean difference across four measurements in b on left pam, for intervention group*

Group	Measurement	2	3	4
Intervention	1	-5.399**	-6.439**	-5.527*
	2		-1.040*	-.128
	3			.912*

**p<.001

4.3.1.6.2 Changes in skin redness in four other regions

Results showed a strong trend towards a significant change in skin redness between the four measurements, for the left inner upper arm, $F(3, 144) = 2.617$, $p = .053$, $\eta_p^2 = .053$. No significant changes in redness for left outer upper arm, $F(2.55, 122.34) = 2.12$, $p = .11$, $\eta_p^2 = .042$; and for left palm, $F(3, 144) = .66$, $p = .58$, $\eta_p^2 = .014$. A significant difference was detected across the four measurements for skin redness at the left shoulder $F(3, 144) = 3.63$, $p = .014$, $\eta_p^2 = .070$. The pairwise comparison using a more sensitive LSD showed significant difference across between measurement 1 and 4 (mean difference = -.74, SE = .297, p = .016); and between measurement 3 and 4 (mean difference = -.697, SE = .241, p = .006). Greenhouse-Geisser corrections were used to correct against all those with violations of sphericity.

A significant difference in skin redness was detected between the two groups for left inner upper arm, $F(1, 48) = 5.124$, $p = .028$, $\eta_p^2 = .096$; left shoulder, $F(1, 48) = 8.56$, $p = .005$, $\eta_p^2 = .151$; and left palm, $F(1, 48) = 10.10$, $p = .003$, $\eta_p^2 = .174$. No significant difference was detected for left outer upper arm, $F(1, 48) = 1.30$, $p = .26$, $\eta_p^2 = .026$ between the two groups. Pairwise comparison revealed that, participants in intervention group showed redder skin tones as compared to the participants in control group, in their left inner upper arm (mean difference = .815, SE = .36, p = .028), left shoulder (mean difference = 1.478, SE = .505, p = .005), and left palm (mean difference = 1.342, SE = .422, p = .003).

No interaction was detected between measurement and group for left inner upper arm, $F(3, 144) = 1.92$, $p = .14$, $\eta_p^2 = .038$; left outer upper arm, $F(2.55, 122.34) = 1.52$, $p = .22$, $\eta_p^2 = .031$ and left shoulder, $F(3, 144) = 1.68$, $p = .174$, $\eta_p^2 = .038$. A significant interaction (between measurement and group) was detected for the skin redness on left palm, $F(3, 144) = 8.95$, $p<001$, $\eta_p^2 = .157$. Greenhouse-Geisser corrections were used to correct against all violations of *sphericity*.

Two repeated measures ANOVA (with skin redness on palm as independent variable, measurement as within subject factor) was conducted to examine the above interaction. Result showed a significant main effect in measurement for intervention group (3, 78) = 5.58, p = .002, $\eta_p^2 = .117$; and

control group (3, 66) = 4.12, p = .01, η_p^2 = .158. Pairwise comparison found an increment in skin redness on palm between the first and third measurement, and between the first and forth measurement, for intervention group. No difference between measurements was detected for control group. The pairwise comparisons of skin redness across 4 measurements, on palm, for the two groups were summarized in Table 4.12.

Table 4.12

Mean difference across four measurements in a on left palm*

Group	Measurement	2	3	4
Intervention	1	-.920	-1.496*	-1.247*
	2		-.576	-.327
	3			.249
Control	1	1.714	1.681	1.213
	2		.033	-.501
	3			-.468

*p<.05 **p<.001

4.3.1.6.3 Changes in skin luminance in four other regions

Result showed no significant changes in skin luminance between the four measurements for the left inner upper arm, F(2.48, 119.12) = .297, p = .79, η_p^2 = .006; left outer upper arm, F(1.54, 73.94) = 1.17, p = .31, η_p^2 = .024; left shoulder, F(1.58, 76.02) = 1.49, p = .23, η_p^2 = .030; and left palm, F(1.57, 75.49)

= .35, p = .66, η_p^2 = .007. Greenhouse-Geisser corrections were used to correct against all violations of *sphericity.*

No significant difference in skin luminance was detected between the two groups for left inner upper arm, F(1, 48) = .22, p = .64, η_p^2 = .005; left outer upper arm, F(1, 48) = .055, p = .82, η_p^2 = .001; left shoulder, F(1, 48) = 1.33, p = .26, η_p^2 = .027; and left palm, F(1, 48) = .61, p = .44, η_p^2 = .012.

No interaction between measurement and group for left inner upper arm, F(2.48, 119.12) = .195, p = .87, η_p^2 = .004; left outer upper arm, F(1.54, 73.94) = .30, p = .68, η_p^2 = .006; left shoulder, F(1.58, 76.02) = 1.11, p = .32, η_p^2 = .023; and left palm, F(1.57, 75.49) = .033, p = .94, η_p^2 = .001. Again, Greenhouse-Geisser corrections were used to correct against all violations of *sphericity.*

All the mean and standard deviation of the measurements (for three colour spaces, four body regions and between the four measurements) have been summarized in three tables in Appendix 3.

4.3.2 Preliminary analysis for dietary assessment

Independent t-tests showed that participants (n=46) in both supplemented and control groups were similar in their body composition. No difference was found between the two groups with respect to the following parameters: age (t=.339, p=.736), body mass index (t=-.784, p=.437), height (t=1.15, p=.256),

body weight (t=.106, p=.916), fat mass (t=-1.404, p=.167), fat-free mass (t=.666, p=.509), muscle mass (t=.666, p=.509), total body water (t=.419, p=.677), bone mass (t=.639, p=.526), basal metabolic rate (t=.410, p=.684), visceral fat rating (t=-.953 p=.346), and degree of obesity (t=-.776, p=.442) between the two groups. The descriptive statistics of the comparison are presented in Table 4.13.

Table 4.13.

General characteristics and body composition parameters among the two groups of participants

	Intervention Group		Control Group		
Measurement	Mean	(±SD)	Mean	SD	p
Age	20.65	1.112	20.52	1.473	.736
BMI	20.543	2.8305	21.339	3.9605	.437
Height (in cm)	167.09	9.020	163.96	9.436	.256
Weight (in kg)	57.561	10.3717	57.970	15.3445	.916
Fat Mass (in kg)	11.126	4.4206	13.617	7.2717	.167
FFM (in kg)	46.435	9.6530	44.352	11.4762	.509
Muscle Mass (in kg)	43.917	9.2302	41.930	10.9231	.509
TBW (in kg)	31.330	6.5521	30.413	8.2083	.677
Bone Mass (in kg)	2.517	.4345	2.422	.5712	.526
BMR (in kJ)	5774.91	1035.754	5630.17	1341.720	.684
Visceral fat rating	2.74	2.816	3.57	3.057	.346

Obese (%)	-6.61	12.844	-3.04	17.957	.442

4.3.2.1 Carotenoids intake and FV consumption

Both carotenoid intake and FV consumption were not normally distributed, and thus non parametric test Spearman's rank correlation was used to examine the relationship between these two variables. One tailed Spearman's rank correlation coefficient found significant association between the carotenoid intake and fruits consumption (Spearmans rho=.319, p = .015); between carotenoids intake and vegetables comsumption (Spearmans rho=.660, p<.001), and between fruits and vegetables consumption (Spearmans rho=.471, p<.001).

4.3.2.2 Main contributors of carotenoids in Malaysian Chinese Diet

Repeated measures ANOVA was conducted to examine the difference in the contribution of different food categories (IV) to the overall carotenoids intake (DV) of the participants. Mauchly's test of sphrericity is significant, and thus Greenhouse-Geisser corrected values are reported below. Results showed a significant difference in the contribution of food category in carotenoids intake F(1.10, 71.91) = 83.571, p<.001, among the six food categories listed (grain, meat and bean, milk and dairy products, fruit, vegetables and others), with fruit (13.25%) and vegetables (81.20%) contributing approximately 94.45% of the overall carotenoids intake. Pairwise comparison (Bonferroni)

showed a significant difference between the contribution of carotenoids among all different food categories, except between grain and dairy products. The percentage of the contribution of each food type are listed in Table 4.14 below.

Table 4.14

Contribution of carotenoids intake by food categories

Item	Mean (in mg)	Percentage
Vegetable	3707.37	81.2
Fruit	604.88	13.25
Meat and Bean	160.76	3.52
Grain	18.77	0.41
Dairy	13.40	0.29
Others	60.60	1.33
Total	4565.78	100

Among all the 151 food items listed in our food frequency questionnaire, nine items contributed more than 80.67% of the overall carotenoids consumed with each contributing more than 2% of the overall carotenoids intake (refer to Table 4.15)

Table 4.15

Contribution of carotenoids intake by food types

Item	Mean (in mg)	SD	Percentage
Carrot juice (non prescribed)	1150.67	2532.68	25.20
Spinach	1001.27	1459.80	21.93
Lettuce	417.15	592.56	9.14
Papaya	254.6	499.22	5.58
Kale	246.57	517.50	5.40
French beans	215.63	284.52	4.72
Carrot	185.54	202.34	4.06
Watercress	111.2	458.78	2.44
Broccoli	100.76	109.63	2.21
Accumulative	3683.367		80.67

4.3.3.1 Pre and Post Supplementation Energy Intake

Mixed ANOVA (with calories as dependent variable, measurement as within participant independent variable, sex and group as between participants IV) was conducted to examine the difference in energy intake between groups, sex and measurement. Even though energy intake was not normally distributed, ANOVA was still selected to analyse the data because it is robust to violations of normality. We found no significant difference between groups, $F(1, 42) = .361$, $p = .551$. However, the energy intake reduced significantly, F (1,

42) =17.958, p=.001, η_p^2 = .300, from 2212 kcal (SD=161.12) at the baseline measurement to 1573 kcal (SD=104.86) taken on week 7. There was also a difference between the sexes F (1, 42) =15.274, p<.001, η_p^2=.267. Male participants reported higher calories intake, 2335.31 kcals (SE=174.27) than female participants, 1450.64kcals (SE=144.46). There was a significant interaction between measurement and sex F (1, 42) =4.145, p=.048, η_p^2=.090 (Fig. 6.5), with males reducing their intake more than females.

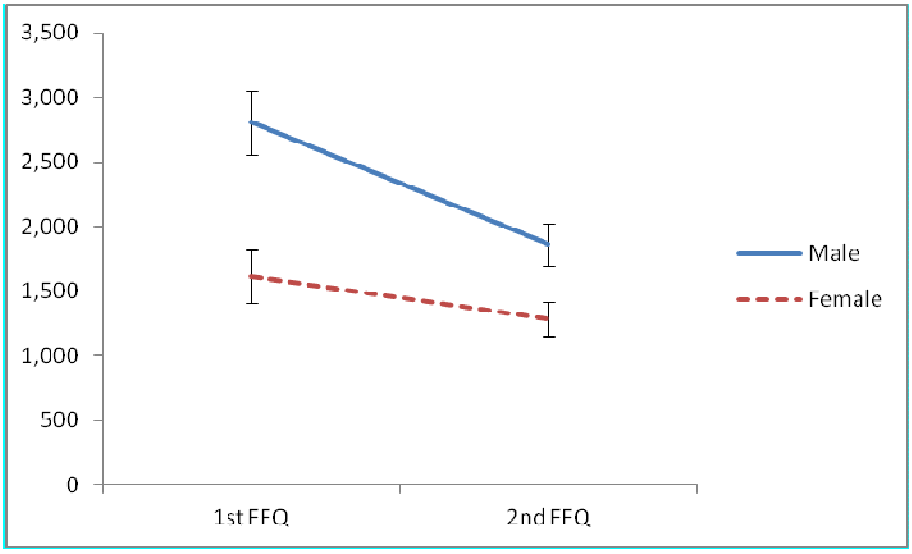


Figure 4.8. Interaction between sex and measurement in energy intake.

No interaction was found between measurement and group F (1, 42) =.003, p=.958, nor interaction between sex and group, F (1, 42) =1.525, p=.224, nor between measurement*sex*group, F (1, 42) =.002, p=.961. The average caloric intakes of the participants according to their sex, group and the measurement are presented in Table 4.16.

Table 4.16.

Energy Intake in Males and Females in Intervention and Control group

Group	Sex	First FFQ (in kcal)		Second FFQ (in kcal)	
		Mean	SD	Mean	SD
Intervention	Male	2592.19	1534.41	1662.96	1033.70
Group	Female	1688.00	632.54	1356.91	571.42
Control	Male	3023.03	1299.72	2063.04	482.11
Group	Female	1544.79	770.63	1212.85	558.50

4.3.3.2 Pre and Post Supplementation's fruit and vegetable Intake

Participants reported in the first food frequency questionnaire that they consumed, on average 689.25 grams of fruit and vegatable per day (SD=391.02), and reported 441.47 grams on the second food frequency questionnaire (SD=297.39), excluding smoothies. Approximately 28.3% of the sample were found to have consumed less than 400 grams that was the minumum amount of daily fruit and vegetable consumption as recommended by the World Health Organization (WHO).

Mixed ANOVA (with fruit and vegetable intake in grams as DV, time as within participant IV, gender and group as between participants IV) found a significant difference in participants' fruit and vegetable intake before and after the supplementation, F (1, 42) =24.392, p<.001, η_p^2 = .367. Participants

reported higher fruit and vegetables intake (mean difference = 250.287, SE=50.68) in their first food frequency questionnaire than the second one. No significant differences were found between the sexes F (1, 42) =2.404, p=.129 and between groups F (1, 40) =1.913, p=.174. There were no significant interactions between measurements and sex F (1, 42) =.288, p=.594, nor interaction between measurements and groups F (1, 42) =.761, p=.388, nor interaction between sex and group F (1, 42) =.201, p=.656, nor interaction between measurement*sex*group F (1, 42) =.885, p=.352.

Controlling for energy intake, and excluding smoothie prescribed to the participants, ANCOVA (with fruit and vegetable intake in grams as DV, time as within participant IV, sex and group as between participants IV and calories intake as covariate) found no significant difference in participants' fruit and vegetable intake before and after the supplementation, F (1, 40) =.019, p=.89. No significant differences were found between the sexes F (1, 40) =.093, p=.762 and between groups F (1, 40) =1.913, p=.174.

There were no significant interactions between measurements and sex F (1, 40) =.876, p=.355, nor interaction between measurements and groups F (1, 40) =.598, p=.444, nor interaction between sex and group F (1, 40) =.002, p=.967, nor interaction between measurement*sex*group F (1, 40) = 1.808, p=.186. The amount of fruit and vegetable intake of the participants, in grams, according to their sex, group and the sequence are presented in Table 4.17.

Table 4.17

Total fruit and vegetable consumption, excluding smoothies, in males and females in intervention and control group

Group	Sex	First FFQ (in grams)		Second FFQ (in grams)	
		Mean	SD	Mean	SD
Intervention	Male	702.65	520.75	527.86	331.79
Group	Female	880.19	109.99	719.48	403.63
Control	Male	421.72	417.50	396.65	215.88
Group	Female	606.17	236.10	404.49	280.20

4.4 Discussion

4.4.1 Changes in facial colours induced by supplementation

The results of this study demonstrated that participants' face colour increased in redness and yellowness after consuming fresh fruit juice for at least 4 weeks, and that the yellowness and redness remained for at least another two weeks even after the supplementation had ceased. No change in facial yellowness and redness was found for participants in the control group who drank normal filtered water labelled as "purified, detoxifying water" and served as a placebo. There was also a slight change in facial lightness between baseline measurement and the third measurement, for participants in both groups. The slight decrement in facial luminance might be due to the increase of melanin as a result of the increment in the exposure to sunlight during the

duration of supplementation. The third measurement was taken between the end of March and early April, by which the temperature and amount of sunlight in Malaysia peaks around March every year (Zain-Ahmed, Sopian, Zainol Abidin, & Othman, 2002), which could explain the increased darkness of the participants' skin tone.

4.4.1.1 Carotenoids concentration, skin yellowness and health

Consistent with previous studies (Perrett, Whitehead, & Ozakinci, 2013; Stephen et al., 2011) (Coetzee & Perrett, 2014c; Stephen et al., 2011), Study 6 discovered an increment in skin yellowness of the participants in the intervention group after 6 weeks of supplementation with a natural source of carotenoids (the fruit and vegetable smoothie). Facial yellowness is determined by both melanin and carotenoid levels (Stephen et al., 2011; Stahl et al., 1998). A significant correlation was detected between the changes in reflectance of skin and the absorption spectra of carotenoids (β-carotene and lycopene), for the participants in the intervention group, suggests that changes in skin colour of the participants in the intervention group in this study may be due to the absorption of carotenoids in the fruit and vegetable smoothies distributed to the participants, rather than to changes in melanin due to sun tanning.

In general, carotenoids and their compounds are beneficial to human health in various ways. They have been associated with human immunity (Hughes,

1999), reproductive success and the prevention of various chronic ilnesses such as cancers (Finley, 2005; Mullick & Gasser, 2004; Tang, Jin, Zeng, & Wang, 2005), cardiovascular diseases (Fraser & Bramley, 2004; Granado, Olmedilla, & Blanco, 2007) and eye diseases (Granado et al., 2007). Carotenoid concentration was also found to be related to women's fertility, whereby beta-carotene peaks in the late follicular phase when women are most fertile (Forman et al., 1996).

The findings of this study further strengthen the suggestion of Alaluf and colleagues (2002) that measuring skin colour using a spectrophotometer can be utilised as a non-invasive technique to monitor the dietary carotenoids of an individual.

Even though several studies had previously been conducted exploring the relationship between skin yellowness and fruit and vegetable intake, these have been either correlational studies (Alaluf et al., 2002; Pezdirc et al., 2015; Stephen et al., 2011), or interventional studies examining the dermal carotenoid level after the supplementation of beta-carotene pills (Heinrich et al., 2003; Stahl et al., 1998). The only intervention study which examined the change in skin yellowness after supplementation of carotenoids in a non-Caucasian population was conducted by Coetzee and colleagues (2014) in Africa. However, these researchers were using a much lower dose of carotenoids (15mg beta-carotene pills) but with a longer duration of

supplementation. Slightly different from what have been discovered in Study 6, out of the six body regions examined by Coetzee and colleagues (2014), only two regions (palm and inner arm) experienced significant increment in skin yellowness.

From previous studies (Stephen et al., 2011; and Study 3 of this thesis), we know that increased in skin yellowness made the face look healthier and therefore should be more attractive. This will be investigated in Chapter 5.

4.4.1.2 Change in skin redness

Participants in the intervention group who consumed fruit juice had an increase in their skin redness after four weeks of supplementation. Since carotenoids provide the yellow-orange-red colouration in fruit (Mortensen, 2006), we suggest that the increment of facial redness in participants in the intervention group may be due to these pigments, especially the reddish pigment of lycopene found in watermelons (Bramley, 2000; Charoensiri, Kongkachuichai, Suknicom, & Sungpuag, 2009). This conclusion is supported by the finding that the change in reflectance spectrum of the skin is consistent with increment in both beta carotene and lycopene.

Certain species of birds, such as the red-winged blackbird *Agelaius phoeniceus*, rely on red keto-carotenoids, such as astaxanthin and canthaxanthin, for the bright red hue of their epaulets (McGraw & Wakamatsu, 2004) while others

such as the northern cardinal were found to even have the ability to use yellow carotenoids in their diet to display bright red plumage (McGraw, Hill, Stradi, & Parker, 2001). The colour of male plumage was proposed to be related to the consumed component of carotenoids, their relative concentrations, and the total concentration of all carotenoids (Inouye et al., 2001). Even though there is literature exploring the association between carotenoids and skin yellowness in humans (Alaluf et al., 2002; Stahl et al., 1998; Stephen et al., 2011), less is known in relation to the contribution of colour pigments in fruits to human skin colour, especially skin redness.

Besides carotenoids, other red-purple pigments found in some of the other underutilised fruits were also present in the smoothie, for example betacyanin, the red-violet-coloured pigment found in dragon fruit (Phebe & Chew, 2009). However, we are not aware of any evidence that betacyanin impacts skin colour. The slight increment of skin redness could also be due to better blood circulation of the participants in the intervention group after taking the fruit juice. To our knowledge, there is no study discussing the changes in skin redness after regular consumption of fruit and vegetable smoothie. More research in the field of bioscience and nutrition is needed to validate this hypothesis.

A series of analyses were administered to examine the changes of skin colours for the other four body regions (inner upper arm, outer upper arm, shoulder

and palm) and found a similar pattern. Skin yellowness increment significantly after four weeks for all four regions, and remain stable thereafter. Minor changes in skin redness was detected in three of the body regions measured, and no change was detected in skin luminance.

4.4.2 Change in calories intake

Previous research reported mixed result in regards to behavioural change induced by diet intervention. While some (for example Young et al., 2004) proposed that individuals engaged in compensatory behaviour when participating in diet-related intervention, others (Coyle et al., 2009; Sullivan & Birch, 1990) showed that repeated exposure to certain types of food increased the individual's preference and willingness to consume that particular kind of fruit.

Participants as a whole reduced their energy consumption over the supplementation period. One of the possible reasons was because the first measurement was taken one week after Malaysian Chinese celebrated their annual Chinese New Year. It may have been that the participants were continuing to indulge in food left over from the festive season and that may have been the reason for a higher energy intake prior to supplementation compared to 6 weeks after. Similar patterns can be observed in the West whereby individuals usually gain weight after the winter holidays after they

have celebrated Thanksgiving, Christmas and New Year (S. Roberts, 2000; Yanovski et al., 2000).

It was predicted that the smoothie as a source of calories might be induce compensatory behaviour, whereby participants in the intervention group might reduce their overall caloric intake after being prescribed with the fruit and vegetable smoothie. However, a reduction in calories at the end of the studies was observed, for both groups, and not just intervention group. Hence, the null hypothesis is not rejected.

4.4.2.1 No change in fruit and vegetables consumption

Result showed a reduction in fruit and vegetable consumption, for participants in both groups on the second survey. However, when controlling for the calories intake, no significant reduction in fruit and vegetable consumption was found, in response to the extra supplementation of the smoothie. Neither did they increase in the fruit and vegetable intake at the end of the intervention. Fruit and vegetable consumption of the participants in the current study remained the same before and after the supplementation, when controlling for caloric intake, and there was no difference between control and intervention groups. This finding was in line with the finding of Acheampong and colleagues (2003) who found that intervention did not increase vegetable consumption, while contradicting the findings of Young

and colleagues (2004) that suggested individuals reduce their diet in relation to the changes in diet resulting from the intervention their participated in.

On an average, participants in our study were consuming higher than the recommended amount of fruits and vegetables. However there were 28.3% of the sample who consumed less than 400 grams that was the minumum amount of daily fruit and vegetable consumption recommended by the World Health Organisation. Karim and colleagues (2003) report that Malaysians on average consumed one cup (150 ml) of cooked green leafy vegetables per day (mainly spinach, sawi and kangkung). Another group of researchers conducted a study in the year 2003 and found that only 40% of Malaysian adults consumed 1 cup of green leafy vegetables, with approximately 96g per day (Norimah et al., 2008). As insufficient fruit and vegetable consumption has been suggested as one of the important risk factors contributing to the non-communicable disease (World Health Organization, 2015), there is a call for an intervention to encourage the community to consume more fruit and vegetables.

Rewards have been suggested as a more efficient approach to changing one's behaviour than punishment (Skinner 1938), thus, individuals might be willing to adopt a healthy lifestyle if they believe that living healthily can bring them advantages such as improving their physical appearance (Whitehead, Ozakinci, Stephen, & Perrett, 2012). Explaining the benefits of practising health

enhancing behaviour, for example by explaining its association with increased attractiveness, will be a much more effective way to encourage healthy behaviours.

One of the main limitations of this study was related to the issues of underreporting of dietary intake which were common in most dietary studies, particularly from obese individuals (Black & Cole, 2001; Goris, Westerterp-Plantenga, & Westerterp, 2000; Hise & Sullivan, 2002). However, it was suggested that the extent of underreporting tends to be relatively consistent within individual (Black & Cole, 2001). Thus, the dietary differences between two observation points are likely to be relatively more accurate as compared to the absolute dietary intakes at a single time point. However, in regards to the case of fruits and vegetables consumption, it is uncertain if the participant might be doing otherwise to exhibit a healthy image.

4.4.3 Source of carotenoids in Malaysian Chinese's diet

In line with what was suggested by the literature (Jaswir, Noviendri, Hasrini, & Octavianti, 2011), fruits and vegetables appeared to be the main sources of carotenoid intake. Among all the different food consumed by the Malaysian Chinese, carrot juice, spinach, lettuce, papaya, kale, French beans, carrot, watercress and broccoli emerged as the main sources of carotenoids in their daily dietary intake. This should not be generalized as reflecting the Malaysian diet, as previous research (Izzah & Fatimah, 2012) had found significant

differences in the amount of fruit and vegetables consumed, and vegetable preferences vary among different ethnic groups in Malaysia. The Malays reported eating more fruit and vegetables as compared to the Malaysian Chinese and Malaysian Indian, while the latter two groups reported eating more leafy and brassica vegetables as compared to the Malays. Furthermore, participants recruited in this study are all young adults and from relatively high socioeconomic groups, so may not be representative of the population as a whole.

4.4.4 Conclusion

This chapter reported the first study conducted in Asian setting, exploring the relationship between changes in skin colour and supplementation with smoothie. Results of the analysis showed that the Malaysian Chinese increased in skin yellowness, after consuming a fruit and vegetable smoothie daily for 4 weeks. There was a change in skin redness, possibly due to skin deposition of lycopene.

Analysis of the dietary patterns revealed that approximately one third of the sample were found to have consumed less than 400 grams or the minumum amount that was recommended by the World Health Organization (WHO). Finally, participants in the intervention group were not found to decrease in their caloric to compensate for the additional supplementation taken.

CHAPTER 5: CAROTENOID COLOURATION AND PERCEIVED HEALTH

5.1 Introduction: Preference for skin yellowness

Human skin with increased redness (a*) and yellowness (b*) was perceived as healthier by observers from various cultures, including in Caucasian (Stephen et al., 2011; Stephen, Law Smith, et al., 2009), African (Coetzee & Perrett, 2014) and East Asian samples (refer to Study 3 of this thesis).

Skin yellowness is primarily determined by melanin concentration (for example from tanning) and deposition of carotenoids (from fruit and vegetable intake) in human skin, while skin luminance is primarily influenced by melanin concentration. Therefore, one can argue that individuals' preference for skin yellowness might be at least partially due to their preference for melanin colouration. To answer that question, Lefevre and Perret (2014) conducted a study, comparing the preference for these two colouration and found that individuals preferred carotenoid colouration over melanin colouration.

Another study by the same group of researchers (Lefevre, Ewbank, Calder, von dem Hagen, & Perrett, 2013) illustrated that this preference for yellowness is not due to a general aesthetic preference for the colour, but

rather is specific to yellowness in skin. Their study showed that while there is an increment in attractiveness in line with the increment of carotenoid colouration in human faces, such an effect was not observed in stimuli consisting of scrambled line images.

5.1.1 Carotenoids and its health functions

Carotenoid colouration has been suggested to be a valid cue to human health as, in addition to being perceived as healthy (Stephen et al, 2011), carotenoids have been found to serve various protective functions, including prevention of age related macular degeneration (Samimi, 2005), protection against cardiovascular diseases (Gammone, Riccioni, & D'Orazio, 2015; Voutilainen, Nurmi, Mursu, & Rissanen, 2006b) and epidermal defence against ultraviolet induced damage (Cesarini & Michel, 2003).

Specifically in human skin, carotenoids such as beta-carotene, lutein, and lycopene are thought to play significant role in the reduction in skin degeneration, and in mediating the risk of skin cancer (Heinen et al., 2007; Stahl & Sies, 2005). Both internal and external factors such as metabolic processes, inflammation, and irradiation with UV light can lead to the production of reactive oxygen species (ROS) in the human body. High levels of ROS cause oxidative cell damage, degeneration of skin, and even skin cancer (Halliwell, 2006; Sander, Chang, Hamm, Elsner, & Thiele, 2004). To combat the

adverse effects of ROS, our body has an anti-oxidative system consisting of carotenoids, vitamins and other active substances.

Preference for individuals with bright and striking ornaments is also observed in non-human animals. The striking colouration of the exposed skin, feathers or scales was found to be related to carotenoid levels in various species of fish (for example, two-spotted gobies and live-bearing fish) and birds (zebra finches and yellow-eyed penguins) (Amundsen & Forsgren, 2001; Blount, Metcalfe, Birkhead, & Surai, 2003; Bourne, Breden, & Allen, 2003; MacDougall & Montgomerie, 2003; Massaro, Davis, & Darby, 2003; Pitcher, Rodd, & Rowe, 2007; Williamson, Surai, & Graves, 2006).

Carotenoid colouration in animals too reflects the actual health and fertility status of the animal. Researchers found associations between ornament colouration and immune function (Blount et al., 2003; K. J. McGraw & Ardia, 2003; Peters et al., 2004; Pike, Blount, Lindström, & Metcalfe, 2007; Saks et al., 2003), whereby individuals with bigger, brighter ornaments tend to have better immunity and a higher level of antioxidant activity. Carotenoid concentration is also associated with higher reproductive success among male northern cardinals (Wolfenbarger, 1999), better sperm quality in *Parus major* and mallards (Helfenstein et al., 2010; Peters et al., 2004) and successful embryonic, post-hatching development and survival of new born of the zebra finch (Williamson et al., 2006).

The handicap principle of sexual selection states that, in order to be an honest signal of quality, signals must be costly so that they cannot be faked (Zahavi, 1975). Because carotenoids cannot be synthesised de novo within the body (Alaluf et al., 2002); to develop such flashy carotenoid-based ornaments, carotenoids must be redirected away from other functions, such as immunity or antioxidant action. Because of this, only individuals who are fit and with good genes can afford to develop such attractive ornaments.

5.1.2 Objective of the study

The three studies reported in this chapter investigate the role of skin carotenoid colouration in the perception of human health. In Study 7, facial photographs taken before and during supplementation with a carotenoid-rich smoothie (refer to Study 6, Chapter 4) were judged by an independent group of participants to see if the perceived health of the faces improves significantly after 4 weeks of intervention. In Study 8, participants were recruited to determine if the amount of facial colour that was observed in Study 6 was appropriate to optimize perceived health of the faces. Finally, by using computer software, participants in Study 9 were asked to indicate the amount of carotenoid colouration required to optimise healthy appearance.

5.2 Study 7: Perceived health of faces before and after intervention

5.2.1 Introduction

Insufficient fruit and vegetable intake is estimated to contribute to more than 2.6 million premature deaths worldwide every year (Lock, Pomerleau, Causer, Altmann, & McKee, 2005). However, the populations of most countries, including Malaysia (Izzah & Fatimah, 2012; Norimah et al., 2008), consume well below the recommended intake of fruit and vegetables (Hall et al., 2009; OECD, 2013). To reduce this preventable morbidity and mortality, it is crucial to design efficient campaigns to encourage and improve fruit and vegetable eating habits. One plausible approach is to highlight and promote the benefits of such behaviour, including the possibility of improving one's physical appearance (Whitehead, Ozakinci, Stephen, et al., 2012).

Higher levels of consumption of food rich in carotenoids increases skin yellowness (Alaluf et al., 2002; Stephen et al., 2011). In line with what was found in the previous studies conducted in the West, Malaysian Chinese participants in the intervention group in Study 6 experienced a significant increment in skin yellowness and skin redness after 4 weeks of supplementation with a fruit and vegetable-based smoothie. Previous studies (Stephen, Coetzee, et al., 2009b; Stephen et al., 2011; Stephen, Law Smith, et al., 2009) showed that increased skin yellowness and redness improves apparent health of both Caucasian and South African samples. However, it is

uncertain if the apparent health of Malaysian Chinese participants (in the intervention group) improves in line with the increment in skin yellowness.

Study 7 aims to determine if changes in facial colour induced by supplementation with smoothie can increase the perceived health of faces. It was hypothesized that facial images taken during mid-intervention will be perceived as healthier than images taken before the intervention.

5.2.2 Method

This is a within subject study whereby participants were asked to compare the health status of two same-face images taken at different times (before and during an intervention). The study was approved by the Ethics Committee at the University of Nottingham (Malaysia Campus). All participants gave both verbal and written informed consent prior to the experiment.

5.2.2.1 Stimuli prepration

Facial photographs taken before and during the intervention study (Study 6) were used in this study. Seventy three participants from Study 6 (30 male, 43 female; 38 from experimental group, 35 from control group) gave informed consent for their photograph to be used in this perceptual study.

For each participant, one photograph was taken before the intervention began (week 0) and another one after four weeks of intervention (week 5). In

total, 146 facial images (two images from each participant) were presented in pairs in this experiment. All photos were taken as described in Chapter 4.

5.2.2.2 Participant

Fifty seven Malaysian Chinese (24 males, 33 females; average age = 22.35, SD= 3.17) were recruited for this study. All participants were students pursuing undergraduate or postgraduate degrees at two private institutions of higher education in Malaysia - University Putra Malaysia (UPM) and Universiti College Sedaya International (UCSI). All participants have normal colour vision.

5.2.2.3 Procedures

The experiments were presented using Psychopy (Peirce, 2009). Participants were given two-alternative forced-choice psychophysics tasks, presented on a computer screen colour-calibrated with a Data Color Spyder3 Pro. Specifically, the participants were shown one face image for 750ms, followed by a visual mask of black dots on a white background for 100ms, followed by the other same face image for 750ms. Participants were asked to decide which of the paired faces shown (pre-intervention and mid-intervention facial images of the same individual) looked healthier, and indicate their choice using the keyboard. There was no explicit indication which of the paired photos was taken before intervention, and which was taken during the intervention.

Images were presented in random order, and order of presentation of before and during images was randomized within trials.

5.2.3 Results

The average frequencies of the pre-intervention and mid-intervention images being chosen as appearing healthier for each comparison was compiled. The descriptive statistics are presented in Table 5.1.

Table 5.1

The frequencies of pre-intervention and mid intervention images chosen as healthier

Group / Preference	Pre Intervention		Mid Intervention	
	Mean	SD	Mean	SD
Intervention (N=38)	21.81	6.343	16.19	6.343
Control (N=35)	19.46	3.123	15.54	3.123

Pearson's chi-square was conducted to examine the association between the group (intervention vs. control) and the preference (before intervention vs. mid intervention) of the participants. No significant association was found between the two variables χ^2 (1) = .096, p = .76.

Paired samples t-tests were conducted to compare the frequencies of the pre-intervention and during-intervention images that were selected as healthier, in both groups. Result showed that participants significantly chose the pre-intervention images as healthier than the mid-intervention images, in both the intervention group, $t(56)=3.341$, $p=.001$ and the control group, $t(56)=4.729$, $p<.001$

5.2.4 Discussion

Contrary to our hypothesis, faces from both the intervention group and the control group were perceived as healthier in the pre-intervention than in the mid-intervention images. Since there is no significant interaction between group and time, we suggest that the perceived changes might not be attributable to the supplementation of the two different types of health drink (smoothie vs. water), but rather to confounding variables.

Various physical attributes can be used to determine judgments of apparent facial health, including its shape, adiposity, texture, colour, facial expression etc. All these facial characteristic change over time, and with changes in dietary pattern, weather, physical and psychological stress, and exercise level. With so many factors changing across time, it is hard to pinpoint which was the main factor that contributed to the decrement in perceived health.

Firstly, we discovered in Study 6 that all participants decreased in their skin luminance during the latter measurement of the intervention, which might be due to the higher exposure to sunlight. The latter measurement was taken between the end of March until early April, when the local temperature and amount of sunlight peaks (Zain-Ahmed et al., 2002). Individuals who had more exposure to sunlight might be darker as compared to individuals who went out door less. Changes in skin luminance gives impact on the apparent health of a person (Stephen et al., 2011). Nonetheless, we found in Study 3 (Chapter 2) that Malaysian Chinese, as whole, decreased skin luminance to optimise the apparent health of faces, so we suggest that this is an unlikely explanation for the current results.

Participants reported consuming more calories before the intervention period than during the intervention period (refer to the dietary assessment in Study 6), as the intervention began a week after Chinese New Year. Changes in calories intake affect the body size and facial weight. Facial adiposity has been shown previously to be influencing the judgment of apparent health and attractiveness of the bearer (Coetzee et al., 2009). Even though there was variation within individuals, with some individuals losing weight while others gained weight, no significant changes in body weight were detected between the two measurements when looking at the sample as whole, suggesting that this is an unlikely explanation.

Another important factor which might be influencing the apparent health of a person is his or her stress level. As the semester progresses, participants which consist of university students tend to experience more academic stressors, including the stress to finish up assignments, to prepare for oral presentations and written examinations. Previous research has shown that long term exposure to stress hormones leads to wear and tear on the body including the deficiency of the immune system (McEwen, 2005, 2008)), which further leads to the risk of contracting disease and getting ill (Kaplan, Madden, Mijanovich, & Purcaro, 2013). Stress affects actual physical health of individuals, as well as impacting on mood and facial expressions, and thus might be reflecting on the perceived health of the participants.

Considering the few extraneous factors which might affect the judgment of apparent health, which is beyond the control of this study, it is hard to conclude which factor was the major influence of such change. In study 9, we examine if such a decrement in the rating of perceived health was due to the changes which was induced by the supplementation, specifically if it was due to the increment in skin yellowness and skin redness that was observed in the intervention group.

5.3 Study 8: Preferences for faces that differ only in carotenoid coloration

5.3.1 Introduction

Since previous studies have suggested that skin red and yellow, reflecting skin carotenoid levels enhance the healthy appearance of faces (Lefevre et al., 2013; Lefevre & Perrett, 2014; Perrett, Whitehead, & Ozakinci, 2013; Stephen et al., 2011, and Study 3 of this thesis), it seems that individuals should perceive faces with increased carotenoid colouration (changes in facial colour which was induced by carotenoid supplementation) as healthier as compared to the initial facial colour. However, in Study 7 participants chose pre-intervention facial images as healthier than mid supplementation faces from both intervention and control groups. Individuals rely on various facial attributes when making judgments of perceived health. Throughout the 4 weeks intervention (of Study 6), participants experienced some minor changes on their facial appearance which might affect their apparent health. Since photographs differed in more ways than simply colour, in Study 8, we compare healthy appearance of pre- and during-intervention faces that differ only in colour.

5.3.1.1 Hypothesis of the study

While Study 7 involved comparison between the overall facial changes (shape, texture and colour) before and after 4 weeks of intervention, Study 8 focused only on the change in facial colour. It compared between the initial facial

colour and the facial colour after 4 weeks of intervention. It was hypothesized that the faces with intervention-induced colouration should be perceived as healthier than the original ones.

5.3.2 Methods

This is a within subjects study in which the participants were asked to compare the health status of two same-face images with different facial colouration (with and without intervention-induced colouration). The study was approved by the Ethics Committee at the University of Nottingham (Malaysia Campus). All participants gave both verbal and written informed consent prior to the experiment.

5.3.2.1 Stimuli preparation

As reported in Study 6 (refer to Chapter 4), after four weeks of dietary intervention with a carotenoid rich fruit and vegetable smoothie, participants in the intervention group increased in skin yellowness ($\Delta b^* = 3.382$), skin redness ($\Delta a^* = 1.077$) and decreased in skin luminance ($\Delta L^* = -.913$), as measured by a spectrophotometer.

Thirty face photographs of the participants in the intervention group in Study 6 were selected randomly to be used in this experiment, with an equal number from each sex. All the facial images were taken before the intervention began. The same images were used to create another set of

same-face images with skin yellowness increase by 3.382 units, skin redness increase by 1.077 units and skin luminance decrease by .913 units, using Psychomorph (Tiddeman et al., 2005). All the facial images were standardized in terms of size and eye level.

5.3.2.2 Participants

Fourty two Malaysian Chinese (20 males, 22 females) participants with average age 22.29 (SD= 3.42) were recruited for the experiment. All participants were students pursuing undergraduate or postgraduate degrees at two private institutions of higher education in Malaysia - University Putra Malaysia (UPM) and Universiti College Sedaya International (UCSI). All participants have normal colour vision.

5.3.2.3 Procedure: Forced Choices Task

The experiments were presented using Psychopy (Peirce, 2009), following the procedure described in Study 8. The only difference was the images used -- between an original face image, and the same face image with changes in facial colour. The pre-intervention image of the participants were manipulated using software to create identical image that differed only in the level of facial skin colour associated with smoothie supplementation. This controls for any change in facial shape, texture or expression during the course of intervention.

Participants were asked to choose the healthier face between paired facial images. Participants were not explicitly informed about the differences between the paired images. The sequence of pairing was randomized and the order of presentation of images within trials was randomized.

5.3.3 Results

The frequencies of each image being selected by the independent sample (n=42) as healthier was compiled, and summarized in Table 5.2.

Table 5.2

The frequency of original photos and colour-adjusted photos being chosen by participants as healthier

	Original images		Colour-adjusted images	
	Mean	SD	Mean	SD
Preference	16.05	6.347	13.95	6.347

Paired sample t-tests were conducted to compare the frequencies of the original and colour-changed images being selected as more healthy. Result showed no significant difference in participants' preference, $t(41)=1.07$, $p=.291$.

5.3.4 Discussion

Contrary with what was being hypothesized, the forced-choice task failed to detect any difference between the preference for original facial image, and preference for colour-adjusted images. One of the plausible reasons might be because the amount of changes in skin yellowness was not appropriate to optimize the apparent health of human face. It might be that the changes were too drastic and beyond the healthy level.

It was found in Study 3 (Chapter 2) that individuals on average increased skin yellowness by 1.31 unit and skin redness by .85 units, when asked to optimum apparent health of human faces. However, the actual increment in skin yellowness that was observed on participants in the intervention group in Study 6 was 158% higher than the preferred value, and increment in their skin redness was also 26.7% higher than the preferred value.

Although faces with increased yellowness and redness were preferred as healthy, the fact that ceiling and floor effects were not observed in Study 3 suggests that overly yellow and red faces may not be perceived as healthy, and may even resemble the symptoms of certain physical illnesses such as jaundice, rosacea and climatecterium (Cohen, Wong, & Stevenson, 2010; Izikson, English, & Zirwas, 2006; Mansor et al., 2012). It has been suggested that selection would favour characteristics that were not too far from the

average of the population (Judith H Langlois & Roggman, 1990). See the General Discussion for further discussion of this idea.

5.4 Study 9: Examination of the desired amount of carotenoid colouration

5.4.1 Introduction

An increment in skin yellowness and skin redness after a supplementation of carotenoid rich smoothie for 4 weeks, was observed in Study 6. Nonetherless, Study 8 failed to detect a preference for the faces with the increment of intervention-induced carotenoid colouration. It may be that the amount of change in skin colouration induced by the supplementation may have exceeded the healthy-appearing range. It was found in guppy that carotenoid colouration was perceived as attractive only to a certain extent, and overly yellow was not seen as attractive (Houde, 1987). One of the objectives of Study 9 was to investigate the amount of intervention-induced colouration that is perceived as optimum (most healthy). It was predicted that participant will increase the intervention-induced colouration, but not to the extent seen in the supplementation study.

Following the same line of argument, faces with higher amount of redness, yellowness, and darkness before the intervention would be predicted to be given less amount of increment in carotenoid colouration, to avoid exaggeration of the characteristic. We predict that faces that are initially low in red and yellow, and high in lightness will be manipulated by observers to increase the amount of smoothie colour more than in faces with higher redness and yellowness and lower lightness.

As male faces tend to be slightly redder, yellower, darker than females (Giacomoni, Mammone, & Teri, 2009), we predict that male faces should receive less increment in intervention-induced colouration, as compared to female faces.

5.4.2 Method

This is a within subjects design, with the amount of colour adjustment chosen to enhance healthy appearance as the dependent variable, initial facial colour and sex as independent variables. The study was approved by the Ethics Committee at the University of Nottingham (Malaysia Campus). All participants gave both verbal and written informed consent prior to the experiment.

5.4.2.1 Stimuli preparation

To create stimuli for Study 9, Matlab was used to produce masks with even colouration representing the skin areas of faces, with a Gaussian blur at the edges. One mask was created to represent average face colour with 4 times the change observed in the supplementation study (henceforth referred to as intervention units; decreased luminance by 3.652 units, increased redness by 4.308 units and increased yellowness by 13.528 units) and another one with average face colour -4 intervention units of colour change (increased luminance by 3.652 units, decreased redness by 4.308 units and decreased

yellowness by 13.528 units). Forty one images were created based on the original images using Matlab. The images were numbered from “0” to “40” with image-0 has facial colour reduced by 4 intervention units, increasing incrementally so that images-20 will be the original image and image-40 has colour increased by 4 intervention units. For all the transformations, participants’ hair, eyes and clothing and the background were remained unchanged.

The colour manipulation of the images was conducted using Psychomorph, and the colour changes of the facial photos were validated by using photo editing software GIMP and online colour converter (http://www.workwithcolor.com/color-converter-01.htm) to ensure that the conversion was accurate. The colour of a specific spot on the face of the first (image labeled 0), last (images labelled 40) and original image of every face were identified with computer software GIMP. The CIEL*a*b values of the same-face image were compared to ensure that the differences between the original and the images on both ends were approximately 4 intervention units (4 times the change seen in the intervention study).

5.4.2.2 Original skin colour

Facial skin colour obtained from Study 6 were used as part of the analysis in this study. The measurements of the skin colour on both cheeks, and forehead of the participants were taken before the intervention with a

spectrophotometer. Details information about the spectrophotmetry were presented in the Method part in Study 6 (refer to Chapter 4).

5.4.2.3 Participant

Fifty seven Malaysian Chinese (24 males, 33 females; mean age = 22.35, SD= 3.17) were recruited for this study. All participants were students from University Putra Malaysia (UPM) and Universiti College Sedaya International (UCSI). All participants had normal colour vision.

5.4.2.4 Procedure: Slider Task

Stimuli were presented using computers which had been colour calibrated using a Data Colour Spyder 4 Pro. Participants were presented with facial images, one image at a time, on computer software, and were asked to adjust the colour of skin portions of the facial images presented. By moving the computer mouse horizontally, the app cycled through the 41 frames of the colour change for a single face identity to give the appearance that the participant was manipulating the colour of the face along the carotenoid intervention colour axis in real time. The sequence of the individual faces presented and the starting frame in the colour transform sequence was randomised. Participants were asked to move the mouse, and “make the face as healthy as possible”.

5.4.3 Results

Participants were asked to determine the optimum facial colour changes that is being perceived as most healthy. One intervention unit of increment in this experiment is equivalent to an increment in skin yellowness of 3.382 units, an increment in skin redness of 1.077 units, and a decrement in skin luminance by -.913 units (the amount of skin colour change seen in the intervention study). One intervention unit of decrement in this experiment is equivalent to 3.382 units of decrement in skin yellowness, 1.077 units of decrement in skin redness and -.913 units of increment in skin luminance.

Result showed, participants, on average increased .34 intervention units of changes (SD=.074) to all faces to make it look most healthy.

5.4.3.1 Adjustment of colouration and initial facial colour

To examine if the adjustment of colours was related with the initial colours of the face, Pearson's correlation was performed. All the involved variables were normally distributed. Results showed a significant correlation between the amount of colour adjustment chosen to optimise healthy appearance and the intial colour of the face (Fig 5.5, 5.6 and 5.7), with faces which were lighter receiving more increment ($r=.542$ $p=.002$), and faces which are redder ($r=-.387$, $p=.038$) and yellower ($r=-.708$, $p<.001$) receiving less increment to optimise healthy appearance.

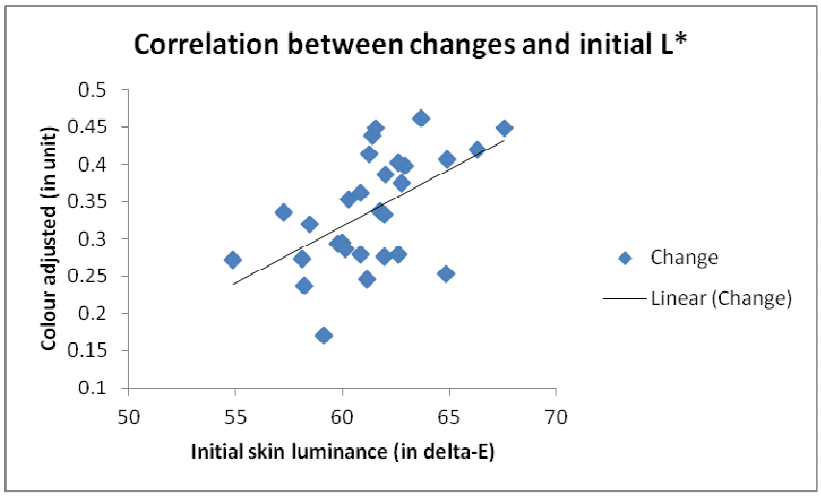


Fig. 5.1. Amount of colour change applied to optimise healthy appearance: Faces with higher levels of luminance received more increment (in intervention-induced colouration) as compared to those with lower levels of luminance.

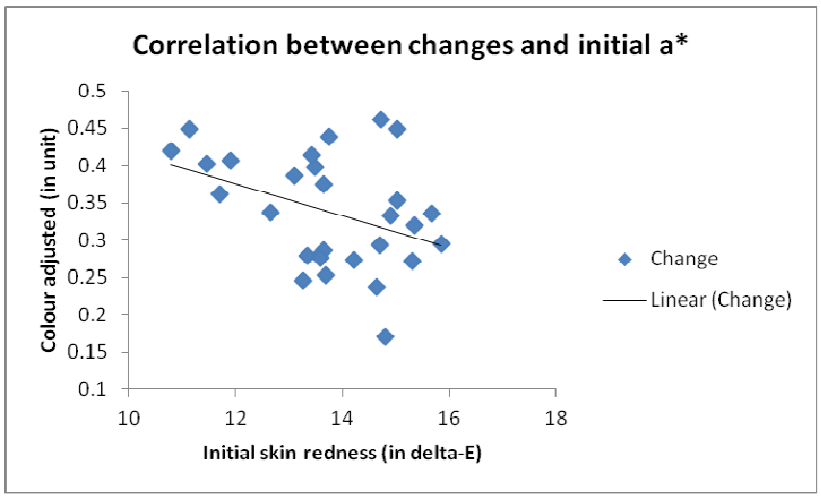


Fig. 5.2. Faces with higher levels of redness received less increment as compared to those with lower levels of redness.

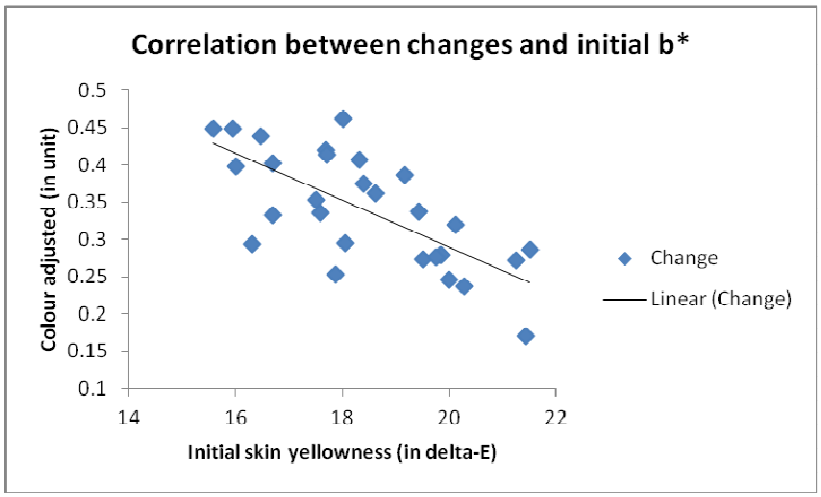


Figure 5.3. Faces with higher levels of yellowness received less increment as compared to those with lower levels of yellowness.

5.4.3.2 Sex difference in the adjustment of colouration

An independent samples t-test was conducted to examine the different between the amount of adjustment being administered to faces of different sexes to optimise healthy appearance. The result was significant, t(28)=2.194, p=.037, where males received less increment (mean=.31, SD=.065) as compared to females (mean=.36, SD=.074).

An ANCOVA was conducted (with degree of adjustment as dependent variable, sex as fixed factor, initial skin colour as covariate) to examine if the sex difference still exist after controlling for the initial facial colours. No significant sex difference was found F (1,25) = .014, p = .908.

Independent samples t-tests were further conducted to validate the sex difference in the initial facial colours (luminance, redness and yellowness). Results showed significant difference in skin luminance, $t(28)=4.30$, $p<.001$ and skin yellowness $t(28)=2.28$, $p = .031$ and a trend to significant difference for skin redness $t(28)=1.98$, $p = .058$. Male faces were found to be darker, redder and yellower than the female faces. The avarage facial colours of the two sexes were presented below in Table 5.3.

Table 5.3

Sex difference in initial colour of the face

	Male		Female	
	Mean	SD	Mean	SD
Luminance	59.64	2.01	62.94	2.17
Redness	14.20	1.17	13.27	1.42
Yellowness	19.18	1.81	17.86	1.33

5.4.4 Discussion

In line with our predictions, participants increased facial colour by .34 intervention units of intervention-induced colouration (SD=.074) to make the faces look healthier. The amount chosen was only one third of the change in facial colour that was experienced by the participants in the intervention group in Study 6. This amount of change is equivalent to an increment in skin yellowness of 1.15 units of b*, an increment in skin redness of .37 units of a*,

and a decrement in skin luminance of .31 units of L*. This preference is much closer to the optimum health colouration chosen in Study 3 (Chapter 2), especially for skin yellowness and skin luminance because the participants chose to increase in skin yellowness by 1.31 units of b*, increased skin redness by .85 units of a*, and decreased skin luminance by .37 units of L* when given an opportunity to adjust the three colour spaces separately (refer Table 5.4).

Table 5.4

*A comparison between the optimum CIEL*a*b chosen in Study 3, changes of CIEL*a*b observed in Study 6 and the preferred CIEL*a*b which was obtained based on the degree of preferred intervention-induced colouration as acquired in Study 9 (measured in Euclidean distant)*

	Study 3 Preference	Study 6 Actual Change	Study 9 Preferred change
Skin yellowness	1.31	3.38	1.15
Skin redness	.85	1.08	.37
Skin luminance	-.37	-.91	-.31

The results of this perceptual study are useful as it gives clues to the appropriate amount of carotenoids that should be consumed by an individual to optimize their healthy appearance. The results suggest that the dose of the

carotenoids that was being prescribed to the participants in Study 6 might be too high, and beyond the level that makes the face look healthy. A smaller dose of carotenoids, that is approximately one third of the previous prescription of 25mg, should be used in future research to see if it can improve the apparent health of individuals. This hypothesis warrants further investigation.

5.4.4.1 Adjustment chosen relates to the initial facial color

Participants were found to have adjusted the colouration according to the initial colour of the faces. Faces with higher levels of yellowness and redness received less intervention-induced colouration (which is increased in yellowness and redness) as compared to those with lower level of yellowness and redness. The opposite was true for skin luminance – the increment in intervention-induced colouration is negatively correlated with skin luminance. As an increment in intervention-induced colouration is equaivalent to a decrement in skin luminance, the result actually suggested that faces which are darker received higher amount of luminance. The negative correlation between the level of adjustment and initial colour of the faces showed that participants added more colour (redness, yellowness, and lightness) to those initial faces which have lower amounts of redness, yellowness and lightness, to optimize the apparent health of the faces.

Male faces were found to have received less adjustment in intervention-induced colouration as compared to the female faces. This sex difference appears to be due to the difference in the initial facial colours between the sexes.

5.5 General Discussion

Contrary to our hypothesis, participants in Study 7 preferred the pre-intervention facial images over the mid-intervention images, regardless of whether the images were from the experimental group or the control group. This is probably due to the influence of some extraneous factors, which were beyond of the control of the experimenters. As mentioned in the discussion presented earlier, this includes the change in weather, dietary habits, and stress levels of the participants within the period of study. All these changes can alter the participants' facial characteristic, such the adiposity, skin texture and skin colours, which may affect the rating of the health judgment.

Controlling for all changes in extraneous factors, a second perceptual study (Study 8) was launched to examine the above hypothesis. Facial images of the participant (in intervention group, Study 6) were used, with a same-image with the change of facial colouration being used as comparison. No significant difference was found between the preference between the original images and colour-adjusted images. We therefore hypothesized that the

change in facial colouration might have overshot the optimum colour change and therefore be too great to be perceived as healthy.

The third perceptual study (Study 9) was designed to identify the amount of intervention-induced colouration that is preferred. In line with the hypothesis, participants in Study 9 increased the intervention-induced colouration, to only one third of the amount of colour change that was observed in the participants in the intervention group in Study 6, to optimize the healthiness of the facial images shown. The result of this perceptual study suggested the carotenoid level of the prescribed smoothies must have exceed the optimum dose. Based on the results of Study 9, we proposed to reduce the carotenoids dose to one third of the current prescribed dose, which should be around 8mg.

5.5.1 Selection for averageness

We know from previous studies (Lefevre, Ewbank, Calder, Hagen, & Perrett, 2013; Stephen et al., 2011) that increment in facial yellowness and redness are perceived as healthy. However, the result from the three perceptual studies (Study 7, 8 and 9) suggested that there is a ceiling in this preference. Faces which are overly red or yellow will no longer be seen as healthy and attractive. This can be attributed to the preference for averageness (Apicella et al., 2007; Rhodes, Zebrowitz, et al., 2001b) which is related with the

evolutionary pressure that favour characteristics close to the mean of the population (Langlois & Roggman, 1990).

Increased carotenoid intake is thought to be beneficial for humans' physical health, such as the improvement of immunity (Hughes, 2001), visual acuity and photoprotection of the skin (Fraser & Bramley, 2004; Krinsky & Johnson, 2005). However, an extremely high level of skin yellowness might be an indication of certain diseases and ailments, such as jaundice (Cohen et al., 2010; Mansor et al., 2012).

Beta carotene may serve as both antioxidant or prooxidant, depending on its own internal properties and the surrounding biological environment (Lowe, Vlismas, & Young, 2003; Palozza, Serini, Di Nicuolo, Piccioni, & Calviello, 2003; Palozza, 2009). Specifically, the prooxidant character of beta carotene will be enhanced if its consumption exceeds the normal dietary level (Palozza et al., 2003; Palozza, 2009). Adverse effects have been specifically observed among smokers (Omenn et al., 1996; The Alpha-Tocopherol and Beta-Carotene Cancer Prevention Study Group, 1994). Nevertheless, there was no such report from fruit and vegetable consumption. Lowe and colleagues (2003) suggested that there is an optimal dose of carotenoids which results in maximum antioxidant effectiveness in human cells, and these optimal levels differ for different type of carotenoids, and for different organs and cells.

The same was found in animals. Studying the mate preference of guppies, Houde (1987) found that female guppies preferred males with more-than-average amounts of orange in their colour patterns. This increment in female responsiveness levelled off with increasing amounts of orange. The result suggests that there may not be an advantage for the increasing amounts of carotenoid colouration above a certain level.

Further, channelling most if not all of the carotenoids into the skin means that a lesser amount can be made available for other bodily functions, including the immune system (Saks et al., 2003).

A healthy, balanced diet includes a range of foods and nutrients. An unbalanced vegetarian diet, for example those which are composed mainly of carotenoid-rich fruits and vegetables, might lead to nutritional deficiencies. Specifically, some scientists have voiced their concern over the issue of insufficiency of certain nutrients such as vitamin B12, vitamin D, omega 3 fatty acids, calcium, iron, zinc and iodine in the vegetarian diet (Craig, 2010; Dietetic American Association, 2003; Key, Appleby, & Rosell, 2007).

High intake of smoothie also will increase the amount of fructose absorbed, which might contribute to an increment in low-density lipoprotein (Stanhope et al., 2011). However, two recent reviews (Sievenpiper et al., 2012; Tappy &

Mittendorfer, 2012) suggested that these effects may not be attributed to fructose, but rather to the increment in caloric intake.

Skin which is overly red is not always perceived as healthy either. Even though increment in skin redness is associated with aerobic fitness and changes in fertility (Armstrong & Welsman, 2001; Jones, Hahn, Fisher, et al., 2015), sudden and temporary flushing of the skin, especially in a greatly exaggerated manner, can also be the symptoms of fever, hyperthermia and neurological diseases (Izikson et al., 2006).

Previous research has established the significance of carotenoids in skin yellowness (Alaluf et al., 2002; Coetzee & Perrett, 2014; Stephen et al., 2011) which is perceived in various cultures as healthy (Stephen et al., 2011, and Study 3 of this thesis). The results of the three perceptual studies presented in this chapter however suggest that there may be a ceiling to this preference for carotenoid colouration in human. Faces with too high levels of carotenoid colouration, after a supplementation of approximately 25mg carotenoids for 4 weeks, were not perceived as healthier than the initial face colouration.

It is uncertain if a different (and possibly significant) result would be obtained if the participants were asked to rate attractiveness instead of health. It is suggested that individual may not have a well-tuned mechanism for making conscious judgments of healthiness, as compared to attractiveness (Rhodes,

2006; Rhodes et al., 2007). Therefore, even if attractiveness is increased because of the effect carotenoids have on health; that might only be detectable if people make attractiveness judgements.

5.5.2 Adjustment of colouration and Intial skin colour and sex difference

Results of the analysis showed a negative correlation between the level of adjustment and initial colour of the faces. Participants added more colouration (redness, yellowness, and lightness) to those initial faces which have lower amount of redness, yellowness and lightness.

There was a sex difference in the amount of adjustment being applied to the faces, which is believed to be related with the sex difference in face colour (initial colour of the faces). As showed by the ANCOVA, the sex difference in amount of adjustment disappeared after we controlled for the starting colours of the faces.

5.5.3 Conclusion

As a summary, we found in Study 7 that participants selected the pre-intervention facial images as healthier as compared to the mid-supplementation images. As the preference was consistent across the two groups which were supplemented with two different health drinks (smoothie and water as placebo), it was suggested that it might be due to the influence of the extraneous factors, instead of the drink itself.

Study 8 was designed to examine if the images with intervention-induced carotenoids colouration was being perceived as healthier as compared to the original ones. Results showed no significant difference in the perceived health levels of the faces with and without the colouration. Based on the figures obtained in Study 3 (Chapter 2) on the optimum healthy facial colouration, it was suspected that the intervention-induced colouration might be too high, and thus no longer perceived as healthy, especially for those faces with high initial face colouration.

Another independent sample of participants were asked to indicate the amount of intervention-induced colouration that is perceived as healthy on faces in Study 9. Results showed that participants increased the colouration, but on average only to one third of the skin colour change observed in the intervention study. Possibly the improvement in perceived health can be obtained with the comsumption of a lesser amount of carotenoids than the amount being prescribed in Study 6. This hypothesis warrants further investigation.

CHAPTER 6: GENERAL DISCUSSION

This thesis set out to investigate the various domains of health perception. Chapter 2 reported three studies (Study 1, 2 and 3) conducted to assess different facial cues involved in health perception, including Study 3 which examined the importance of different facial colours in defining perceived health. Following on from that, Chapter 3 explored the sensitivity threshold of human vision to changes in different colour axes, by asking participants to judge the changes in different facial colours (Study 4), and compared it with the sensitivity threshold when determining colour changes in non-face objects and patches (Study 5). Chapter 4 reported the outcome of a dietary intervention that aimed to assess the facial colour changes associated with consuming a carotenoid-rich fruit and vegetable smoothie daily for 6 weeks. I examined both the changes in skin colour and the dietary pattern of participants (Study 6). Finally, Chapter 5 investigated if the face images taken during the smoothie intervention were perceived as healthier than those taken before (Study 7), and if the changes in facial colouration induced by the intervention were appropriate to enhance apparent health of faces (Study 8). The last perceptual task in Chapter 5 (Study 9) aimed to study the amount of intervention-induced colouration that is appropriate to enhance perceived face health.

All the empirical work presented in this thesis will be summarized in the following sections, followed by a brief discussion of the findings. Suggestions for future studies, along with the theoretical and practical implications of the findings will be discussed in the final part of this chapter.

6.1 Importance of skin condition in health perception (Study 1 and 2)

Previous studies on health perception have been conducted mainly as a compliment to the studies on attractiveness (Henderson, 2003; Pound et al., 2014; Rhodes, Chan, Zebrowitz, & Simmon, 2003a; Rhodes, Zebrowitz, et al., 2001). These studies of health have been predominantly focused on facial shape such as symmetry (Fink, Neave, Manning, & Grammer, 2006; B. Jones & Little, 2001; Little, Apicella, & Marlowe, 2007; Rhodes, Yoshikawa, et al., 2001a; Wade, 2010; Zaidel & Hessamian, 2010), averageness (Apicella, Little, & Marlowe, 2007; Langlois & Roggman, 1990a; Rhodes et al., 2007; Rhodes, Yoshikawa, et al., 2001b; Rhodes, Zebrowitz, et al., 2001; Thornhill & Gangestad, 1993), sexual dimorphism (Frederick & Haselton, 2007; Gray & Boothroyd, 2012; Puts, Welling, Burriss, & Dawood, 2012; Rhodes, Chan, Zebrowitz, & Simmons, 2003; Scott, Pound, Stephen, Clark, & Penton-Voak, 2010; Smith et al., 2006) and facial adiposity (Coetzee et al., 2009; Coetzee, Re, Perrett, Tiddeman, & Xiao, 2011). Recently, some studies highlighted the relative importance of skin condition as an indicator of perceived health and attractiveness (Jones, Little, Burt, & Perrett, 2004; Stephen, Coetzee, Law Smith, & Perrett, 2009; Stephen, Coetzee, & Perrett, 2011; Stephen, Law

Smith, Stirrat, & Perrett, 2009), with some authors suggesting that skin condition might be more important than facial shape in defining one's apparent health and attractiveness (Scott et al., 2010; Stephen et al., 2012). Most of these studies were conducted in the West.

Two studies reported in Chapter Two were conducted to examine the physical facial attributions that Malaysian Chinese participants used when making judgments about perceived health. Both studies suggested that skin condition, both texture and colour, serve as an important criterion of perceived health.

Study 1 and Study 2 of this thesis showed that individuals perceived skin with even texture, and with fewer pores and fewer red spots as healthier. Skin with slightly yellower skin tones was also perceived as healthier. The importance of skin texture (Jones et al., 2004; Samson, Fink, & Matts, 2010), skin colours (Fisher, Hahn, DeBruine, & Jones, 2014; Lefevre, Ewbank, Calder, Hagen, & Perrett, 2013; Stephen, Coetzee, et al., 2009; Stephen et al., 2011) and the distribution of colours (Fink, Grammer, & Matts, 2006; Fink & Matts, 2008; Matts, Fink, Grammer, & Burquest, 2007) in determining perceived health and attractiveness of faces have been reported in several studies conducted in the West, and one African sample (Coetzee et al., 2012). This is the first empirical evidence of the importance of skin condition in defining perceived health conducted on an Asian sample, specifically among the Malaysian Chinese.

While facial shape was found to be associated with health perception in a previous study (Coetzee et al., 2009), various studies (Scott et al., 2010; Stephen et al., 2012) suggest that its importance might not be as large as the influence of skin condition. Skin condition, specifically skin texture and colour, has a significant role in determining the apparent health of a person.

Human skin is in a continual state of renewal (Schwartz, Marsh, & Draelos, 2005). While the shape (including facial adiposity) and the features of human faces tend to remain relatively stable across time, facial colour and physical condition of facial skin change with slight changes of the environment and changes in our physiological function.

Take facial colour for instance. Humans' facial redness can change rapidly with changes in our emotions (Drummond et al., 2007; Drummond & Lazaroo, 2012), and our activity level (González-Alonso et al., 2008), while facial luminance can be altered with exposure to sunlight (Robin, 1991). As it was found in several studies, skin yellowness also fluctuates with changes in the consumption of fruit and vegetables over a few days or weeks (Coetzee & Perrett, 2014c). Skin carotenoid level, which is related to skin yellowness, also has been found to experience changes within days in response to changes in dietary intake (Stahl et al., 1998).

Despite the strong influence of genotype (including hormones), human skin's physical condition, for example the growing of acne, can be influenced by external environmental factors such as diet and humidity. A meta-analysis, examining the findings of 27 empirical studies (Spencer et al., 2009) concluded that dairy products and food with high glycemic-index worsened the severity of acne on the human face. Meanwhile, Egawa and colleagues (2002) showed that a short exposure of skin to a dry environment (for just 3 hours and 6 hours) induced changes in the moisture content of the *stratum corneum* and skin surface pattern, which contributed to more fine wrinkles.

In Study 2, participants' ratings of skin health (obtained from the left cheek part of their face images) were found to be associated with perceived face health. This result is in line with what was found in Jones and colleagues (2004)'s study where apparent health of facial skin was found to be correlated with the attractiveness of male faces.

Study 2 also further strengthen the importance of skin yellowness in determining perceived health, when it emerged as the sole skin colour that was correlated with both skin health and face health. The same hypothesis has been validated several times in the West in Caucasian samples (Lefevre, Ewbank, Calder, von dem Hagen, & Perrett, 2013; Stephen et al., 2011) and also in an African sample (Coetzee et al., 2012).

6.2 Cultural differences in preferences for optimal skin health (Study 3)

Previous studies have highlighted the relative importance of skin colour in defining attractiveness, more than some other physical cues such as masculinity (Scott et al., 2010; Stephen et al., 2012). Testing on two different populations, Caucasian and African, Stephen and colleagues (2011) showed that individuals from both populations increased the luminance and yellowness of the facial skin to enhance the apparent health of faces. They also showed in another study (Stephen, Coetzee, et al., 2009b) that increased facial redness, which is related with skin blood perfusion, was judged as healthier by Caucasians.

We hypothesized that the Malaysian Chinese would increase facial redness and yellowness the same way as the Caucasian and African sample did. However, it is uncertain whether the same preference for increased luminance can also be observed in a population which is living nearer to the equator (Kuala Lumpur is 349.23 km from the equator). Jablonski (2011) suggested that different levels of skin darkness evolved to adapt to different needs in different geographical environments. Higher concentration of melanin (which leads to a darker skin tone) might be beneficial for individuals who are living near to the equator who are constantly exposed to large amounts of sunlight, and not so much to those living far away from the equator and exposed to lower amounts of sunlight (Jablonski & Chaplin, 2000).

Study 3 was designed to examine the skin colour preferences of an Asian population. To enhance the perceived health of the faces, Malaysian Chinese participants increased skin yellowness and skin redness in agreement with the Caucasian and African samples in previous studies (Stephen, Coetzee, et al., 2009b; Stephen et al., 2011). However, contrary to what was found by Stephen and colleagues (2011) on the preference of increased skin lightness, Malaysian Chinese decreased skin luminance to enhance the apparent health of the faces.

Higher levels of skin redness may have been preferred as it indicates better blood perfusion which is found to be associated with better aerobic fitness (J. M. Johnson, 2010) and fertility (Jones, Hahn, & Fisher, 2015). Increased skin yellowness, on the other hand, reflects higher levels of carotenoid intake (Alaluf et al., 2002; Stahl et al., 1998) which further predict a better functioning of the immune system (Fraser & Bramley, 2004; Peters, Denk, Delhey, & Kempenaers, 2004).

The difference between the preferences for skin luminance by the three different samples (Malaysian Chinese vs. Caucasian and African) can be explained by the differences in health needs of individuals living in different geographical locations, as mentioned earlier. Communities which are living further away from the Equator (such as UK-based Caucasians and South Africans) are expected to perceive a lighter skin tone as healthier as higher

concentration of melanin might filter too much sunlight which is needed for the synthesis of vitamin D. Communities which are living around the Equator (such as the Malaysian Chinese) on the other hand should have enough sunlight for the synthesis of Vitamin D, but if they were over exposed to ultra violet radiation, it might bring upon several other skin related illnesses such as sunburn and skin cancer (Gloster & Neal, 2006). Therefore, darker skin tones with higher levels of melanin are preferred for photo protective reasons and were perceived as healthier by the sample in Study 3. This is contra to Frost (1994) however, argues that preference for lightness is universal, but light skin evolves when selection for UV protection is relaxed away from the equator.

We also found in Study 3 that participants increased skin yellowness to a much higher level as compared to the increment in skin redness and the decrement in skin luminance. This finding is consistent with the results of a previous study (Lefevre & Perrett, 2015) which further supports the importance of carotenoids colouration in defining both perceived health and attractiveness.

In short, despite sharing the same preference for increased skin redness and skin yellowness in optimizing perceived health between the current sample and those of Stephen and colleagues (2011)'s study, the findings of Study 3 suggests a cross-cultural difference in skin health perception. Malaysian

Chinese decreased skin luminance to optimize apparent health of faces, in contrast with what was being done by the Caucasian and African samples – that is increased skin luminance to enhance perceived health.

6.3 Colour vision as a mechanism for detecting changes in skin colouration (Study 4 and 5)

As skin colour has been found to be an important criteria for the assessment of perceived health, which can be utilized for the screening of potential mates, it may be hypothesized that our perceptual mechanisms, specifically colour vision, has been selected to help us make accurate judgments about changes in facial colour. Are we more sensitive to changes in certain skin colours, especially the colours that provide us with cues about others' health, than those colours that are less informative? Study 4 was designed to examine this hypothesis. Participants were requested to determine either the redder, yellower or lighter face between a pairs of otherwise identical faces, in three different sets of trials. Results of the study revealed that individuals made more accurate judgments when analysing changes in chrominance on the face, than changes in luminance. Participants had the highest accuracy when determining changes in facial redness, followed by facial yellowness and lastly facial luminance.

However, one can argue that this extra-sensitivity to chrominance might not be specific only to the perception of human faces. To further test our

hypothesis, Study 5 was designed in a similar way, except that the stimuli were replaced by colour patches and images of objects instead of human faces. Comparisons were made, and results indicated that individuals' sensitivity towards changes in luminance remain the same across three different stimuli (face vs. patch vs. object), whereas the extra sensitivity to chrominance was found only in faces.

Trichromatic colour vision has been proposed to be selected for humans and certain primates to enable us to detect minor changes in facial colours which provide important information for mate selection and socialization (Changizi et al., 2006). Humans are sensitive to changes in skin redness as it provides us information about others' health status, including aerobic fitness (Armstrong & Welsman, 2001; González-Alonso et al., 2008) and fertility (Jones et al., 2015). Jones and colleagues (2015) recently found an association between women's facial redness and the level of estradiol. As estradiol is normally high during the late follicular phase of the menstrual cycle (Hambridge et al., 2013), facial redness should be able to provide information about female fertility.

Facial redness also serves as a social cue (Drummond et al., 2007; Drummond & Lazaroo, 2012; Drummond, 1997). Individuals' facial redness fluctuates according to their emotion, for example people may blush (increased facial redness due to increased blood flow) when they are angry and embarrassed (Drummond, 1999; Drummond et al., 2007; Drummond & Lazaroo, 2012;

Drummond, 1997). This social information can be very useful for humans' daily socialization as it gives clues whether one should fight or flight.

Indicating dermal carotenoid level, recognising skin yellowness is also important as it serves as a predictor of others' immune system (Blount, Metcalfe, Birkhead, & Surai, 2003; Hughes, 1999; Krinsky & Johnson, 2005). It may therefore be useful for helping us to avoid contact with carriers of harmful pathogens.

Minor changes in skin luminance on the other hand, provide very little information about one's health status, except their susceptibility to UV related ailments, and deficiency in the formation of vitamin D.

6.4 Increment in skin yellowness and skin redness induced by smoothie supplementation (Study 6)

Previous studies have established the relative importance of skin yellowness in defining perceived health (Lefevre & Perrett, 2014; Stephen et al., 2011). A similar preference was discovered in two of the studies reported earlier. In Study 2, skin yellowness was found to be correlated with perceived health. And in Study 3, participants increased skin yellowness to a much higher level as compared to the increment in skin redness and the decrement in skin luminance, to optimize perceived health of the faces shown.

Consumption of carotenoids, which are primarily obtained from fruit and vegetables in the diet, was found to be associated with the skin yellowness in several studies (Stahl et al., 1998, 2000; Stephen et al., 2011). However, the studies conducted before either used correlational methods (Stephen et al., 2011) or prescribed supplementary pills (Alaluf et al., 2002). Additionally, control groups were not used. It is not known whether the same effect can be achieved using natural source of carotenoids. Furthermore, there is no previous study in an Asian population. Prescribing a carotenoid rich smoothie, with the inclusion of control group, Study 6 was designed to answer the above questions. Skin colour of participants, both in the intervention group and control group, were measured four times throughout the supplementation period.

There was a slight change in skin luminance between baseline measurement and the third measurement, for participants in both groups, which was probably due to an increase in skin melanin levels, with a higher exposure to sunlight during the duration of intervention. The third measurement was taken between the end of March and early April, at which time the temperature and amount of sunlight in Malaysia peaks (Zain-Ahmed et al., 2002). As for skin yellowness and skin redness, only participants in the intervention group experienced an increment in each colour axis when comparing the baseline measurement with all the subsequent measurements.

We therefore suggest that such an increment can be attributed to the content of the prescribed smoothie.

In line with what has been established in previous studies conducted in the West (Stahl et al., 1998, 2000; Stephen et al., 2011), the significant amount of increment in skin yellowness should be due to an increased intake of carotenoids, which can be found in the smoothie. However, the skin of the participants in experimental group also experienced a slight amount of increment in redness. This increment in skin redness may be due to the influence of red carotenoids such as lycopene (found in watermelon for example). Other red pigments such as betacyanin (found in dragon fruit) (Phebe & Chew, 2009) were also present in the smoothie, but we are unaware of studies finding an influence of these pigments on skin colour. A spectral analysis was conducted and significant correlations were detected between the changes in reflectance of skin of participant in the intervention group and the absorption spectra of common carotenoids, specifically β-carotene and lycopene. This further validates that the changes observed was due to the intervention and not extraneous factors.

It was suggested that sometimes, health intervention might result in behavioral compensation; that is a decrement in energy intake in response to the extra energy consumed in an intervention (Young et al., 2004). This compensatory behavior adjustment had also been observed in exercise-

related intervention studies (King et al., 2008; Staten, 1991) whereby participants were found increase in their energy intake over the course of the exercise intervention. A simple analysis was conducted to examine if participants change their fruit and vegetable consumption during the period of supplementation. Results indicate that participants in the experimental group did not adjust their fruit and vegetable intake even with the extra smoothie intake, after controlling for calorie intake. Nonetheless, there was a slight decrement in overall calorie intake for participants in both groups, which was probably because the participants were indulging themselves in food during Chinese New Year, which was celebrated by the Malaysian Chinese slightly before the start of the study.

6.5 Preference for smoothie induced skin coloration (Study 7, 8 and 9)

Even though increment in skin redness and yellowness were associated with a better perceived health (as found in Study 3), and facial skin of the participants in the supplementation study increased in yellowness and redness during 4 weeks of smoothie consumption (as found in Study 6), it is not known if these changes contribute to the actual improvement in the faces' apparent health. An experiment was designed (Study 7) to explore this hypothesis, asking an independent sample of observers to identify the more healthy face between pairs of before-and-after-intervention same-individual-faces. Contrary to our prediction, participants chose pre-supplementation facial images as healthier than the mid supplementation facial images, for

images from both the intervention group and control group. It was suggested that this might be due to the influences of extraneous variables such as the changes in dietary pattern and stress levels of the participants over the course of the intervention.

Despite the fact that the images before and during supplementation were taken in a well-controlled setting, there were inevitably some visible differences between the two faces between the 4 weeks duration, besides the changes in colour that was induced by the supplementation, thus we cannot conclude that the changes in facial colours make no difference in improving apparent health. With that in mind, a second perceptual task was designed by creating a series of intervention-induced-coloured facial images using software Psychomorph and the before intervention facial images as a template. These images differed only in colour and were identical in all other respects. Another independent sample of participants was recruited for Study 8. However, participants in Study 8 did not show any preference between the increased supplementation-induced-colour-changes images and the original ones.

As the facial colour changes induced in Study 6 (+0.95 units of redness and +3.26 units of yellowness) were much yellower and redder than the preferred colours chosen by the Malaysia Chinese in Study 3 (+0.85 units of redness and +1.31 units of yellowness), it was suspected that the lack of preference for

carotenoid colour in Studies 7 and 8 might be due to the overshooting of optimal levels of skin redness and yellowness in the face. Study 9 was designed to test this hypothesis. Participants in Study 9 were given a slider task to indicate the amount of carotenoid colouration preferred, and the value obtained was only one third of the amount of change observed among the participants in the supplementation group in Study 6. It is suggested that a future study should reduce the amount of carotenoids prescribed and observe if there is an improvement in the perceived health of the faces after the supplementation with lower doses.

6.6 Theoretical Implications

It is well established that certain birds and fish select potential mates by attending to the colour of the feathers or scale of the opposite sex, specifically carotenoid coloration, as the colouration reflects the health and fertility status of that particular individual. In this thesis, it was revealed that humans might have a similar tendency whereby increased skin yellowness and skin redness was perceived as healthier. This is in line with what was being reported by Stephen and colleagues (2009, 2011).

The selection of adaptive attributions might be different within the species owing to differences in the needs of the individuals living in different environments. For example, even though individuals living in the West prefer a body shape corresponding to a BMI within the range of 19-24 and with a

waist-to-hip ratio around .7 (B. J. Dixson, Dixson, Bishop, & Parish, 2010; Singh, 2002; Swami & Tovée, 2005a, 2005b; M. Tovée & Maisey, 1999), those societies living in a rural environment with limited food sources are reported to have a preference for a larger body size (Marlowe & Wetsman, 2001; Sorokowski, Kościński, Sorokowska, & Huanca, 2014; Tovee et al., 2006) and perceived it as healthier (Johnston & Franklin, 1993). Cultural variations in preferences for attractive facial shape (Danel et al., 2012; D. Perrett, May, & Yoshikawa, 1994) have also been explored previously (Little et al., 2007; D. Perrett et al., 1994). A cultural variation in the preference of healthy skin colour was discovered here, as different from the Caucasians and Africans who perceived lighter skin tones as healthier (Stephen et al., 2011), Malaysian Chinese preferred skin tone with a lower luminance. Lighter skin tone and a preference for such colouration is selected for communities at high latitudes with limited UV light, to increase the one's ability to synthesis vitamin D; while darker skin tone is selected for the population living near to the equator as the melanin has UV protective properties. This was in line with Jablonski's (2011) hypothesis about the selection of different skin tone across different populations.

Even though individuals from various cultures (Lefevre, Ewbank, Calder, Hagen, & Perrett, 2013; Lefevre & Perrett, 2014; Stephen et al., 2011), including Malaysian Chinese reported in this thesis, consistently showed a preference for increased carotenoid coloration in the face to be perceived as

healthy and attractive, faces with too high an amount of yellowness were not judged as attractive (as shown in Study 8 and 9). Instead, participants chose less exaggerated amounts of carotenoid coloration when given the opportunity to do so (in Study 10). Humans showed a preference for averageness (Judith H. Langlois & Roggman, 1990; Rhodes, Yoshikawa, et al., 2001b) and for a slight exaggeration of attractive traits, but not for those which are highly exaggerated (Rhodes, Hickford, & Jeffery, 2000). Rhodes and colleagues (2000) found no support for a preference for faces with exaggerated sex typical traits over those average faces in their study. Preferences for averageness may have evolved because it reflects one's health (Rhodes, Zebrowitz, et al., 2001). Facial distinctiveness (a converse measure of averageness) at the age of 17 was found to be associated with poor childhood health in males and poor current (adult) and adolescent health in females.

Further, building on the hypothesis proposed by Changizi and colleagues (2006), that colour vision is selected to detect changes in skin colour, research conducted in this thesis (Study 4) also showed that human are indeed more sensitive to the changes in skin colours which are useful for health evaluation. Whilst Changizi and colleagues (2006) came up with such a hypothesis based on their observation on chromatic capacity of primates and their exposed skin coverage, his hypothesis is further supported with the evidence collected from humans, by examining the sensitivity threshold of our colour vision on

changes in facial colours. Individuals may be better at detecting changes in facial chrominance more than facial luminance because it provides cues to the subtle changes in the bearer's physical fitness, fertility and immunity. This provides more evidence of how important our colour vision is, for the purpose of mate selection.

6.7 Practical Implications

The implications for health perception in the modern world are manifold. In particular, our results shed light on the chronic illness challenge that is increasingly serious. The 57th World Health Assembly (WHA) endorsed the World Health Organization's Global Strategy on Diet, Physical Activity and Health in May, 2004, highlighted the growing burden of non-communicable (chronic) diseases (World Health Organization, 2015). Non-communicable diseases, such as cardiovascular diseases, diabetes and cancers are now the leading causes of mortality worldwide, accounting for 63% of all deaths, and are predicted to incur approximately US$ 47 trillion of output loss in the next two decades (Bloom & Cafiero, 2012). According to the latest Malaysia National Health Morbidity Survey obtained from a nationally representative sample of 28,498 Malaysians, approximately 15.2% adults aged 18 years and above were diagnosed with diabetes, and another one third (32.7%) were diagnosed with hypertension (Malaysia Ministry of Health, 2011).

Unhealthy behaviours that contribute to the leading causes of morbidity and mortality are often established in childhood and are preventable (Eaton, Kann, & Kinchen, 2012; Grunbaum & Kann, 2002). It has been found that in 2001, approximately 2.7 million deaths and 26.7 million disabilities across the world have been associated with low fruit and vegetable consumption (Lock et al., 2004). Many countries have adopted the recommendation to consume at least 5 portions or 400 grams of fruit and vegetables a day (Agudo, 2005). However, all around the world, people are found not eating enough fruit and vegetables. In Malaysia for example, 92.5% of adults reported eating less than 5 portions of fruit and vegetables per day (Malaysia Ministry of Health, 2011).

Fruit and vegetables contain various vitamins, minerals, fibre, energy and micronutrients which are beneficial to our health. Various studies have shown that high intake of fruit and vegetables is associated with a reduced risk of chronic diseases such as cardiovascular diseases (Hung et al., 2004; Mirmiran, Noori, Zavareh, & Azizi, 2009; Rissanen et al., 2003; Voutilainen, Nurmi, Mursu, & Rissanen, 2006a), stroke (He, Nowson, & MacGregor, 2006) and certain types of cancers (Tapiero, Townsend, & Tew, 2004; World Cancer Research Fund Panel, 2007).

Preventing chronic diseases is a vital investment. And, since fruit and vegetable consumption reduces the risk of preventable mortality and morbidity, effort should be made to highlight the importance of this healthy

practice and to encourage society as a whole to consume more fruit and vegetables. Effective strategies need to be identified, with more programs needed to promote the consumption of fruits and vegetables (Coyle et al., 2009)

6.7.1 Behavioral Image Model as an approch to improve fruit and vegetable consumption

Individuals' health behaviours can be influenced by one's own public image (Ginis & Leary, 2004; Martin & Leary, 2007). Research on consumer behaviours revealed that individuals spend a great deal of money on products and services related to appearance. Americans, for example, were found to have spent approximately $400 million on clothing, personal care products and services in 2008, which accounted for 5% of the all consumer spending that year (Care, 1955). Since grooming is an important aspect of modern life, appealing to vanity might be an effective strategy to change one's behaviour, specifically, in this context, to increase one's consumption of fruit and vegetables.

As discussed earlier, carotenoids which can be found in dark coloured fruits and vegetables have been shown not only to be important in human's immunity, visual acuity, and skin protection (Darvin et al., 2011; Hughes, 1999; Rao & Rao, 2007; Samimi, 2005) but also to play a significant role in enhancing the perceived health and attractiveness of the face, as shown in

Study 10 and several other previous studies (Lefevre & Perrett, 2014; Stephen et al., 2011; Whitehead, Coetzee, Ozakinci, & Perrett, 2012). Individuals working in health related professions can utilize this information to encourage individuals to consume appropriate amounts of fruit and vegetables, to improve one's physical appearance.

The Behaviour-Image Model has been adopted to promote healthy behaviour by using salient images of oneself or others, combined with either gain- or loss-framed messages (Werch, 2007). Even though both positive- and negative framing messages can be used to modify an individual's behaviour, it has been suggested that positive framing works more efficiently as compared to the negative framing messages (Gallagher & Updegraff, 2012; Werch, 2007; Skinner, 1938). Nonetheless, some researchers highlighted the importance of considering various other factors while deciding the framing of the message, for example the risk involved (Rothman & Martino, 1999), the internal belief system of an individual (Broemer, 2002; George, Fritz, Bialosky, & Donald, 2003) and his or her previous personal experiences (Finney & Iannotti, 2002).

6.8 Strengths, Limitations and Suggestions for Future Studies

There are several limitations to the studies reported in this thesis, and suggestions, for future research, to overcome these limitations will be discussed in the following section.

The data collected from the studies reported in this thesis were solely the perception of the Malaysian Chinese, and therefore, may not generalize to other populations. Malaysia is a multi-cultural country, comprised of three main ethnic groups that have different skin colour. It would interesting to conduct a cross-cultural comparison between the preference for healthy skin colours between these three main ethnicities in Malaysia, namely the Malays, the Chinese and the Indian. It is uncertain too if the three ethnic groups would obtain similar changes in skin colour with the same consumption of smoothie. Studying skin colour changes in African sample, Coetzee and colleagues (2014) found an increment in skin yellowness on only two body regions (out of 6 regions examined) of the African sample, after the participant being prescribed with beta-carotene capsules for 8 weeks.

Studies 1 and 2 in this thesis focused mainly on establishing a connection between visual cues and subjective perception of an individual's health status. While previous studies have established the connection between the cues and dietary behaviours, we are uncertain if the cues and the subjective perception of health do really predict actual physical and physiological health of a person. Future research can further dive into this aspect by exploring the interconnection between the abovementioned factors with the actual health status of the individual, for example by collecting data about their blood pressure, aerobic fitness, or even one's medical records. A similar study in the West, examined the association between symmetry, averageness and sexual

dimorphism of human face and actual health of a person (Rhodes et al., 2003b; Rhodes, Zebrowitz, et al., 2001)

As skin condition appeared to be a more significant factor in defining perceived health than facial shape, one would predict that the observer should spend more time scanning and fixating on the cheek and forehead part of the face, rather than the common fixation on eyes, mouth and nose when asked to judge the health of faces (Tan et al., 2012). To examine this hypothesis, one can incorporate eye tracking technique into the health rating task. Finding of the eye-track study would definitely further validate the importance of skin evaluation as part of health assessment.

A few limitations have been observed from the supplementation studies (Study 6) and the perceptual studies conducted based on the stimuli collected from the supplementation studies (Study 7-9). However, due to the time and financial constraints, we were not able to conduct another follow up study.

Results from Study 6 revealed that the increment of the skin colour changes were stagnant from week-5 onwards (as compared to the measurements on week-7 and week-9). As the first mid-supplementation measurement was taken after 5 weeks of supplementation, it is possible that the plateau might be achieved earlier than that. Future research should therefore adjust the dose of the supplementation (reduce to one third of the amount that being

prescribed in Study 6, or even lower) and the timing of measurement (slightly earlier, and with short intervals), to estimate the minimum dose and duration needed to induce a change in healthy facial colouration.

Contrary to what was predicted, raters in Study 7 did not choose the after supplementation image over the before supplementation image. Neither did the rater from study 8 choose images with supplementation-induced-coloration over the original facial images. Result from study 9 showed that individual preferred a much lower amount of carotenoids coloration, which is approximately 3 times lower than the amount of skin yellowness observed in Study 6. It seems as the amount of increment of skin yellowness was too great to optimise healthy appearance. Thus, we suggest that the by decreasing the carotenoid level of the smoothie to one third, one might be able to observe an increase in preference of the after supplementation facial photos, especially if all the other variables are held constant. Perhaps a future study can shed light on this matter by prescribing participants with smoothies with lesser amounts of carotenoids, for example approximately 8mg.

Previous research has identified an influence of menstrual cycle phase on women's facial colour (Jones, Hahn, Fisher, et al., 2015) and their attractiveness (Roberts et al., 2004). Female skin colour was found to be slightly redder when their estradiol level is high (Jones, Hahn, Fisher, et al., 2015), and they were judged by men as more attractive during the follicular-

phase of their menstrual cycle, when they are most fertile (Roberts et al., 2004). Further, women were found to have different preferences for men's facial masculinity across different phases of their menstrual cycle (Penton-Voak et al., 1999). However, due to the slightly more conservative culture of Malaysian society, this particular aspect was not included in the current investigation of heath perception. The impact that women's menstrual cycle has on skin colour, and on women's perception of health in male faces warrant further investigation.

6.9 Conclusion

In summary, findings from the work presented in this thesis revealed that, despite that various cues that have been proposed to be adopted as criteria for spontaneous health assessment; individuals tend to choose those cues which are able to reflect current health status of an individual rather those reflecting future health risk. Skin condition emerged to be an important criterion for spontaneous visual health assessment, specifically skin textures and skin colour. Whilst the Malaysian Chinese chose to increase skin yellowness and skin redness to enhance perceived health of faces, the same way as what the Caucasians and Africans did in previous studies (Stephen et al., 2011), they decreased the facial luminance instead of increasing it like the Caucasians and Africans. This provides preliminary evidence of a cultural difference in skin colour preference.

The supplementation study showed that one can increase their facial colour to a desirable level by changing their fruit and vegetable consumption. However, the current study failed to prescribe an appropriate amount of carotenoids which enhanced the health perception of the face. Findings from three of perceptual studies conducted suggested that the dose was probably too high, and thus future research should try with a lower level.

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APPENDICES

Appendix 1: HPLC-UV/VIS analysis (method) of carotenoid content of smoothies

Representative smoothie samples were collected on all study days and stored at -20° C until analysis. Smoothie samples were freeze dried and carotenoids were extracted using ethanol/hexane (4:3, v/v) in 5 consecutive extraction cycles. Each smoothie sample was extracted and analyzed in triplicate. Extracts were kept at -20° C until HPLC-UV analysis. A Varian HPLC was used comprising of two solvent pumps (Varian 212-LC, CA, USA), a degasser (Degassit, MetaChem, Ludenscheid, Germany), an autosampler (Varian 410, CA, USA), and a UV-VIS detector (Varian 325, Agilent Technologies, Germany). Mobile phase A (MPA) consisted of methanol/water/ammonium acetate (1M) (97:3:0.2, v/v) and mobile phase B (MPB) consisted of MTBE. Carotenoids were separated on a Phenomenex Ultracarb ODS (30) HPLC column, with a length of 150 x 4.60 mm and 5 µm particle size (Phenomenex, CA, USA). Analysis was conducted at room temperature at a flow rate of 1 mL/min using a gradient from 0 to 10% MPB from 0 to 15 min, 10 to 50% MPB from 15 to 45 min, followed by a wash step consisting of 50 to 100% MPB from 45 to 50 min, 100 to 0% MPB from 50 to 51 min and an equilibration phase from 51 to 65 min at 0% MPB. Carotenoids were identified based on their co-elution with authentic standards. β-carotene was purchased from Nacalaitesque Inc. (Kyoto, Japan), while α-carotene and lycopene (and other carotenoids) were received from DSM (The Netherlands/Germany). Carotenoid quantification was based on absorption at 450 nm and calibration curves were prepared using standard solutions whose carotenoid content was precisely determined following quantitative spectrophotometry and the Beer Lambert law. Varian Workstation version 6.9.3 was used to integrate carotenoid peaks from collected chromatograms.

Appendix 2: HPLC analysis of carotenoid content of smoothies:

Representative smoothie samples were collected every study day and stored at -20° C until analysis. Quantity of carotenoids was determined by HPLC-UV. Each smoothie sample was extracted and analyzed in triplicate. Six different smoothie recipes (A – F) were created to deliver variations in taste, and depending on availability of raw ingredients, smoothie A – F were prepared on 9, 8, 2, 7, 7 and 4 study days respectively. Values in the table are average carotenoid content in each of the 6 smoothie variations used in the study (± SD).

Carotenoids (in mg)	smoothie A	smoothie B	smoothie C
β-carotene	26.32 ± 8.73	20.95 ± 4.03	16.07 ± 0.90
α-carotene	5.40 ± 2.43	3.65 ± 1.12	2.38 ± 0.27
Lycopene	-	-	0.57 ± 0.07
Total carotenoids	31.88 ± 10.98	24.60 ± 5.08	19.59 ± 0.94

Carotenoids (in mg)	smoothie D	smoothie E	smoothie F
β-carotene	16.35 ± 3.70	25.65 ± 4.72	20.78 ± 2.49
α-carotene	3.56 ± 0.65	4.74 ± 1.29	4.18 ± 1.33
Lycopene	-	-	1.92 ± 0.37
Total carotenoids	19.76 ± 3.50	30.59 ± 5.56	26.68 ± 4.04

Appendix 3: Mean and standard deviation of the measurements (for three colour spaces, four body regions and between the four measurements)

3.1. Mean and standard deviation of skin luminance on four body regions between four measurements

		Intervention		Control	
Region	Time	Mean	SD	Mean	SD
R1	1	66.76	3.15	66.95	2.60
R1	2	66.82	3.71	67.20	2.42
R1	3	66.77	2.85	67.21	2.44
R1	4	66.77	2.75	67.21	2.43
R5	1	60.84	4.54	61.58	6.56
R5	2	60.32	4.80	60.50	4.32
R5	3	60.79	4.15	60.72	4.12
R5	4	60.82	4.40	61.10	3.96
R6	1	64.17	4.25	66.07	6.39
R6	2	63.91	3.04	65.04	3.47
R6	3	64.16	3.45	65.38	3.62
R6	4	64.07	3.51	64.18	3.10
R7	1	67.65	3.88	68.34	6.76
R7	2	68.28	3.11	68.83	2.10
R7	3	68.00	2.13	68.64	2.87
R7	4	68.05	2.16	68.41	2.79

3.2. Mean and standard deviation of skin redness on four body regions between four measurements

		Intervention		Control	
Region	Time	Mean	SD	Mean	SD
R1	1	8.39	1.65	7.91	1.46
R1	2	8.84	1.59	8.10	1.17
R1	3	8.98	1.29	7.98	1.18
R1	4	8.92	1.38	7.88	1.29
R5	1	11.75	1.99	11.58	1.68
R5	2	12.53	1.99	11.64	1.81
R5	3	12.13	1.67	11.53	1.87
R5	4	12.15	1.78	11.64	1.80
R6	1	11.78	2.39	10.85	2.25
R6	2	12.66	1.50	10.81	2.11
R6	3	12.28	2.22	10.37	2.41
R6	4	12.66	2.14	11.43	1.69
R7	1	10.60	2.37	11.33	2.55
R7	2	11.52	2.08	9.61	2.17
R7	3	12.10	1.63	9.64	1.80
R7	4	11.85	1.69	10.11	2.18

3.3. Mean and standard deviation of skin yellowness on four body regions between four measurements

		Intervention		Control	
Region	Time	Mean	SD	Mean	SD
R1	1	16.64	2.39	16.69	1.83
R1	2	18.94	2.09	16.84	1.77
R1	3	19.29	1.81	16.58	2.17
R1	4	19.28	1.97	16.91	1.79
R5	1	20.99	1.70	21.18	2.12
R5	2	22.18	1.93	20.93	1.50
R5	3	22.82	1.48	20.94	1.92
R5	4	22.49	1.80	20.73	1.92
R6	1	19.28	2.60	18.54	2.50
R6	2	21.04	2.41	18.07	1.93
R6	3	21.48	1.99	18.35	1.78
R6	4	21.10	2.24	18.68	2.08
R7	1	15.45	1.94	16.65	2.38
R7	2	20.85	1.65	15.87	1.90
R7	3	21.89	1.42	16.22	1.70
R7	4	20.98	1.70	16.30	2.18