Stability of curcuminoids in turmeric oleoresin: effects of light exposure, antioxidants and metal chelation

By

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DECLARATION

I, Nishanie Moonaisur, declare that the dissertation that I hereby submit for the degree MSc Food Science, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Nishanie Moonaisur, has obtained, for the research described in this work, the applicable research ethics approval. I declare that I have observed the ethical standards required in terms of the University of Pretoria's *Code of ethics for researchers* and the *Policy guidelines for responsible research*.

SIGNATURE: 

DATE: 25 April 2016

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DEDICATION

To my parents - I have never taken any compliments to heart because deep down inside, I know that all of them actually belong to you both. Thanks for everything, mom and dad.

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"Education is the most powerful weapon which you can use to change the world"

-Nelson Mandela

ABSTRACT

Stability of curcuminoids in turmeric oleoresin: effects of light exposure, antioxidants and metal chelation

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Nishanie Moonaisur

Supervisors: Prof HL de Kock
Co-supervisor: Prof KG Duodu

Despite the increasing demand for natural colourants, their sensitivity to light is a major disadvantage and could pose restrictions to their utilization as food colourants in industry. Turmeric (*Curcuma longa* L.) is a tropical plant native to southern and south eastern tropical Asia. The most active colour components in turmeric roots are curcuminoids, which are used in many food and pharmaceutical preparations. However, curcuminoids are unstable and have been replaced by stable synthetic dyes such as sunset yellow. Hence, the purpose of this study was to evaluate the effect of storage of turmeric oleoresin with and without protection from external environmental conditions, including sunlight during daytime, on the stability of curcuminoid pigments, measured by high-performance liquid chromatography and correlated with $L^*a^*b^*$ colour space values and colour evaluation by a trained sensory panel.

Turmeric oleoresin powders were placed in labelled, clear plastic jars and exposed to external environmental conditions during the day for a period of 10 weeks. Control samples were stored in a refrigerator at 4 °C. To determine the effect of protection of turmeric oleoresin powders from external environmental conditions on curcuminoid stability, jars were covered with aluminium foil and stored outside and exposed to external environmental conditions for a period of 10 weeks. Control samples were refrigerated at 4 °C.

Over time of exposure to external environmental conditions, curcuminoid pigment degradation was evidenced, with 100% degradation observed after 9 weeks of storage, relative to the control. Curcuminoids were less sensitive to degradation when shielded from external environmental conditions, with a 22% reduction in degradation observed relative to turmeric oleoresin powders

without aluminium foil protection after 10 weeks of exposure to external environmental conditions. Significant sensory differences were observed between turmeric oleoresin powders with and without protection from external environmental conditions compared to the control samples.

In an attempt to enhance curcuminoid stability in the presence of external environmental conditions, the effect of antioxidants, tertiary butylhydroquinone (TBHQ) and ascorbic acid on the stability of curcuminoid pigments in turmeric oleoresin, was studied. The effect of divalent ion-curcuminoid complexation on the stability of curcuminoid pigments in turmeric oleoresin when exposed to external environmental conditions, was also investigated.

Antioxidants were homogenously distributed onto the powdered turmeric oleoresin base at 0.02% (m/m). Samples were exposed to external environmental conditions for a period of 10 weeks. According to the total colour difference results (L^*, a^* and b^* values), the addition of both TBHQ and ascorbic acid did not improve the colour stability of the curcuminoid powders after 10 weeks of exposure to external environmental conditions. The presence of ascorbic acid had no significant influence on curcuminoid stability, with the degradation trend following that of the control, which did not contain antioxidants.

The effect of complexation of curcumin with divalent ion (Mg^{2+}) by mechanical mixing was also investigated. After 10 weeks of exposure to external environmental conditions, no significant improvement in curcuminoid stability was observed after the addition of magnesium ions to turmeric oleoresin powders when compared to the control which did not contain magnesium ions.

Correlation analysis was conducted to determine the relationship between percentage curcuminoid degradation, sensory scores and colour ratings of turmeric oleoresin powders that were exposed to external environmental conditions. A strong negative linear relationship was observed between percentage curcuminoid degradation and rating of colour. A perfect positive relationship was observed between chroma and b^* values. Chroma also had a strong positive relationship with L^*. Percentage curcuminoid degradation showed a weak positive relationship with both a^* and hue angle values.

Exposure to external environmental conditions negatively affected the colour stability of curcuminoid pigments in turmeric oleoresin powders and the level of pigment degradation was

dependent on time of storage. Stabilization strategies investigated in this study, such as Mg^{2+}-curcuminoid complexation as well as the addition of antioxidants (TBHQ and ascorbic acid) did not significantly improve curcuminoid stability in turmeric oleoresin powders.

Constant storage of ingredients and products containing turmeric oleoresin at low temperature (<5 °C), together with light protection is vital to retard colour degradation.

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CHAPTER 1

1 Introduction and Problem Statement

Despite the increasing demand for natural colourants, their sensitivity to light, heat and oxygen is a major disadvantage and could pose restrictions to their utilization as food colourants in industry (Tang and Norziah, 2007). Stability of naturally coloured pigments in thermally processed and stored foods has been a major challenge (Clydesdale, Fleischman, and Francis, 1970; Ihl, Monslaves and Bifani, 1998). This significantly limits the use of natural colourants in the food processing industry (Hanne and Thorsteinn, 2002).

The colour of food is important for consumers as it is the first characteristic to be noticed and one of the main ways of visually assessing quality of a food product before it is consumed (Sampathu, Krishnamurthi, Shivasankar, Shankarnarayan, Roa, and Lewis, 1981; Santanakrishnan, 1981; Pattnaik, Roy, and Jain, 1999). Colour also helps in the recognition of food and makes it appealing to consumers (Ahmed and Sandu, 2002).

Turmeric (*Curcuma longa* L.) is a tropical plant native to southern and south eastern tropical Asia. The most active colour component in turmeric roots is curcumin. Curcumin is used in many food and pharmaceutical preparations, amongst others (Sampathu, Lakshminarayanan, Sowbhagya, Krishnamurthy, and Asha, 2000) and imparts an attractive bright yellow-orange colour to food (Joe, Vijaykumar, and Lokesh, 2004). It is used to colour a variety of foods including dairy products, fat emulsions, confectionery, soups and sauces. Furthermore, it possesses antioxidant, anti-inflammatory, antimicrobial and anti-cancer properties (Ali, Marrif, Noureldayem, Bakheit, and Blunden, 2006).

However, as a natural colouring agent, curcumin is known to be unstable and has been replaced by stable synthetic dyes e.g. sunset yellow, whenever possible. This limits its use in many food applications because it loses its colour upon storage and under alkaline conditions (Ali *et al*., 2006).

Various factors are responsible for the loss of pigment and colour during processing and storage of food products. These include non-enzymatic and enzymatic browning reactions and process conditions such as acidity, pH, oxidation, packaging material, temperature of storage and light exposure (Meyer, 1987). Colour degradation is a particularly important parameter to be

considered when applying thermal food treatments such as pasteurization, cooking and boiling (Ali *et al*., 2006).

Although visual colour and pigment degradation kinetics of food products are complex phenomena, they are dependable models that can be used to predict experimental colour variation. Kinetics of pigment and colour degradation of fruits, vegetables and spices such as saffron and paprika during thermal processing have been studied by numerous researchers (Shin and Bhowmik, 1994; Steet and Tong, 1996; Kajuna, Bilanski, and Mittal Kajuna, 1998; Weemaes, Ooms, Loey, and Hendrickx, 1999). The kinetic parameters, namely rate constant and activation energy, provide useful information on the quality changes which occur during thermal processing. Several studies have been reported in the literature on quality aspects of turmeric based mainly on curcumin loss during thermal processing and under alkaline conditions (Ahmed and Sandu, 2002; Meyer, 1987).

Little information is available on the pigment and visual colour degradation kinetics of curcumin pigments during light exposure. The visual colour measurement has been accepted by the processing industries as an on-line quality control technique. However, measurement of pigment compound concentration could provide a better quantitative assessment of the actual colour degradation during processing. Hence, there is an urgent need to establish the significance of the correlation between pigment concentration and the visual colour of food products during processing (Ahmed and Sandu, 2002).

Therefore, this research is an attempt to quantify and investigate the effect of external environmental conditions, including exposure to sunlight, on the stability of curcuminoid pigments in turmeric oleoresin powders, as well as to investigate the effect of curcuminoid pigment degradation on the colour of the turmeric oleoresin powder. This work also attempts to explore strategies that may be used to stabilize curcuminoid pigments in turmeric oleoresin powder, with the aim of enhancing the application of this natural colourant in the food industry.

CHAPTER 2

2 Literature Review

Consumers are increasingly avoiding foods containing synthetic colourants (Azeredo, 2009), which lead food industries to replace them with natural pigments such as carotenoids, betalains, anthocyanins and curcuminoids. This literature review will discuss the chemistry and sources of natural food colourants, particularly turmeric, and its usage in the food processing industry. Attention has also been given to the intrinsic and extrinsic factors affecting pigment stability and colour, such as temperature, the presence of sunlight and pH. Natural pigment stabilization strategies and the principles behind the analytical methods used in this study will also be reviewed.

2.1 Importance of food colour

Apart from flavour, texture and economic considerations, colour is one of the most important attributes affecting consumer acceptance of food (Azeredo, 2009).

Colour is an important feature of any food item (Rymbai, Sharma, and Srivastav, 2011). The colour of food is important for consumers as it is the first characteristic to be noticed and one of the main ways of visually assessing a food product before it is consumed (Sampathu *et al*., 1981, Santanakrishnan, 1981; Pattnaik, Roy, and Jain, 1999). The perceived colour provides an indication of the expected taste of a food product. If the flavour of a food product is inconsistent with the colour, then the flavour can very often be perceived incorrectly; for example an orange flavoured drink coloured green could be perceived to taste of lime. Hence, attractive colours of food products like strawberry jams or raspberry jellies are an important quality parameter which influences consumer behaviour (Mohammadi, Rafiee, Emam-Djomeh, and Keyhani, 2008).

Colour helps in the recognition of food and makes it appealing to consumers (Stintzing and Carle, 2004). Therefore, colour has a direct bearing on the flavour of a food product. As an example in terms of food manufacturing, a bright strawberry colour can indicate to the consumer a high quality product, whilst a “washed out” or “faded” colour can indicate poor product quality (Aberoumand, 2011).

Many raw foods such as fruit and vegetables have a bright attractive colour. Other foods such as confectionery items and flavoured soft drinks would be grey or colourless if colour was not added to them during the manufacturing process (Mohammadi *et al*., 2008).

2.2 History of food colour

The earliest written record of the use of natural dyes in food processing dates back to 2600 BC in China. It has also been reported that around 1500 BC in Egyptian cities, confectioners added natural extracts and wine to improve the appearance of candies (Aberoumand, 2011).

The first synthetic colour, mauvine, was developed by Sir William Henry Perkin in 1856. The beginning of the 19th century was notable for the bulk of production and recovery of synthetic colours from petroleum derived products like aniline. Up to the middle of the 19th century, ingredients such as saffron were added to decorate certain food products (Naidu and Soubhagya, 2012).

Several sociological, technical and economic factors have influenced the food industry over the last 20 or so years. Over this time, the food market has changed rapidly, with a much larger proportion of food being processed or modified before sale and ready prepared to meet the needs of new consumers such as working mothers, single parent families and the elderly. The challenge to the food industry is to deliver visually appealing foods that taste good and meet the consumers' demands in terms of both product quality and price (Downham and Collins, 2000).

2.3 Demand for food colourants

The demand for food colourants in the global market in the year 2000 was 2400 MT and was expected to increase to 15000 MT by the year 2015 (Figure 2.1). The investment in the natural food colour market across the globe has approached US $ 1 billion and is continuously growing as there is a higher consumer demand for natural food colours compared to synthetic food colours (Downham and Collins, 2000).

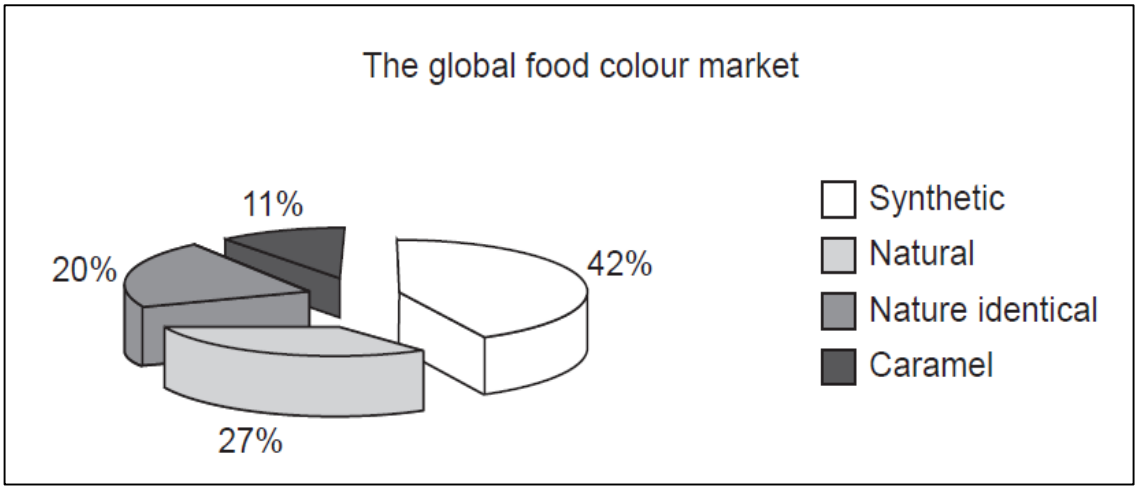


Figure 2.1: Percentage market share of food colours globally (Downham and Collins, 2000)

2.4 Classes of food colourants

Food colourants may be classified into natural, nature-identical, synthetic and inorganic colourants (Aberoumand, 2011).

Most often, the colourants are extracted from plant material, as well as other sources such as insects, algae, cyanobacteria and fungi. They are extracted and concentrated using either water or lower alcohols for water-soluble pigments and organic solvents for lipophilic pigments (Downham and Collins, 2000). Usually, pigments made by modification of materials from living organisms such as caramel, vegetable carbon and Cu-chlorophyllin, are also considered natural, though they are in fact (except for carbon) not found in nature (Aberoumand, 2011).

Nature-identical colours are man-made pigments which are identical to those found in nature. Examples are β-carotene, canthaxanthin and riboflavin (Downham and Collins, 2000).

Synthetic colours are man-made colours which are not found in nature—these are often azo-dyes e.g. tartrazine, erythrosine and indigo carmine. Examples of inorganic colours are titanium dioxide, gold and silver (Aberoumand, 2011).

In South Africa, legislation [Foodstuffs, Cosmetics and Disinfectants Act of South Africa, 1972 (Act 54 of 1972)] restricts which colourants are allowed, what sources may be used for that

particular colourant, what solvents may be used to extract it and the required purity of the pigment (Aberoumand, 2011).

Although synthetic pigments exhibit evident advantages compared to their natural counterparts with respect to stability, they are increasingly rejected by consumers due to health concerns. As a consequence, natural colourants are widely preferred as food colourants (Collins and Timberlake, 1993; Giusti and Wrolstad, 2005).

2.5 Natural colourants

Natural colourants play an important role in sensory and consumer acceptance of food products (Giusti and Wrolstad, 1996). Substantial developments have occurred with natural colours since their wider commercialization around 25 years ago. Today, natural dyes and colourants have growing importance, not only in food applications, but also due to their medicinal properties. Consumer awareness towards natural dyes and their therapeutic uses are increasing due to their non-toxic properties and fewer side effects compared to synthetic dyes (Rymbai *et al*., 2011).

Colour is spread widely throughout nature in fruit, vegetables, seeds and roots. Natural dyes are derived from naturally occurring sources such as plants (e.g., indigo, annato and saffron); insects (e.g., cochineal beetles); animals (e.g., some species of molluscs or shellfish); and minerals (e.g., ferrous sulphate, ochre, and clay) without any chemical treatment. The turmeric plant for example, measures up to 1 m high, with a short stem and tufted leaves (Aggarwal, Kumar, Aggarwal, and Shishodia, 2005). The parts used are the rhizomes from which the curcuminoids are extracted (Nagarajan, Kubra, and Rao, 2010).

A spectrum of beautiful natural colours ranging from yellow to black exists in the above sources. These colours are exhibited by various organic and inorganic molecules (pigments) and their mixtures due to the absorption of light in the visible region of 400-800 nm (Chengaiah, Rao, Kumar, Malagusundaram, and Chetty, 2010).

Today, natural dyes are commonly used in the cosmetic industry due to few side effects, UV protection effects and anti-aging properties (Adinew, 2012). In India, there are more than 450 plants that can yield dyes. Some of these plants also possess medicinal value (Chengaiah *et al*., 2010). Natural dyes are generally also environmentally friendly, for example, turmeric, the

brightest of naturally occurring yellow dyes, is a powerful antiseptic which revitalizes the skin, while indigo gives a cooling sensation (Adinew, 2012).

Natural pigments, however, vary widely in their physical and chemical properties. Many are sensitive to oxidation, pH change and light and their inherent solubility varies widely (Downham and Collins, 2000).

With these drawbacks in mind, suppliers of natural colourants have focused the development on currently permitted pigments in three main areas: formulation technology, processing technology and alternative sources of pigments (Downham and Collins, 2000). These approaches have proved very successful and have contributed to the increase in usage of natural colourants throughout the food and drink industry (Chaitanya, 2014).

2.5.1 Turmeric as a natural colourant

Turmeric (*Curcuma longa* L.) is extensively used as a spice, food preservative and colouring material in India, China and South East Asia (Lal, 2012).

The turmeric plant is a perennial herb, belonging to the ginger family. It measures up to 1 m high, with a short stem and tufted leaves (Figure 2.2) (Aggarwal, Kumar, Aggarwal, and Shishodia, 2005). The parts used are the rhizomes from which the curcuminoids are extracted. The most active component in turmeric is curcumin, which may make up 2 to 5% (w/w) of the total spice (Nagarajan, Kubra, and Rao, 2010).

Curcuminoids are groups of phenolic substances present in turmeric powder/oleoresin, namely curcumin, [molecular weight (MW) of 368] accounting for 60–80%, demethoxycurcumin (MW of 338) accounting for 15–30%, and *bis*-demethoxycurcumin (MW of 308) with a level of 2–6% (Figure 2.3) (Wichitnithad, Nimmannit, Wacharasindhu, and Rojsitthisak, 2011).

India is the main country exporting turmeric and its production is approximately 80% of the world supply. Today, the species is widely cultivated in some African countries e.g. Madagascar, South Africa and Ethiopia (Parthasarathy, Chempakam, and Zachariah, 2008). As a food additive, turmeric can improve the deliciousness, aesthetic appeal and shelf life of food products (Joe *et al*., 2004). Moreover, the powder of turmeric is expansively used as a preservative and colouring agent (Abdeldaiem, 2014).

Figure 2.2: *Curcuma longa* is primarily cultivated for turmeric rhizomes and their products. The upper picture shows the plants cultivated in Nacogdoches, Texas, USA and the lower picture shows rhizomes and ground turmeric (Li, Yuan, Deng, Wang, Yang, and Aggarwal, 2011)

2.5.2 Chemical composition of turmeric

Turmeric rhizomes, on average, contain protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13%) (Ruby, Kuttan, Babu, Rajasekharan, and Ruby, 1995). The essential oil content (5.8%) obtained by steam distillation of rhizomes has *a*-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpenes (53%) (Kapoor, 1990). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour and comprises curcumin (94%), de-methoxy (6%) and *bis*-demethoxy (0.3%) (Ruby *et al*., 1995).

De-methoxy and *bis*-demethoxy derivatives of curcumin have also been isolated (Vopel, Gaisbaver, and Winkler, 1990) (Figure 2.3). Curcumin was first isolated (Vogel and Pelletier) in 1815 and its chemical structure was determined by Roughley and Whiting in 1973. It has a melting point of 176 –177°C and forms a reddish-brown salt (Lal, 2012).

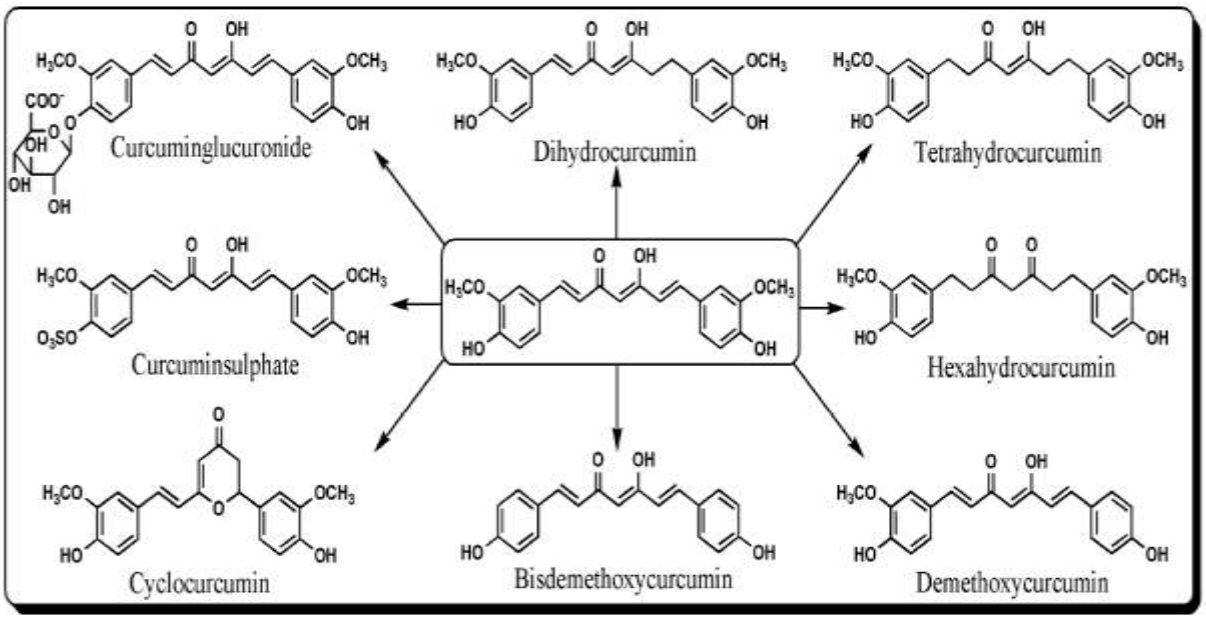


Figure 2.3: The chemical structures of curcumin, demethoxycurcumin, and *bis*-demethoxycurcumin (Rohman, 2012) and other natural metabolites of turmeric and curcumin (Lal, 2012)

2.5.3 Curcuminoids

Curcuminoids are hydrophobic in nature and freely soluble in dimethylsulphoxide, acetone, ethanol, chloroform, oils and insoluble in water. They are sensitive to light, moderately stable to heat, and are unstable at neutral-basic pH (Hanne *et al*., 2002). In addition to this, curcuminoids are relatively stable at acidic pH, but rapidly decomposes at a pH above neutral (Adinew, 2012). Hence, in basic media, curcuminoids undergo degradation reactions (Tonneson, Mason, and Loftsson, 2002).

2.5.3.1 Sources of curcuminoids

Curcuminoids are found in:

- Turmeric (*Curcuma longa*) - 22.21 - 40.36 mg/g in the rhizomes and 1.94 mg/g in the tuberous roots and in other *curcuma* species such as *Phaeocaulis* (0.098 mg/g) (Gowri, Narayanan, Maheswaran, Harshapriya, and Mathachan, 2014).
- Common ginger (*Zingiber officinale*) and shampoo ginger (*Zingiber zerumbet*) (Gowri *et al*., 2014).

Commercially available extracts of curcumin may not be wholly curcumin, but instead a blend comprising of 77% curcumin, 17% demethoxycurcumin, 3% *bis*-demethoxycurcumin and the last 3% not yet classified (Gowri *et al*., 2014).

2.5.3.2 Extraction of curcuminoids from turmeric

Curcuminoids are extracted from the dried rhizome *Curcuma longa* (Figure 2.4) (Zebib, Mouloungui, and Noirot, 2010). The process of extraction requires the raw material to be ground into powder and washed with a suitable solvent that selectively extracts colouring matter (Adinew, 2012). This process, after distillation of the solvent, yields an oleoresin with colouring matter content in the region of 25-35 % along with volatile oils and other resinous extractives. The oleoresin obtained is subjected to further washes using selective solvents that can extract the curcumin pigment from the oleoresin. This process yields a powdered, purified food colourant, known as curcumin powder, with over 90% colouring matter content and very little volatile oil and other dry matter of natural origin. The selection of solvents is done with care to meet extractability and regulatory criteria (Priyadarsini, 2014).

Figure 2.4: Rhizomes of *Curcuma longa* L. plants (Zebib *et al*., 2010)

2.6 Therapeutic benefits of turmeric

Scientific research spanning over more than four decades has confirmed the diverse pharmacological effects of turmeric, and has established its ability to act as a chemo-preventive agent, as well as a potential therapeutic agent against several chronic diseases (Lal, 2012).

Turmeric has been used as a traditional medicinal agent in order to prevent several diseases (Chattopadhyay, Biswas, Bandyopadhyay, and Banerjee, 2004). The biological activities include antioxidant (Kalpravidh, Siritanaratkul, Insain, Charoensakdi, Panichkul, Hatairaktham,

Srichairatanakool, Phisalaphong, Rachmilewitz, and Fucharoen, 2010), anti-inflammatory (Skrzypezac-Jankun, McCabe, Selman, and Jankun, 2000), anti-atherogenic, anti-psoriatic (Heng, Song, Harker and Heng, 2000), anti-diabetic (Arun and Nalini, 2002), immunostimulatory (Antony, Kuttan, and Kuttan, 1999), antibacterial (Singh, Chandra, Bose, and Luthra, 2002) and anticancer effects as reviewed by Aggarwal *et al*. (2003). This also contributes to its incorporation to the healing of dermal wounds (Gopinath, Ahmed, Gomathi, Chitra, Sehgal, and Jayakumar, 2004) as well as the prevention of Alzheimer's disease (Kalpravidh, Siritanaratkul, Insain, Charoensakdi, Panichkul, Hatairaktham, Srichairatanakool, Phisalaphong, Rachmilewitz, and Fucharoen, 2010).

While the majority of researchers have been pursuing the biological aspects, a few others have been interested in understanding the chemistry behind curcumin's unique biological activity. Inorganic chemists have used its metal chelating abilities through the β-diketo group to form new structural entities with modified biochemical activities. Physical chemists have focused on the highly sensitive spectroscopic properties of curcumin to study its interactions with microheterogeneous systems and biomolecules (Priyadarsini, 2014). Analytical chemists have been employing curcumin's unique absorption spectroscopic properties to identify and quantitatively estimate trace elements such as boron, as a red coloured product (Subhan, Alam, Rahaman, Rahman, and Awal, 2014). Other chemistry studies that are useful in understanding the biological activity of curcumin are its chemical reactivity with reactive oxygen species (ROS), addition reactions, degradation and formation of nanoconjugates (Priyadarsini, 2014).

2.7 Disadvantages of using natural colourants in food products

Despite the increasing demand for natural colourants, their sensitivity to light, heat and oxygen is a major disadvantage and could pose restrictions to their utilization as food colourants in industry (Tang *et al*., 2007). Maintenance of naturally coloured pigments in thermally processed and stored foods has been a major challenge in food processing (Clydesdale *et al*., 1970; Ihl *et al*., 1998). Various factors are responsible for the loss of pigment and colour during processing of food products. These include non-enzymatic and enzymatic browning reactions and process factors such as pH, acidity, oxidation, temperature of storage and light exposure (Mesnier, Gregory, Fança-Berthon, Boukobza, and Bily, 2014).

Special care must be taken to produce food that retains a bright, attractive colour during and after processing (Meyer, 1987). Change in colour during thermal processing and product storage may therefore be used as a tool to evaluate the product quality (Chaitanya, 2014).

2.8 Factors affecting natural colourant stability

2.8.1 The effects of light on natural colourant stability

Degradation of purified curcumin has been observed to be caused by UV (280-350 nm) as well as visible (>400 nm) radiation in liquid and dry preparations (Tonnesen, Karlsen, and Vanhenegouwen, 1986).

Protection against exposure to light above 500 nm by storage in brown glass containers protects curcuminoids against degradation. Use of clear UV or yellow filters slightly reduces colour fading (Crews and Reagen, 1987). Light wavelengths from 280 to 450 nm initiate singlet oxygen production, which contributes to sensitizing curcumin. This results in auto-oxidation, as well as undefined mechanisms independent of oxygen participation (Tonneson, Karlsen, and Vanhenegouwen, 1986). Singlet oxygen quenchers such as β-carotene retard, but do not prevent degradation of curcumin. Exposure to triplet oxygen has been shown to have a minor effect on stability (Crews and Reagen, 1987).

Preventing exposure to light, especially to wavelengths <500 nm appears to be the best protection against degradation. Complexing curcuminoids with aluminium ions reduces but does not prevent degradation. Other metal cations such as copper, iron and zinc also reduce colour fading. However, these cations cause a change in hue (Maing and Miller, 1981).

Molecules of all types are excited by and selectively absorb radiant energy at specific wavelengths across the electromagnetic spectrum (Maing and Miller, 1981). Glass, which allows visible light to pass through it without absorption, is not as transparent to ultraviolet (UV) light and is virtually opaque to UV light of the shorter wavelengths (Azeredo, 2009).

2.8.2 Oxygen

Natural pigments react with oxygen (Attoe and von Elbe, 1985). Storage of solutions containing the natural pigments under low oxygen levels resulted in decreased pigment degradation compared to storage under air atmosphere, since low oxygen levels favour partial recovery of the

pigment after degradation (Huang and von Elbe, 1987). A deviation from the first-order degradation kinetics of natural pigment betanin in the absence of oxygen was attributed to reaction reversibility (Azeredo, 2009). The mechanisms of curcuminoid degradation are discussed further is section 2.9.

2.8.3 pH

Curcuminoids are yellow from pH 3 to pH 7 in aqueous solutions, turning brownish-red above pH 8. Shifts in light absorption occur between pH 8 and 9.9 and 10, and 11 and 12 that coincide with changes in curcuminoid hue. The change in colour at alkaline pH is accompanied by reduced stability and a rate of degradation several times greater above pH 8 than in acid solution (Figure 2.5) (Adinew, 2012).

Curcumin is unstable at basic and neutral pH and degrades within 30 min to *trans*-6-(40-hydroxy-30-methoxyphenyl)-2, 4- dioxo-5- hexanal, ferulic acid, feruloylmethane and vanillin. The initial degradation products are formed after 5 minutes and the Chromatographic pattern obtained after 28 hours at pH 8.5 is representative for alkaline degradation. Ferulic acid and feruloylmethane are formed initially. Feruloylmethane rapidly forms coloured (mostly yellow to brownish-yellow) condensation products. Degradation products formed by hydrolysis of feruloylmethane are vanillin and acetone and their amount increase with incubation time. Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1 hour (Kumavat, Chaudhari, Borole, Mishra, Shenghani, and Duvvuri, 2013). Figure 2.5 illustrates the structure of curcumin at different pH values.

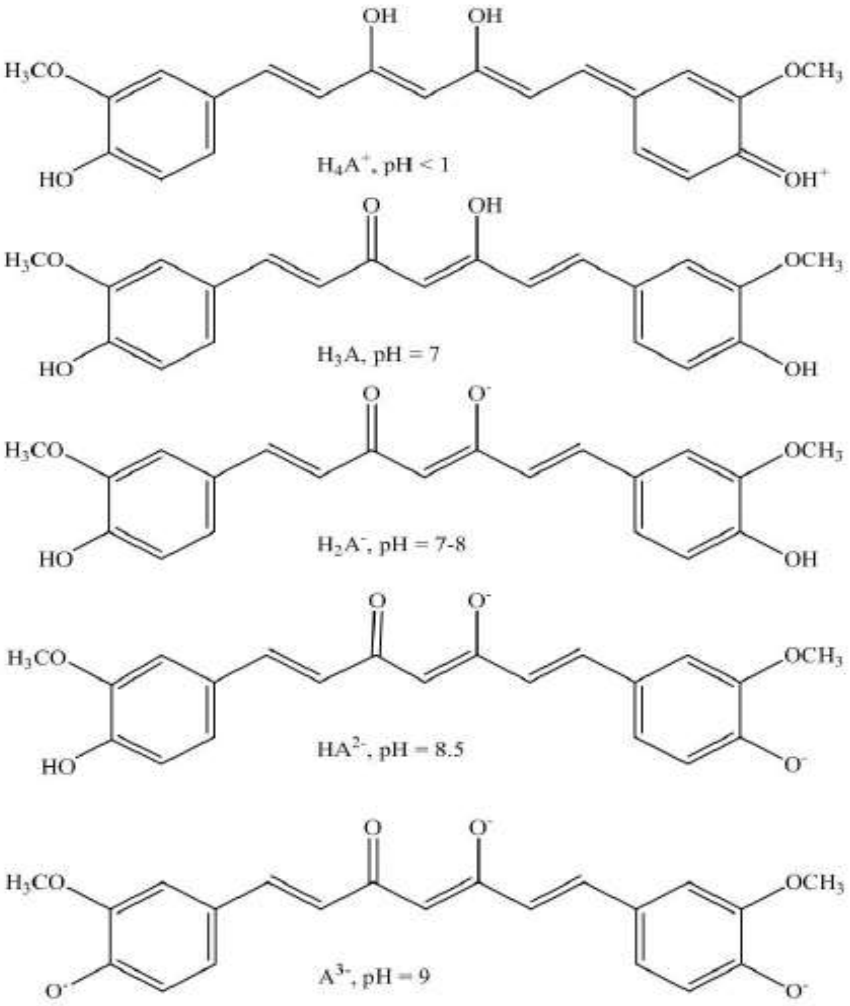


Figure 2.5: Structure of curcumin at different pH values (Adinew, 2012)

2.8.4 Temperature

Temperature is one of the most important factors affecting natural pigment stability during food processing and storage. Some studies reported increasing pigment degradation rates resulting from increasing temperatures (Saguy, Kopelman, and Mizrahi, 1978; Garcia Barrera, Reynoso and Gonzalez de Mejia, 1998).

During heat processing, pigments may be degraded by isomerisation, decarboxylation or cleavage, resulting in a gradual reduction of colour, and eventually the appearance of a light brown colour (Huang and von Elbe, 1985).

In a study conducted by Wongthongdeea and Inprakhona (2013), a significant effect of temperature on curcumin stability was observed after 1 month of storage. It was found that the decomposition rate at room temperatures was faster than at the refrigerated temperatures. The

initial content of curcumin (52.5 ± 5.1 mg/g) decreased rapidly to 31.3 ± 1.9 mg/g at room temperature, a percentage loss of 40%, while only 25% of loss was observed at the refrigerated temperature. No significant effect of storage temperature on curcumin content was observed until 6 months, with the retention only of about 20 mg/g, or an approximate loss of 60% of the curcumin. The curcumin loss during storage seems to be common to other natural colourants. To retard the loss during the study, the turmeric powders were kept at −20 °C and the curcumin content was measured prior to performing each experiment.

2.9 The mechanisms of dye degradation

The first two decades of research on the photocatalytic degradation of dyes were characterized by the dominance of titanium dioxide, a UV active photocatalyst. The general degradation scheme of dyes by the UV-active titanium dioxide consisted of photon absorption by the photocatalyst, charge separation and the generation of active species on the surface of the photocatalyst. Generally speaking, the main active species under this mechanism are OH radicals formed by oxidation of water molecules by the photo-generated holes, hence the primary attack of the dye molecules is oxidative (Hustert and Zepp, 1992; Ojani, Raoof, and Zarei, 2012).

Evidence for direct oxidative attack by holes was also recorded (Hustert and Zepp, 1992). Likewise, irreversible reductive decolourization initiated by electrons or by superoxides formed on the photocatalyst's surface can be quite efficient, as was found for azo dyes (Vinodgopal, 1994).

Decolourization can take place also by a self-sensitization mechanism. Here, the light is absorbed by the dye molecule. Charge transfer from the excited dye molecule to the conduction band of the semiconductor results in the formation of an unstable dye cation radical and in parallel an active specie on the semiconductor surface that attacks the destabilized dye molecule (Rochkind, Pasternak, and Paz, 2015).

One of the first demonstrations of this mechanism, published as early as 1977, described highly efficient *N*-de-ethylation of rhodamine B adsorbed on cadmium sulphide. Likewise, the fact that de-colouring kinetics of methylene blue under solar light in the presence of (undoped) titanium dioxide was faster than de-colouring kinetics under UV light, was explained by this self-sensitization mechanism (Rochkind, Pasternak, and Paz, 2015).

2.9.1 The mechanism of curcuminoid degradation by UV light

Although the mechanism of photodegradation is yet to be elucidated, it is evident that the presence of phenolic hydroxyl groups or lack of them does not play any distinctive role in curcumin photo-degradation. Instead, it is more likely that the β-diketone moiety is involved in scavenging of the hydroxyl radical and redox reactions, thus forming smaller phenolic compounds (Tonnesen *et al*, 1986). Under the influence of light, curcumin acts as photo-sensitizer of oxygen radicals and undergoes self-sensitized decomposition (Tonnesen *et al*., 1986). According to Tonnesen *et al.*, (1986), ferulic aldehyde, ferulic acid, 4-vinylguaiacol, vanillin and vanilic acid are formed as a consequence of various photochemical reactions between curcumin and oxygen radicals (Figure 2.6).

In addition, other by-products such as benzaldehyde, cinnamaldehyde, 2'- hydroxy-5', 6'-benzochalcone, and unknown photoproducts were also identified from the photo-degradation of curcuminoids (Sundaryono, Nourmamode, Gardrat, Grelier, Bravic, Chasseau, and Castellan, 2003).

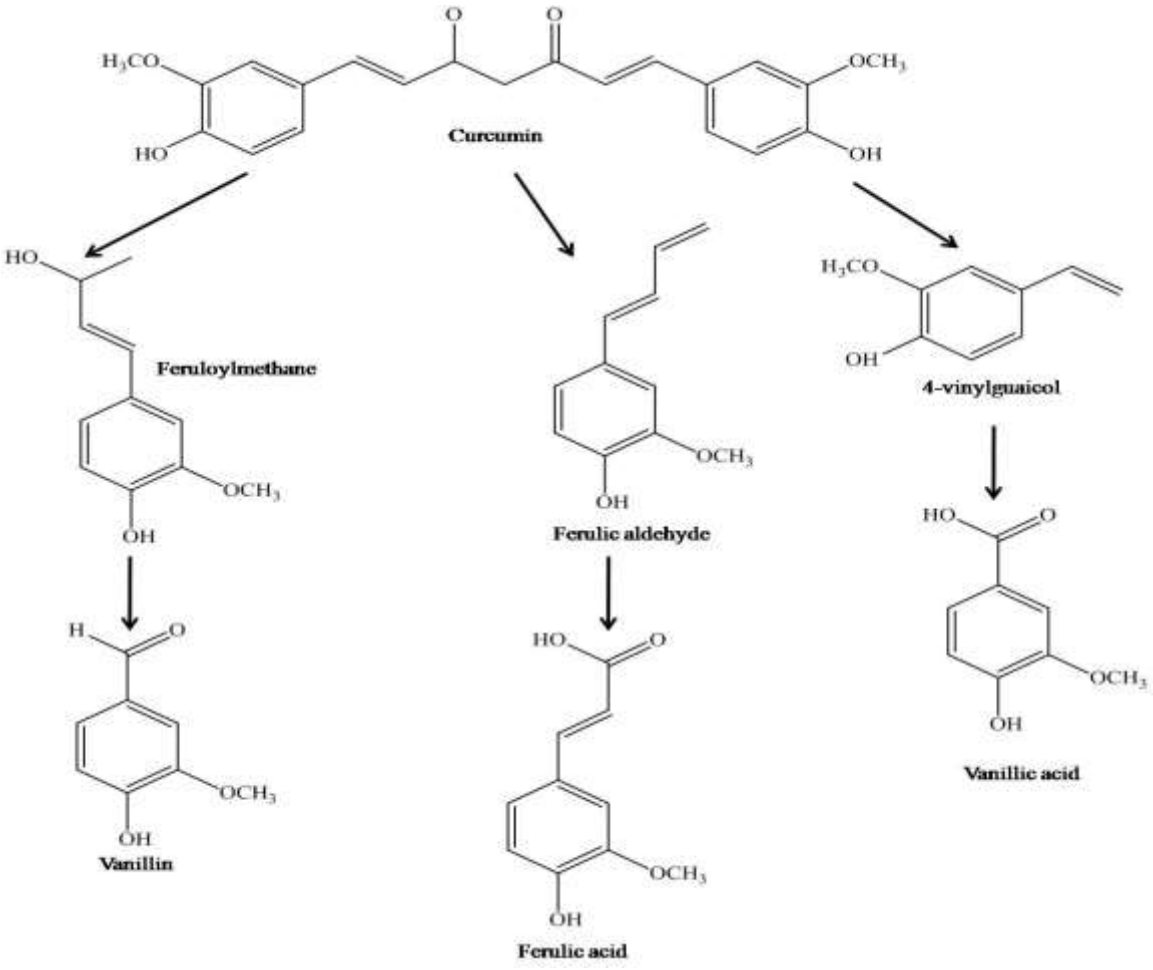


Figure 2.6: Photo-degradation products of curcumin (Tonnesen *et al*., 1986).

2.10 Curcuminoid stabilization strategies

Efforts have been made to achieve colour stability of curcumin (Wang, Zhaoxin, Fengxia, and Xiaomei, 2009); where attempts to prepare water-soluble curcumin by complex formation or interaction with various macromolecules (e.g. gelatine, polysaccharides) have been reported (Maing and Miller, 1981).

2.10.1 Use of cyclodextrin

Cyclodextrin is able to bind various kinds of low molecular organic compounds into its cave structure, thereby stabilizing these compounds by the inclusion effect (Loftsson, 1995). Curcumin and turmeric oleoresin have been encapsulated with β-cyclodextrin using suspension and co-crystallization techniques by Szente and co-workers (1998). Szente reported that β-cyclodextrin complexation provided shelf life improvement for curcumin and turmeric oleoresin (Soxhlet-extraction using *n*-hexane). However, it was shown that the degradation rate of turmeric increased compared to curcumin solution in organic solvent as the cavity size, charge and bulkiness of the cyclodextrin side chain influenced the stability of complexation and subsequent degradation rate of curcumin (Tonnesen, Masson, and Loftsson, 2002).

One alternative for improving curcumin stability is the microencapsulation technique, which entraps a sensitive ingredient inside a coating material. Encapsulation techniques have been widely used to reduce interactions of food components with environmental factors, such as temperature, light, moisture and oxygen (Kanakdande, Bhosale, and Singhal, 2007). Several studies undertaken dealt with encapsulated natural colourants and suggested that encapsulation significantly increased the shelf life of the core materials and prevent the degradation of some natural colourants (Serries and Biliaderis, 2001).

Microencapsulation provides a number of benefits – it protects oleoresin against destructive changes, converts it into a free-flowing powder, thereby delivering more flavour impact in finished products, separates reactive materials from one another, alters surface properties of the materials, controls the release of materials, reduces volatility or flammability of liquids and masks the bitter taste of certain compounds (Shaik, Bhosale, and Singhal, 2006).

Of the techniques available for microencapsulation, both spray drying and freeze-drying are well-established technologies in the food industry and are most commonly used (Wang *et al*., 2009).

Spray drying (Figure 2.7) has paved the way for the production of powder colourants with high storage stability, easier handling for some applications and minimized transportation weight in comparison with liquid concentrates (Dobry, Settell, Baumann, Ray, Graham, and Beyerinck, 2009). Some advantages of spray drying include the ability to quickly produce a dry powder (e.g., as compared to freeze-drying) and the ability to control the particle size distribution (Re, 1998).

The powder produced by spray drying may have some drawbacks in their properties, such as stickiness and high hygroscopicity due to presence of low molecular weight sugars and acids, which have low glass transition temperatures. This problem can be partly resolved by the addition of some carrier or encapsulating agents to the product before atomization. Such agents, besides increasing the glass transition temperature, can also protect sensitive ingredients against adverse conditions, increase their stability or even promote controlled release (Re, 1998).

Different types of encapsulating agents have been used for spray drying. These include polysaccharides (starches, maltodextrins, corn syrups and gum arabic), lipids (stearic acid, mono- and diglycerides) and proteins (gelatin, casein, milk serum, soy and wheat) (Szente and Szejtli, 1986; Shahidi and Pegg, 1991; Bhandari, Dumoulin, and Richard, 1992). Carbohydrates, such as starches and maltodextrins have properties that are desirable in an encapsulating agent such as low viscosity at high solids contents and good solubility. The advantage of using microencapsulated products is that their coating stays intact and therefore colours do not migrate as do conventional ones. In addition, their brightness is enhanced, thus increasing their marketability for commercial use (Dickinson, 2005).

The encapsulating agent, gum acacia, is a well-known natural plant polysaccharide, widely used and remains a respectable choice due to its stable emulsion formation and good retention of volatiles (Chranioti and Tzia, 2014). Gum acacia, arising from its compact structure, is also excellent for encapsulation of flavours as it emulsifies, has low viscosity, bland flavour and protects against flavour oxidation (Gharsallaoui, Roudaut, Chambin, Voilley, and Saurel, 2007). The excellent solubility in aqueous solution, low viscosity, bland taste, neutral odour, fibre source and low calorie of gum acacia has lent itself to many nutritional applications (Gabas, Telis, Sobral, and Telis-Romero, 2007). This is due to the hydrophobic nature of proteinaceous chains which absorb at the surface of the oil droplets, while the hydrophilic carbohydrate blocks attached to the protein chain protrude into aqueous solution preventing droplet aggregation and coalescence (Dickinson, 2009).

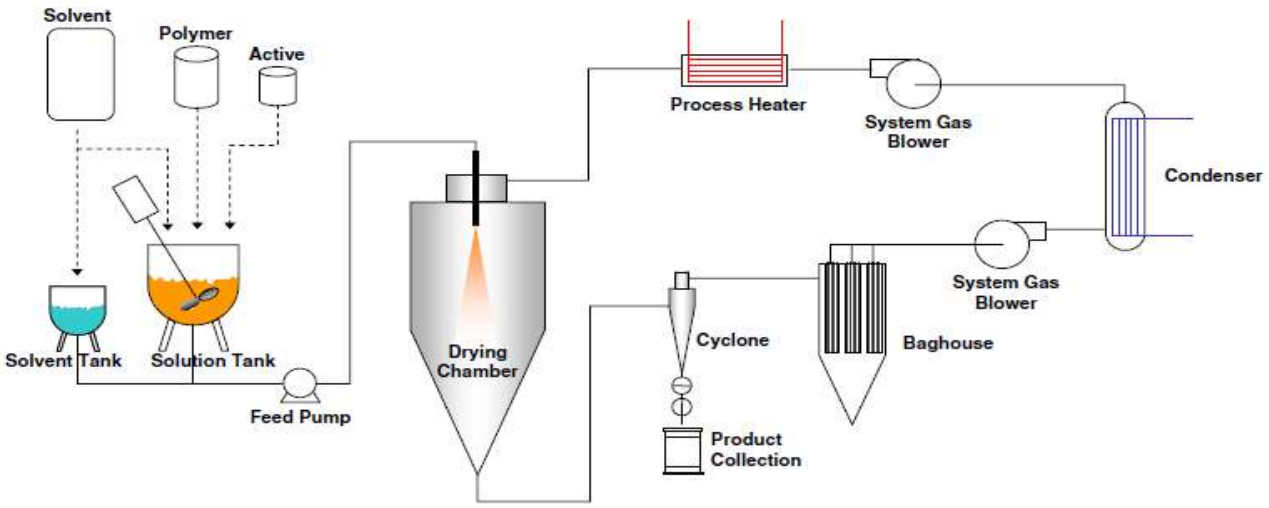


Figure 2.7: General spray drying equipment configuration (Dobry *et al.*, 2009)

Another polysaccharide used for encapsulation is maltodextrin, which is produced from starch by partial hydrolysis and is usually found as a creamy-white hygroscopic spray dried powder (Kilmartin, Reid, and Samson, 2004). Maltodextrin is easily digestible, is absorbed as rapidly as glucose and is either moderately sweet or almost flavourless. It is also water- soluble and protects encapsulated ingredients from oxidation (Shahidi and Han, 1993). Maltodextrins have low viscosity at high solids ratio and are available in different molecular weights, providing different wall densities around the sensitive materials (Cai and Lorke, 2000).

2.10.2 Use of antioxidants

In foods that may undergo oxidation, antioxidants, endogenous or exogenous, function as an inhibitor to oxidation reactions through various mechanisms (Eunok and Min, 2009). Antioxidants were found to protect lipids against oxidation by either quenching free radicals or scavenging oxygen, amongst others (Decker, 2002).

Although there are many compounds that have been proposed to inhibit oxidative deterioration processes, only a few can be used in food products. Antioxidants for use in food processing must be inexpensive, non-toxic, effective at low concentrations (0.001–0.02%), capable of surviving processing (carry through), stable in the finished products and must be devoid of undesirable colour, flavour, and odour effects (Jadhav, Nimbalkar, Kulkarni, and Madhavi, 1996).

Food antioxidants such as ascorbic and isoascorbic acids have been documented to enhance natural pigment stability (Attoe and von Elbe, 1982; Cai and Corke, 1999; Herbach, Rohe, Stintzing, and Carle, 2006). With anthocyanins, ascorbic acid has a protective effect, e.g. when it absorbs available oxygen and thus prevents oxidation of the anthocyanin (Attoe and von Elbe, 1982). Additionally, ascorbic acid can produce dehydroascorbic acid and hydrogen peroxide when oxidized. The hydrogen peroxide may then degrade anthocyanin into malvones, a colourless pigment (Herbach, Rohe, Stintzing, and Carle, 2006).

There are also discrepancies regarding the replacement of ascorbic with isoascorbic acid. Some studies reported that isoascorbic acid had a better retention effect on natural pigments like betanin than ascorbic acid (Bilyk and Howard, 1982; Attoe and von Elbe, 1985; Garcia Barrera *et al*., 1998), while results by Herbach *et al*. (2006) indicated a higher pigment retention with ascorbic than with isoascorbic acid (Herbach *et al*., 2006). The structure of ascorbic acid is shown in Figure 2.8 below.

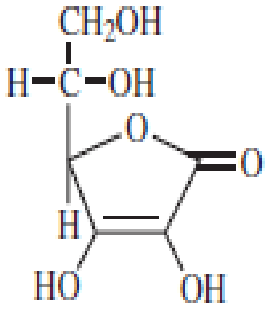
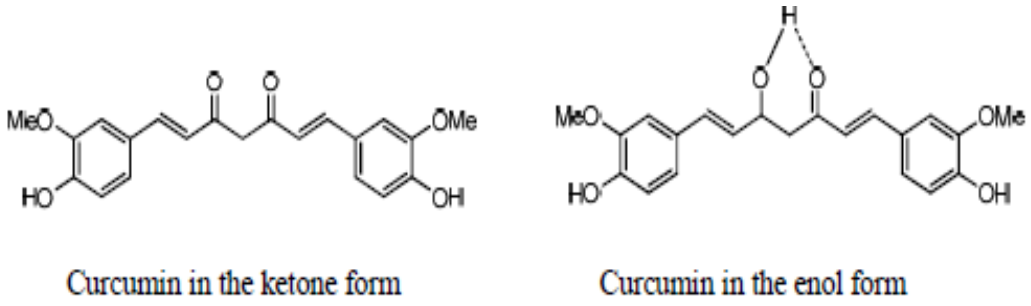


Figure 2.8: Chemical structure of ascorbic acid (Shahidi and Zhong, 2005)

2.10.3 Heavy metal complexation of curcuminoids with cations

Metal bound organic compounds are known to possess potential activities in the areas of biological, clinical, analytical, catalytic, microbial, insecticidal, antibiotic, growth factors, food additive, tumour inhibitor and cell division, amongst others (Subhan, Alam, Rahaman, Rahman, and Awal, 2014). This is due to either the unused coordination sites present on the metal and ligand systems, or due to the selective oxidation state of the complexed metal ions in the coordination sphere (Figure 2.9) (Cotton and Wilkinson, 1966). The usefulness of metal chelates in various branches of theoretical and applied chemistry is now generally recognized. These reagents which form metal chelates are used extensively in both qualitative and quantitative analyses (Subhan *et al*., 2014).



Curcumin in the ketone form Curcumin in the enol form

Figure 2.9: Structure of curcumin in ketone and enol forms (Subhan *et al*., 2014)

Curcumin-metal complex synthesis has opened a new path for stabilization of active ingredients in the food and pharmaceutical industry. Complexation of curcumin with transition metals has been conducted in the past and has attracted much interest over the past years as one of the useful requirements for the treatment of Alzheimer's disease and in vitro antioxidant activity (Subhan *et al*., 2014). Moreover, several metallo-complexes of curcumin have been synthesized, characterized and evaluated for various biological activities (Song, Xu, Ding, Hou, Liu, and Zhu, 2009).

2.11 Analytical methods for curcuminoids

Qualitative and quantitative analyses of curcuminoids in turmeric samples are very important in order to determine the quality of the raw materials or its finished products (Jiang, Somogyi, Jacobsen, Timmermann, and Gang, 2006).

A variety of methods for quantification of the curcuminoids were reported (Jayaprakasha, Rao, and Sakariah, 2002). Most of these are spectrophotometric methods, expressing the total colour content of the sample. Commercially obtained *Curcuma* products contain mixtures of curcumin, de-methoxycurcumin and *bis*-demethoxycurcumin. For an exact determination of the curcumin content, a pre-separation of the three curcuminoids is essential. The curcuminoids isolated from C. *longa* exhibit strong absorption between 420 - 430 nm in organic solvents (Adinew, 2012).

The official methods for assaying curcumin or *curcuma* products as food colour additives are based upon direct spectrophotometric absorption measurements. The evaluation of the total amount of curcuminoids in a sample by use of direct absorption measurements is only valid if the calculations are based on reference values obtained from pure standards. However, it should

be noted that the presence of other compounds absorbing in the region of 420-430 nm influence the results strongly (Karasz, DeCocca, and Bokus, 1973).

The analysis of curcuminoids in food and pharmaceutical products is very important, not only for quality control aspects, but also for ensuring the efficacy and effectiveness of curcuminoids as active compounds in several pharmaceutical dosage forms and functional food preparations. Spectroscopy, chromatography, and electrophoresis-based methods are analytical techniques which are continuously developed for quantification of curcuminoids. In the future, the use of instruments capable of providing on-site application, fast, reliable and inexpensive is highly needed (Rohman, 2012).

Food industry and regulatory authorities require reliable validated techniques for determination of curcuminoids for the scope of the various range of food products stated in the European Colour Directive (Scotter, 2009). For instance, curcumin is allowed to be used in smoked fish with maximum limit of 100 mg/kg. From the perspective of regulatory compliance, it is necessary to determine the levels of curcumin in certain foods (Rohman, 2012). Numerous analytical methods have been reported for quantitative analysis of curcuminoids. Some of the methods are spectrophotometric-based techniques, expressed as the total colour content of the sample (Jayaprakasha, Rao, and Sakariah, 2002).

However, using this technique, it is not possible to separate and to quantify the curcuminoids individually (Jayaprakasha, Rao, and Sakariah, 2002). For this reason, chromatographic-based techniques and electrophoresis are among the methods of choice for determination of curcuminoids attributed to their separation capacities (Rohman, 2012).

2.11.1 Spectrophotometric techniques

2.11.1.1 UV-Vis spectrophotometry

UV-VIS spectrophotometry relies on the direct measurement of a sample in certain solvents (Jayaprakasha *et al.*, 2002). In some organic solvents, curcuminoids show the intensive absorption intensity at wavelength of 420 – 430 nm (Rohman, 2012). However, it should be taken into account that the presence of other species having the chromophoric groups absorbing at this wavelength will influence the accuracy of the results (Jayaprakasha *et al.*, 2002). The results obtained from quantification of curcuminoids using UV-visible spectrometry are usually

expressed as the total curcuminoids content (Rohman, 2012). In 2006, Pothitirat and Gritsanapan determined the curcuminoid content in *C. longa,* which was obtained from 13 regions in Thailand and measured at 420 nm. The calibration curve was made by weighing 2.00 mg curcumin (purity 60–70%), dissolving in methanol and adjusted to a final concentration of 0.8, 1.6, 2.0, 2.4 and 3.2 mg/ ml.

2.11.1.2 Chromatography-based methods

Chromatography-based methods are analytical techniques in chemical analyses which are appropriate for qualitative and quantitative determination of a wide variety of compounds. Besides, these techniques also offer the separation capacities of analytes of interest into their constituent components (Cserháti, Forgács, Deyl, and Miksik, 2005).

Due to its associated advantages such as low cost in operation, ease in sample preparation and the availability of several detection systems, thin-layer chromatography (TLC) was regularly used for the identification, separation, quantification or semi-quantification of natural pigments, including curcuminoids (Forgacs and Cserhati, 2002). However, high-performance liquid chromatography (HPLC) is a method of choice for curcuminoids due to the high precision and accuracy offered and low detection limit achieved. Furthermore, in order to improve the separation power, multi-development in TLC and gradient elution in HPLC are the preferred methods for analysis of samples. Capillary electrophoresis was developed as an optional technique for the analysis of curcuminoids (Sun, Yang, and Wang, 2005).

Because of their low volatility and thermally labile properties, curcuminoids do not lend themselves to determination using gas chromatography and related techniques. Therefore, several methods including HPLC and its coupling with mass spectrometry (LC/MS) and capillary electrophoresis (CE) have been developed for determination of curcuminoids in foods or in pharmaceutical products (Jiang *et al*., 2006).

HPLC is the most reported method for analysis of curcuminoids due to its versatility and ease in use (Jiang *et al*., 2006; Jadhav *et al*., 2006). In most cases, HPLC methods using detectors such as UV/VIS spectrophotometer or photodiode-array detector (PDA) at wavelengths of around 260 or 450 nm have been used since these techniques involve simple instrumentation and are sufficiently capable of determining curcuminoids in some products (Jadhav *et al*., 2006).

2.12 Methods for colour measurement

Human colour vision has been found to be trichromatic, which means a single perceived colour may be regarded as resulting from the effect of three separate stimuli on the visual cortex (Weatherall and Coombs, 1992). These tristimulus values can be converted to CIELAB colour space values (Weatherall and Coombs, 1992). The tristimulus colourimeter hence measures the colour of reflected light and provides a digital output of chromaticity in CIE L^*, a^* and b^* coordinates (Hunter in 1975, according to Madeira, Ferreira, Varennes, and Viera, 2003).

L^* is a measure of lightness which is the reflectance factor given as a percentage (Weatherall and Coombs, 1992). The lightness index L^* ranges from no reflection ($L^* = 0$, black) to perfect diffuse reflection ($L^* = 100$, white). The coordinates a^* and b^* can take positive and negative values: on the horizontal axis, $+a^*$ and $-a^*$ indicate hues of, respectively, red and green; and, on the vertical axis, +b* and -b* represent hues of, respectively, yellow and blue (Figure 2.10). As a^* and/or b^* increases, chromaticity increases. Numerical values of a* and b* are converted into the saturation index or C ($C = (a^{*2} + b^{*2})^{1/2}$), a measure of chromaticity and the hue angle ($H° = \arctan b^*/a^*$) (Voss, 1992).

H° indicates the colour of the material surface and is an angle in a colour wheel of 360°, with 0, 90, 180 and 270° representing the hues of red, yellow, green and blue, respectively. Together, L*, H° and C give an accurate description of the colour of a sample (Madeira *et al*., 2003).

In 2014, Hirun and co-workers investigated the effect of microwave power (2,400–4,000 W) and drying times (10–30 min) on the quality of dried turmeric in terms of colour (L^*, a^*, b^*). From their studies, it was evidenced that increasing drying time from 10 min to 30 min significantly increased the L^* value of dried turmeric. In addition, the results suggested that products might remain brighter in colour when increasing microwave-vacuum power to up to 4000 W (Hirun, Utama-ang, and Roach, 2014).

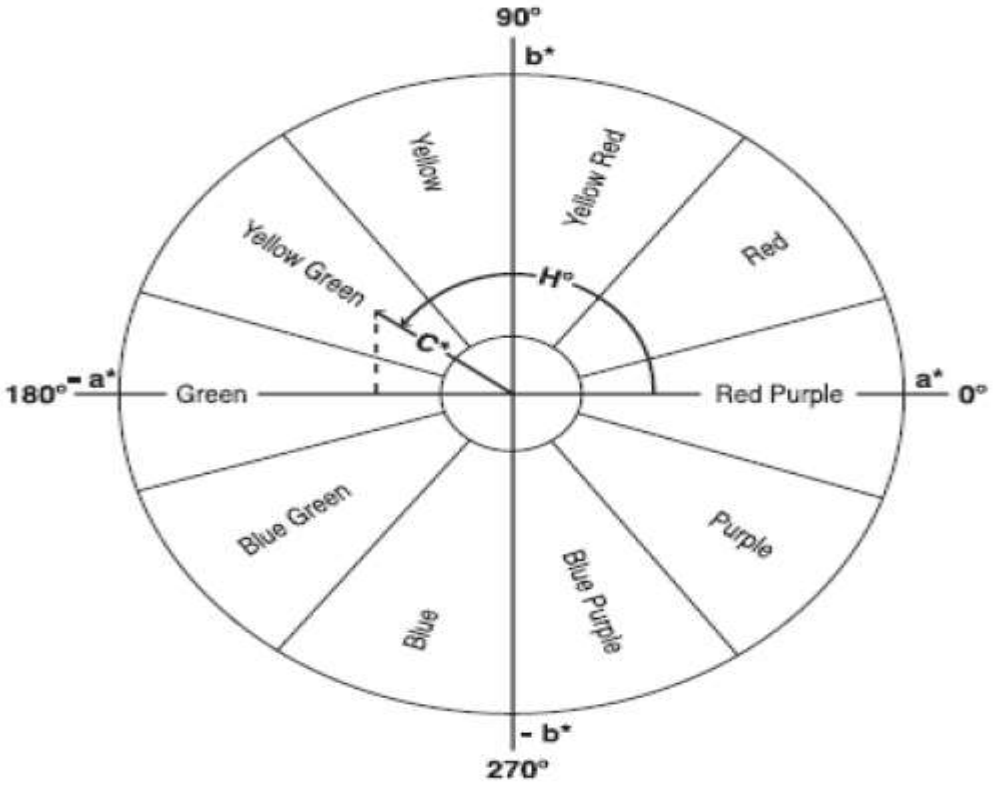


Figure 2.10: Representation of the hue angle and saturation index on a* and b* colour space diagram (Madeira *et al*., 2003)

2.13 Sensory evaluation of foods

The role of sensory evaluation has changed considerably over the years. Initially, it was a service provider supplying data, but now its role is, in partnership with R&D and marketing, to provide insights to help guide development and commercial strategy (Kemp *et al*., 2009).

Human senses, in particular the chemical senses, are tuned to act as composite gatekeepers for food intake. This biological function protects us from eating spoiled or otherwise unfit items and encourages eating nutritious or otherwise beneficial items (Breslin and Spector, 2008).

The consequences of this function to food production and marketing are extensive. No food or beverage is worth producing, distributing or marketing without at least an approximate idea that its sensory quality is accepted by consumers. Thus, sensory evaluation or appraisal of products has long been incorporated into the quality control of commercially noteworthy foods and beverages, such as dairy and wine (Pangborn, 1989). For the same reason, modern product

development and competition within food industry require clear understanding of sensory aspects of foods and adequate sensory techniques (Tuorila and Monteleone, 2009).

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone and Sidel, 1993). This definition has been accepted and endorsed by sensory evaluation committees within various professional organizations such as the Institute of Food Technologists and the American Society for Testing and Materials. The field of sensory evaluation has grown rapidly in the second half of the 20th century. Nowadays, sensory evaluation becomes a tool irreplaceable in food industry while interacting with the key sectors in food production (Tuorila and Monteleone, 2009).

When a consumer buys a food product, they can buy nutrition, convenience and image. Nevertheless, most importantly consumers are buying sensory properties/performance and sensory consistency. Therefore, sensory evaluation should be an integral part in defining and controlling product quality. Every company committed to quality should support, develop and operate a QC/sensory program (Tuorila and Monteleone, 2009).

Sensory attributes, whether the flavour of coffee, the smell of an air freshener, the texture of fabric or even the sound of a car door closing, are key determinants of product delivery including quality, functional and emotional benefits. Thus, a considerable proportion of product failure can be attributed to a mismatch between sensory properties and consumer needs or expectations. When integrated within the product development process, sensory and consumer testing allows cost-effective delivery of acceptable products to consumers and thus reduces the risk of failure (Lawless and Heymann, 1998).

In 2012, Manoharan and co-workers investigated the acceptable level of curcumin as a natural colouring agent in ice cream and also assessed the sensory score of the resultant product. In their study, curcumin powder was incorporated at different levels in butterscotch flavoured ice cream. Prepared ice cream was then subjected to sensory analysis and the optimal level of inclusion of curcumin powder in the ice cream preparation was determined (Manoharan, Ramasamy, Dhanalashmi, Gnanalashmi, and Thyagarajan, 2012).

2.14 Concluding Remarks

Natural colourants from plant sources are receiving growing interest from both food manufacturers and consumers in the continuous replacement of synthetic dyes (Stintzing and Carle, 2004; Francis, 1989).

However, replacing synthetic dyes with natural colourants offers a challenge because the colour and stability of plant pigments are dependent on several factors, which include structure and concentration of the pigment, pH, temperature, light intensity, presence of metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products (Ali *et al.*, 2006).

Visual colour and pigment degradation kinetics of food products are complex phenomena and dependable models to predict experimental colour change. The kinetic parameters, namely rate constant and activation energy, provide useful information on the quality changes which occur during thermal processing. Several studies have reported on quality aspects of turmeric, based mainly on curcumin loss during thermal processing and under alkaline conditions (Ahmed and Sandu, 2002).

However, little information is available on the pigment and visual colour degradation kinetics of curcuminoid pigments during exposure to sunlight. Visual colour measurement has been accepted by the processing industries as an on-line quality control technique. However, measurement of pigment concentration could quantify the actual colour degradation during processing. Hence, there is an urgent need to establish correlation between pigment concentration and the visual colour of food products during processing (Ahmed and Sandu, 2002).

Therefore, this research is an attempt to quantify and investigate the effect of external environmental conditions, including sunlight, on the stability of curcuminoids in turmeric oleoresin powders, as well as to investigate the effect of curcuminoid pigment degradation on the colour of turmeric oleoresin powders. This work also attempts to explore methodologies such as the addition of antioxidants and metal chelation that may stabilize curcuminoids, thus enhancing the application of natural colourants in the food industry.

CHAPTER 3

3 Hypotheses and Objectives

3.1 Hypotheses

Hypothesis 1

The rate of degradation of curcuminoid pigments in turmeric oleoresin powders will be greater when exposed to external environmental conditions compared to control samples that are protected with aluminium foil against light, and the degradation rate will increase with increasing time of storage. Photo-degradation is a phenomenon that affects natural colourants exposed to sunlight. In the presence of radiation, curcumin breaks down because of its unstable keto-enol structure into various non-coloured compounds and as a result, the turmeric oleoresin assumes a discoloured or faded appearance (Schnabel, 1981). The effect of discolouration increases with exposure time (Purseglove, Brown, Green, and Robbins, 1998).

Hypothesis 2

The synthetic antioxidant additive, tertiary butylhydroquinone (TBHQ), will enhance the stability of curcuminoid pigments in turmeric oleoresin powders to a greater extent compared to ascorbic acid when exposed to external environmental conditions over time. TBHQ is a hydroxyl substituted phenol, and as such, acts as a radical scavenger by donating hydrogen atoms, thereby forming more stable phenoxy radicals that do not contribute to the lipid oxidation mechanism (Allam and Mohamed, 2002). TBHQ is more stable to heat than ascorbic acid, which can be easily destroyed during processing as a result of susceptibility to heat and light (Chung, Lee, and Choe, 2004).

Hypothesis 3

The stability of curcuminoid pigments in turmeric oleoresin powders will be enhanced by its complexation with the divalent ion, Mg^{2+} when exposed to external environmental conditions compared to control samples that are not complexed with Mg^{2+}. Curcumin forms strong complexes with most metal ions. The α, β-unsaturated β-diketo moiety of curcumin makes it an excellent chelating agent (Priyadarsini, 2014). Metal coordination of curcumin occurs through the enolic group, where the enolic proton is replaced by the metal ion and the o-methoxy phenolic moiety remains intact in the complexes. The metal ion is able to promote the dissociation of the

enolic proton and the formed complex remains stable in a wide range of pH, confirming the ability of these ligands to act as metal chelators (Ferrari, Benassi, Sacchi, Pignedoli, Asti, and Saladini, 2014).

3.2 Objectives

To determine the effect of exposure to external environmental conditions on the stability of curcuminoid pigments in turmeric oleoresin powders as a function of time, with the aim of measuring the rate of curcuminoid pigment degradation.

To determine the effect of antioxidants (TBHQ and ascorbic acid) on the stability (shelf life) of curcuminoid pigments in turmeric oleoresin powders when exposed to external environmental conditions, with the aim of increasing pigment stability in order to enhance their application in the food industry.

To determine the effect of Mg^{2+}- curcuminoid complexation on the stability of curcuminoid pigments in turmeric oleoresin powders when exposed to external environmental conditions, with the aim of increasing pigment stability in order to enhance their application in the food industry.

CHAPTER 4

4 Experimental design

The main aim of this research was to determine the stability of curcuminoid (*Curcuma longa* L.) pigments in turmeric oleoresin powders using physico-chemical and sensory analyses.

Figure 4.1 shows the experimental design for the research project.

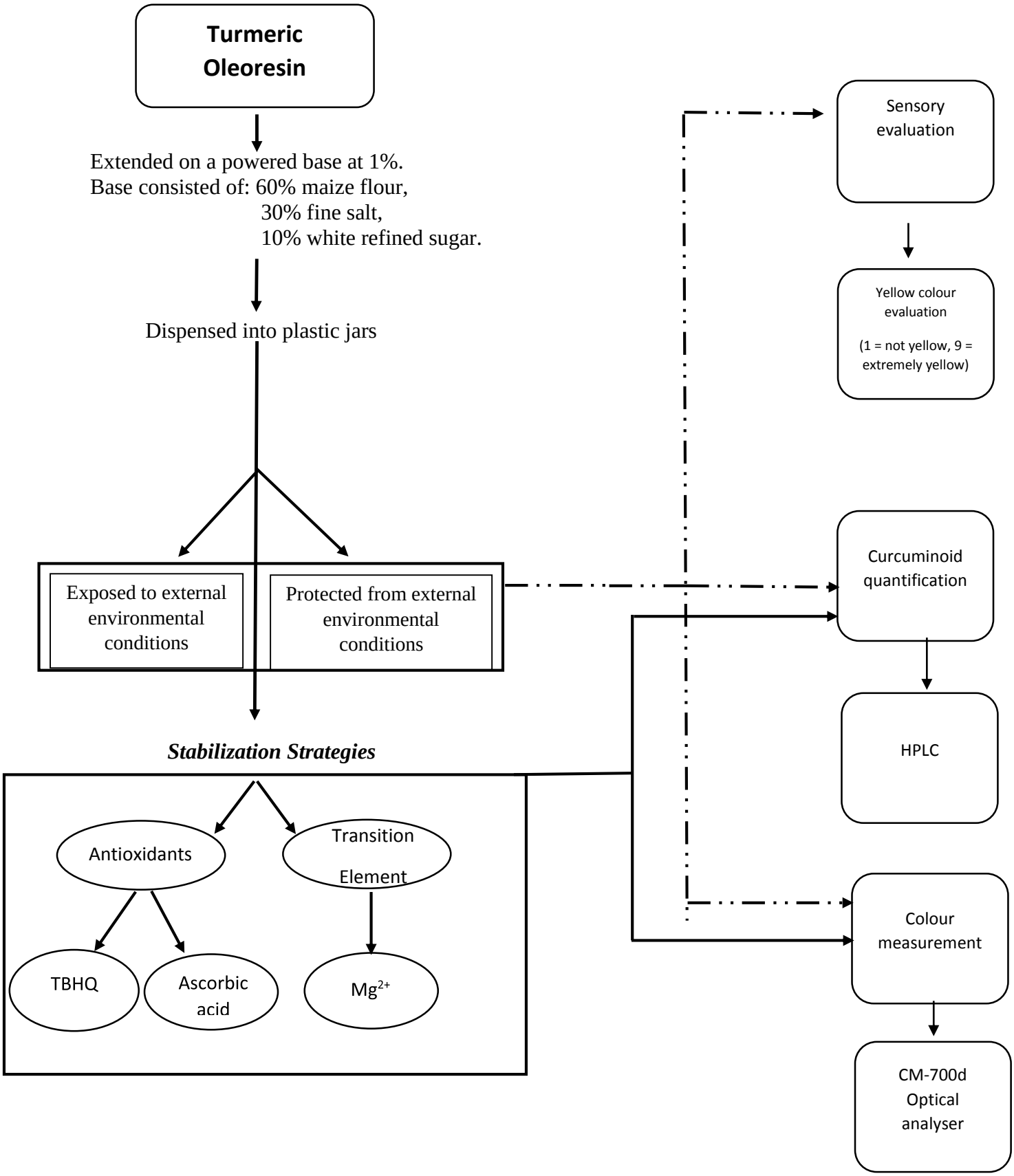


Figure 4.1: Schematic representation of experimental design used for relating the physico-chemical properties of curcuminoid (*Curcuma longa* L.) pigments with sensory quality

4.2 Materials and methods

4.2.1 Reagents, solvents and core materials: All reagents and solvents used in this study were of analytical or HPLC grade. Filtered water was obtained from a Milli-Q Ultrapure water purification system (0.22 µm) (Merck Millipore), and was used throughout the study. All reference stock solutions were stored at 4 °C. Diluted working solutions were freshly prepared for all HPLC analyses. Core material was turmeric oleoresin (Plant Lipids (Pty) Ltd, India). TBHQ and ascorbic acid were procured from Sigma Aldrich, SA. Plastic jars were procured from Polyweld Plastics, Durban, South Africa.

4.2.2 Preparation of experimental samples: Turmeric oleoresin (8% purity) was homogenously mixed into a powdered base at a concentration of 0.2% (m/m). Homogeneity of samples were assessed visually. The powdered base was developed to mimic the matrix of a soup and consisted of 60% maize flour, 30% fine salt and 10% white, refined sugar all on % (m/m) basis. Experimental samples were exposed to external environmental conditions (sunlight and outside environmental temperature). Average daytime temperature of the surroundings was ± 24 °C. All samples were prepared in duplicate and subjected to the conditions described below. Control samples were included in each experiment.

- **Samples exposed to external environmental conditions:** 200 g samples were placed in 60 mm diameter clear labelled plastic jars and exposed to external environmental conditions for a period of 10 weeks. Control samples were stored in a refrigerator at 4 °C in the dark. Samples were monitored over a 10 week period for total curcuminoid content and visual colour.

- **Samples protected from external environmental conditions:** 200 g samples were placed in labelled plastic jars. The jars were covered with aluminium foil and exposed to external environmental conditions for a period of 10 weeks. Average daytime temperature of the surroundings was ± 24 °C. Control samples were refrigerated at 4 °C in the dark. Samples were monitored over a 10 week period for total curcuminoid content and visual colour.

- **Samples treated with antioxidants:**

  The curcuminoids and stabilizing agents mentioned below were interacted by mixing of solid powders only. Solvent systems for metal complexation were not used. This is because practical food processing conditions were being simulated.

  - **TBHQ**: TBHQ was homogenously distributed into the powdered turmeric oleoresin powdered base at 0.02% (m/m). 200 g of these samples were placed in labelled plastic jars. Samples were exposed to external environmental conditions for a period of 10 weeks. Control samples did not contain any antioxidants.

  - **Ascorbic acid**: Ascorbic acid was homogenously distributed into the powdered turmeric oleoresin powdered base at 0.02% (m/m). 200 g of these samples were placed in labelled plastic jars. Samples were exposed to external environmental conditions for a period of 10 weeks. Control samples did not contain any antioxidants.

- **Metal complexation**: Anhydrous magnesium sulphate ($MgSO_4$; 62-70%) was mechanically mixed with curcumin at a molar ratio of 1:1 until a homogenous powder was obtained. 200 g samples were placed in labelled plastic jars and exposed to external environmental conditions. Control samples did not contain metal complexes.

4.3 Physico-chemical analyses

4.3.1 Quantification of curcuminoid pigments in turmeric oleoresin

4.3.1.1 Instrumentation: An Agilent 1200 Series HPLC system equipped with an SL 1 binary pump, auto sampler and diode array detector was used in this study. Data acquisition and analysis was performed using Agilent Lab Advisor software (version B.01.01, November 2008).

Table 4.1: Linear gradient parameters used for HPLC

Time (minutes)	% Solvent A	% Solvent B
0	60	40
8	40	60
9	20	80
10	60	40
End time = 11 minutes, 2 minutes post time.		

4.3.1.2 Curcuminoid extraction: Curcuminoid constituents were extracted from 2 g of powdered base with 10 mL methanol. Samples were vortexed for 10 s and sonicated in an ultrasonic water bath for 10 min (heated at 25 °C with a power level of 5). 2 ml of this solution was placed in a micro-centrifuge tube and centrifuged for 5 min. Samples were placed in amber auto sampler vials and subjected to HPLC analysis. The percentage curcuminoid degradation was calculated from the following equation,

$$\% \text{ Degradation} = \frac{\text{Final curcuminoid concentration} - \text{initial curcuminoid concentration}}{\text{Initial curcuminoid concentration}} \times 100$$

4.3.1.3 HPLC method development: A Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm) was used at 45.0 ^{o}C. The optimized mobile phase consisted of phosphoric acid (pH 1.8) (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL min^{-1} and delivered in a linear gradient as shown in Table 4.1. The mobile phase was filtered through a Pall SolVac filter system using white cellulose filters (0.45 µm, 47 mm) before use. The elution was monitored at 425 nm. An injection volume of 1 µl was used in the study. Peak areas were used for quantitative curcuminoid calculations. To determine the concentration of curcuminoids, the peak area was plotted versus the curcuminoid concentration.

4.3.1.4 Specificity, precision and accuracy: The specificity of the method was ascertained by analysing the curcuminoid standards and the samples. To examine precision of the method, six injections at three different concentration of curcuminoids (100, 500, 1000 ppm) prepared in methanol was made. Accuracy of the method was determined by recovery experiments. The recovery of the method was determined at a single level by adding a known quantity of

curcuminoids to food products and the mixtures were analysed according to the developed HPLC method.

4.3.2 Measurement of visual colour: Colour was measured using a CM-700d optical sensor (Konika Minolta Sensing Inc., Japan), and expressed in terms of "L^*" (lightness), and "b^*" (yellowness and blueness), hue angle (H°) and chroma (C) (Maruatona *et al*., 2010); where H° = $\tan^{-1}(b^*/a^*)$ and $C = (a^2 + b^2)^{0.5}$ (Mohammadi *et al*., 2008). The instrument (45°/0° geometry, 10° observer) was calibrated with a standard white tile (L = 90.55, a = -0.71, b = 0.39). A glass Petri dish containing the powdered sample was placed above the light source, and covered with a white plate. Post-process Hunter L^*, a^*, and b^* values were recorded (Ahmed *et al*., 2002).

4.4 Sensory evaluation

4.4.1 Rating of colour intensity: Samples were prepared as described in section 4.2.2. Rating of colour intensity was conducted on turmeric oleoresin powders stored under the following conditions:

I. Samples exposed to external environmental conditions;
II. Samples protected from external environmental conditions

Sensory evaluation was carried out in Kerry SA sensory evaluation laboratory. A panel of six (3 female and 3 male) trained, non-smoking, self-confessed healthy individuals participated in the evaluation. The evaluation was performed by panellists seated at individual evaluation booths under white light. Samples (20 g) were prepared out of sight of the assessors and in an identical manner and were presented at room temperature (±25 °C) in 85 mm diameter white, round-shaped porcelain vessels. The trained panellists assessed the samples in duplicate over two evaluation sessions. Each sample was identified by a randomly selected 3-digit code. Assessors were presented with the samples in a randomised order and asked to rate the yellow colour intensity on a category scale of 1 to 9 (1= not yellow, 9 = extremely yellow). A Pantone colour swatch book (4^{th} edition, Pantone, Inc., 2006) (Figure 4.2) was provided to assist assessors in the rating process. Pantone 102 C was linked to 9 on the category scale. These swatches were used as a reference guide only and did not necessarily represent the optimum level for the descriptor. All assessors worked under the same test conditions and were asked to evaluate the samples and rate them for the designated attribute.

Figure 4.2: Pantone formula guide swatch book (4th edition, Pantone, Inc., 2006) that was used in the sensory evaluation

4.5 Statistical analyses: To determine the effect of time of storage on curcuminoid degradation of samples stored either exposed to external environmental conditions, protected from external environmental condition or at 4 °C in the dark, separate one-way analyses of variance (ANOVA) were conducted. Separate one way ANOVA was also conducted per treatment (with and without protection to external environmental condition) to determine the effect of external environmental condition on the colour of turmeric oleoresin powders.

To determine the effect of antioxidants and metal complexation on the stability of turmeric oleoresin powders after exposure to external environmental conditions, a two-way ANOVA analysis with interactions was conducted per treatment.

To determine the effect of time of storage on colour (L^*, a^*, b^*, C, H°) degradation of turmeric oleoresin samples stored either exposed or protected from external environmental conditions, a separate one-way ANOVA was conducted per treatment. A two-way ANOVA (without interaction) was conducted in order to test the effect of each treatment and time on L^*, a^*, and b^* colour values.

Correlation analysis was conducted to investigate the relationship between sensory and physico-chemical data. The dependent variable was % curcuminoid degradation (denoted by "*y*") and the independent variable was sensory colour rating scores (denoted by "*x*"). The Pearson correlation test was used to determine whether a significant ($p<0.05$) positive or negative correlation existed between two parameters.

For all tests, where significant differences ($p<0.05$) was noted by ANOVA, the least significant difference (LSD) test was performed to compare means. All data, including regression and correlation analyses was analysed using the statistical programme XLSTAT, version 2015 (Addinsoft, New York, USA).

CHAPTER 5

5 Results

5.1 Curcuminoid degradation profiles

5.1.1 Effect of external environmental conditions on degradation of curcuminoids during storage of turmeric oleoresin powders over a 10 week period

Figure 5.1 shows the effect of exposure to external environmental conditions, refrigeration at 4ºC and covering with aluminium foil on degradation of curcuminoids during storage of turmeric oleoresin powders over a 10 week storage period. Figure 5.2 shows the appearance of turmeric oleoresin powders exposed to external environmental conditions over a 10 week storage period while Figure 5.3 shows the appearance of turmeric oleoresin powders covered with aluminium foil and exposed to external environmental conditions over a 10 week storage period. One-way ANOVA was conducted per treatment in order to determine the effect of time of storage on curcuminoid degradation of samples. Results as shown in Figures 5.1, 5.2 and 5.3 reveal that exposure to external environmental conditions and the higher temperature of storage had a negative impact on the stability of curcuminoid pigments in turmeric oleoresin powders. Figure 5.4 shows the HPLC chromatogram of turmeric oleoresin powder (0.2%) at day 0.

During the first week of storage, the % degraded curcuminoid in turmeric oleoresin powders exposed to external environmental conditions increased significantly, with further significant increases in degradation by week 3 and week 9 (Figure 5.1). Maximum % degradation of curcuminoids occurred after four weeks of storage. These results were supported by visual sensory analysis of the powdered turmeric oleoresin samples (photographs shown in Figure 5.2).

Curcuminoids were less sensitive to degradation when turmeric oleoresin powders were shielded from external environmental conditions (covered with aluminium foil), with a 22% reduction in degradation observed relative to samples without aluminium foil protection after 10 weeks (Figures 5.1, 5.2 and 5.3). However, the level of curcuminoid degradation was significantly higher in all weeks of analysis compared to samples that were refrigerated at 4 °C. Figure 5.5 shows the HPLC chromatogram of turmeric oleoresin powder covered with aluminium foil and exposed to external environmental conditions after 10 weeks, whilst Figure 5.6 shows the HPLC chromatogram of turmeric oleoresin powder after 10 weeks of refrigeration. Percentage

curcuminoid degradation of samples exposed to external environmental conditions after 9 and 10 weeks were statistically similar ($p>0.05$).

Turmeric oleoresin samples refrigerated at 4 °C showed less curcuminoid degradation compared to samples that were covered with aluminium foil and exposed to external environmental conditions, with a four times lower percentage degradation observed after 10 weeks for samples that were refrigerated at 4 °C (Figure 5.1). For turmeric oleoresin powders that were covered in foil and exposed to external environmental conditions, a 70% increase in curcuminoid pigment degradation was observed between weeks 1 and 10, with 8% and 78% degradation occurring in those weeks, respectively. Curcuminoid degradation in refrigerated samples were statistically similar ($p>0.05$) in weeks 7 and 8 of the experiment.

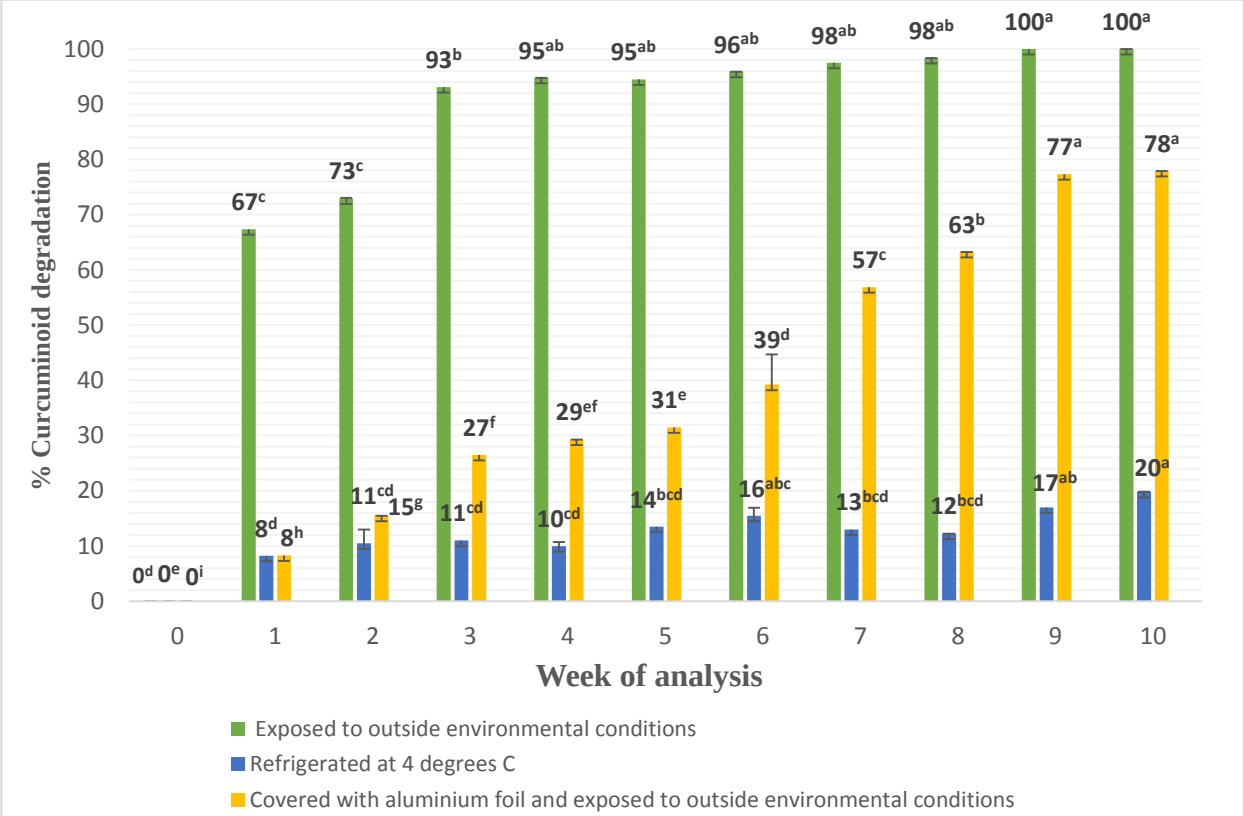


Figure 5.1: Percentage degradation of curcuminoid pigments in turmeric oleoresin powders exposed to and protected from external environmental conditions over a ten week period

[a,b,c,d,e,f,g,h,i] Per treatment, values with different superscripts differ significantly at $p<0.001$

Week 0: 0%[d] Degradation | **Week 1: 67%[c] Degradation** | **Week 2: 73%[c] Degradation**

Week 3: 93%[b] Degradation | **Week 4: 95%[ab] Degradation** | **Week 5: 95%[ab] Degradation**

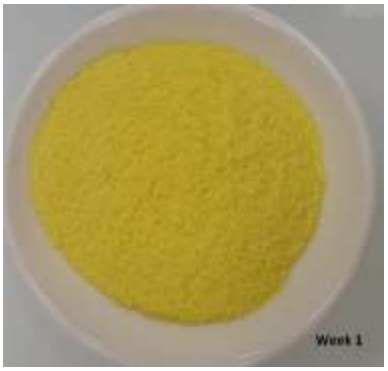
Week 6: 96%[ab] Degradation | **Week 7: 98%[ab] Degradation** | **Week 8: 98%[ab] Degradation**

Week 9: 100%[a] Degradation | **Week 10: 100%[a] Degradation**

Figure 5.2: Photographs of turmeric oleoresin powders exposed to external environmental conditions over a ten week period and weekly percentage curcuminoid degradation

[a,b,c,d]Values with different superscripts differ significantly at $p<0.001$

Week 0: 0%^{i} Degradation



Week 1: 8%^{h} Degradation

Week 2: 15%^{g} Degradation

Week 3: 27%^{f} Degradation

Week 4: 29%^{ef} Degradation

Week 5: 32%^{e} Degradation

Week 6: 39%^{d} Degradation

Week 7: 57%^{c} Degradation

Week 8: 63%^{b} Degradation

Week 9: 77%^{a} Degradation

Week 10: 78%^{a} Degradation

Figure 5.3: Photographs of turmeric oleoresin powders covered with aluminium foil and exposed to external environmental conditions over a ten week period and weekly percentage curcuminoid degradation

^{a,b,c,d,e,f,g,h,i}Values with different superscripts differ significantly at $p<0.001$

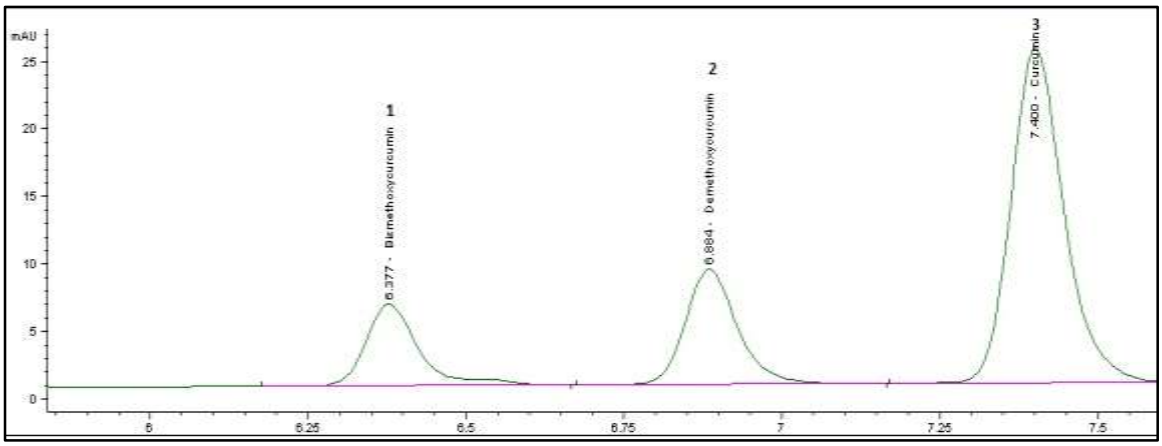


Figure 5.4: HPLC chromatogram of turmeric oleoresin powder (0.2%) at day 0. (1) (6.37 min), *bis*-demethoxycurcumin (2) (6.88 min), demethoxycurcumin (3) (7.40 min), curcumin.

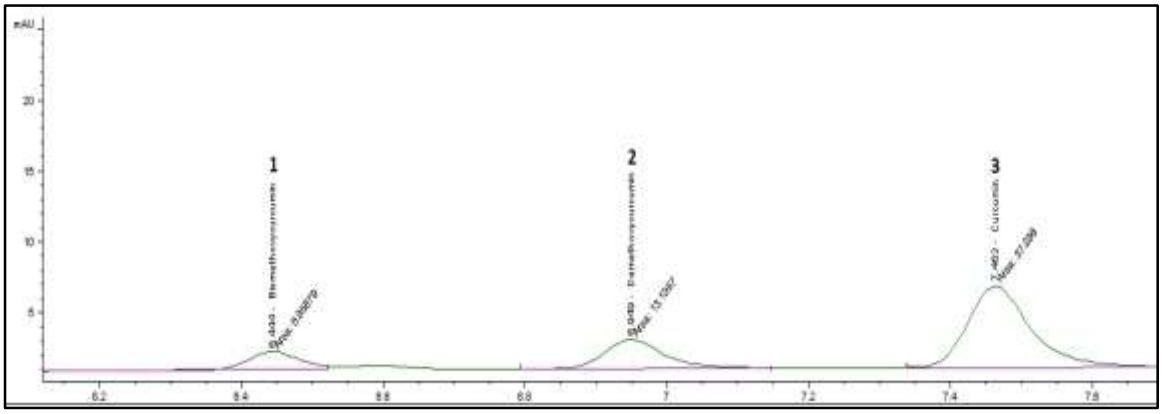


Figure 5.5: HPLC chromatogram of turmeric oleoresin powder covered with aluminium foil and exposed to external environmental conditions after 10 weeks. (1) *bis*-demethoxycurcumin (2) demethoxycurcumin (3) curcumin.

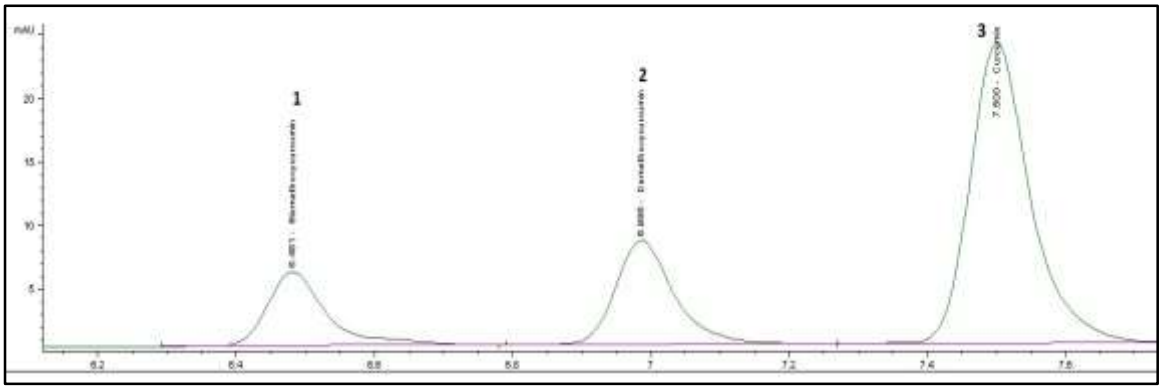


Figure 5.6: HPLC chromatogram of turmeric oleoresin powder refrigerated for 10 weeks. (1) *bis*-demethoxycurcumin (2) demethoxycurcumin (3) curcumin.

5.1.2 Effect of antioxidants (ascorbic acid and TBHQ) on degradation of curcuminoids in turmeric oleoresin powders exposed to external environmental conditions over a 10 week period

The effects of antioxidants ascorbic acid and TBHQ added to turmeric oleoresin powders on curcuminoid degradation are represented in Figures 5.7, 5.8 and 5.9. To determine the effect of antioxidants on the stability of curcuminoids in turmeric oleoresin powders exposed to external environmental conditions for 10 weeks, a two-way ANOVA analysis with interaction was conducted. Ascorbic acid and TBHQ were added separately to the turmeric oleoresin powders. Control samples did not contain any antioxidants.

As shown in Table 5.1, there was no significant difference ($p>0.05$) in % curcuminoid degradation for turmeric oleoresin powders with and without ascorbic acid. This result indicates that the presence of ascorbic acid had no impact on curcuminoid stability, with the degradation trend following that of curcuminoid samples without antioxidants (Figure 5.7). However, the % curcuminoid degradation for treatment with TBHQ was significantly lower compared to treatments with ascorbic acid and without any antioxidant.

Table 5.1: Percentage curcuminoid degradation of turmeric oleoresin powders with and without antioxidants and exposed to external environmental conditions over a 10 week period

Treatment	Means (%)
Turmeric oleoresin powders	83.0^{a} (±0.3)
Turmeric oleoresin powders with ascorbic acid	83.0^{a} (±0.1)
Turmeric oleoresin powders with TBHQ	75.0^{b} (±0.2)

^{a,b}Means followed by different letters are significantly different at level $p<0.05$

Figure 5.7 shows the percentage degradation of curcuminoids in turmeric oleoresin powders with and without antioxidants and exposed to external environmental conditions over a ten week period. Figures 5.8 and 5.9 show photographs of turmeric oleoresin powders with ascorbic acid

and TBHQ, respectively, exposed to external environmental conditions over a ten week period and weekly percentage curcuminoid degradation.

As illustrated in Figures 5.7, 5.8 and 5.9, the degradation process was inhibited in the presence of TBHQ in the first eight weeks of external environmental conditions exposure, with a 17% lower degradation observed after 1 week, relative to samples containing ascorbic acid.

After 10 weeks of exposure to external environmental conditions, 100% curcuminoid degradation was observed in all three treatments, i.e. turmeric oleoresin powders containing ascorbic acid, TBHQ and no antioxidant. For turmeric oleoresin powders without antioxidants, significant differences ($p<0.05$) were noted in samples in weeks 2 and 3, whereas samples in weeks 9 and 10 were statistically similar ($p>0.05$).

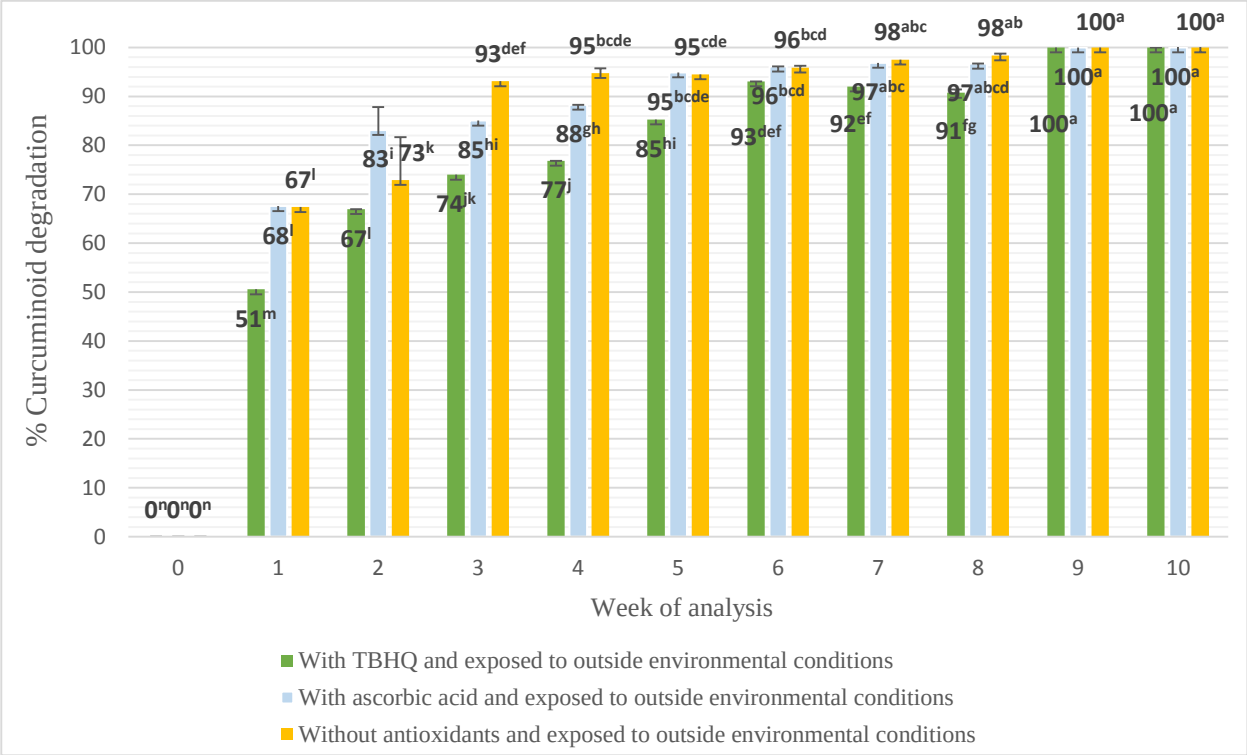


Figure 5.7: Percentage curcuminoid degradation of turmeric oleoresin powders with and without antioxidants (ascorbic acid and TBHQ) and exposed to external environmental conditions

[a,b,c,d,e,f,g,h,i]When comparing treatments, values with different superscripts differ significantly at $p<0.001$

Week 0: 0%[a] Degradation

Week 1: 68%[b] Degradation

Week 2: 83%[c] Degradation

Week 3: 85%[c] Degradation

Week 4: 88%[d] Degradation

Week 5: 95%[e] Degradation

Week 6: 96%[e] Degradation

Week 7: 97%[e] Degradation

Week 8: 97%[e] Degradation

Week 9: 100%[f] Degradation

Week 10: 100%[f] Degradation

Figure 5.8: Photographs of turmeric oleoresin powders with ascorbic acid and exposed to external environmental conditions over a ten week period and weekly percentage curcuminoid degradation

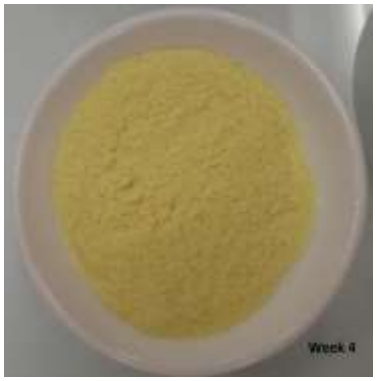
[a,b,c,d,e,f]Values with different superscripts differ significantly at $p<0.001$


Week 0: 0%[a] Degradation

Week 1: 51%[b] Degradation

Week 2: 67%[c] Degradation

Week 3: 74%[d] Degradation



Week 4: 77%[e] Degradation

Week 5: 85%[f] Degradation

Week 6: 93%[g] Degradation

Week 7: 92%[h] Degradation

Week 8: 91%[i] Degradation

Week 9: 100%[j] Degradation

Week 10: 100%[j] Degradation

Figure 5.9: Photographs of turmeric oleoresin powders with TBHQ and exposed to external environmental conditions over a ten week period and weekly percentage curcuminoid degradation

[a,b,c,d,e,f,g,h,i]Values with different superscripts differ significantly at $p<0.001$

5.1.3 Effect of divalent Mg^{2+} complexation on degradation of curcuminoids in turmeric oleoresin powders exposed to external environmental conditions over a ten week period

Table 5.2 shows mean percentage curcuminoid degradation of turmeric oleoresin powders with and without Mg^{2+}- complexes and exposed to external environmental conditions over a 10 week period. Significant differences ($p<0.05$) in curcuminoid stability was observed between Mg^{2+}-complexed curcuminoids and samples that did not contain Mg^{2+}- complexed curcuminoids after exposure to external environmental conditions (Table 5.2).

Table 5.2: Mean (±SD) percentage curcuminoid degradation of turmeric oleoresin powders with and without Mg^{2+} complexes and exposed to external environmental conditions over a 10 week period

Category	Means (%)
Turmeric oleoresin powders	83.0^{a} (±0.3)
Turmeric oleoresin powders with Mg^{2+}- complexed curcuminoids	77.0^{b} (±0.2)

^{a,b}Means followed by different letters are significantly different at level $p<0.05$

Figure 5.10 shows percentage curcuminoid degradation of turmeric oleoresin powders with and without Mg^{2+}-complexed curcuminoids and exposed to external environmental conditions over a 10 week period. Figure 5.11 shows photographs of Mg^{2+}-complexed turmeric oleoresin powders after exposure to external environmental conditions over a ten week period, as well as weekly percentage curcuminoid degradation.

As illustrated in Figure 5.10, a steady increase in curcuminoid pigment degradation was observed in both Mg^{2+}- complexed and uncomplexed oleoresin powders over the duration of the study, with a 36% and 33% increase in degradation observed from week 1 to week 10 of the experiment, respectively. In terms of % curcuminoid degradation, turmeric oleoresin powders with Mg^{2+} complexation were not different ($p>0.05$) between weeks 4 to 8. Significant differences ($p<0.05$) in % curcuminoid degradation were noted for turmeric oleoresin powders without Mg^{2+} complexation in weeks 1, 2 and 3 (Figure 5.10).

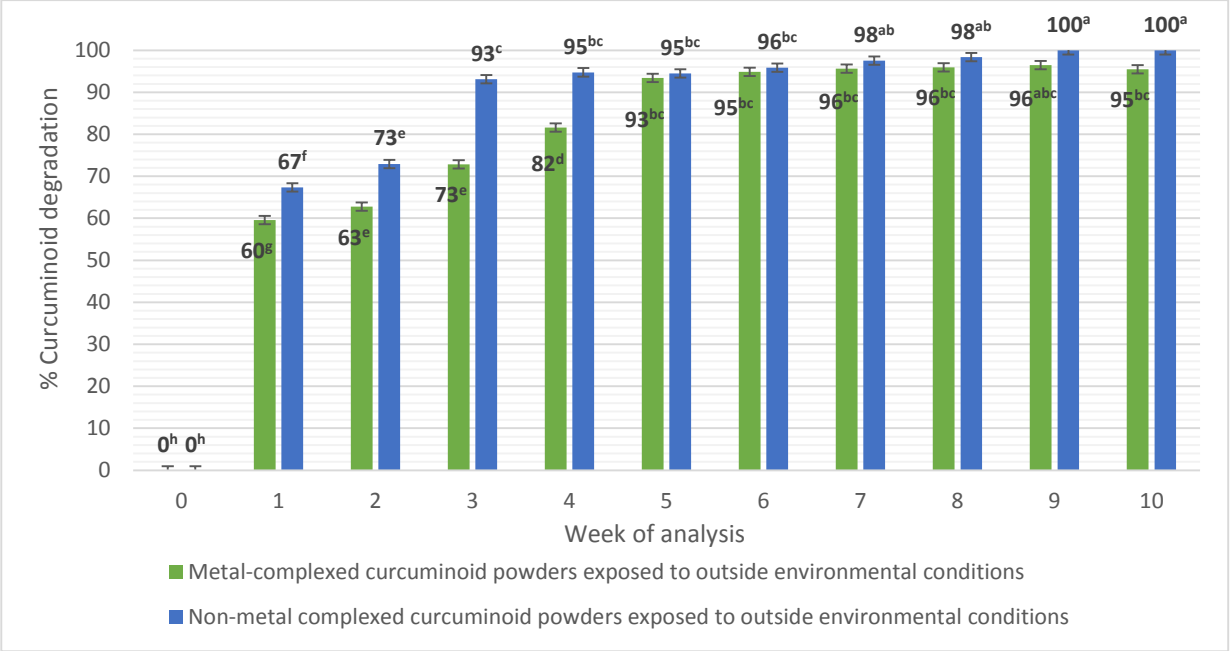


Figure 5.10: Percentage curcuminoid degradation of turmeric oleoresin powders with and without Mg^{2+} complexes and exposed to external environmental conditions

[a,b,c,d,e,f,g,h]When comparing treatments, values with different superscripts differ significantly at $p<0.001$


Week 0: 0%[a] Degradation

Week 1: 60%[b] Degradation

Week 2: 63%[c] Degradation

Week 3: 73%[d] Degradation

Week 4: 82%[d] Degradation

Week 6: 95%[e] Degradation

Week 7: 96%[f] Degradation

Week 8: 96%[f] Degradation

Week 9: 97%[h] Degradation

Week 10: 97%[h] Degradation

Figure 5.11: Photographs of Mg^{2+}- complexed turmeric oleoresin powders exposed to external environmental conditions over a ten week period and weekly percentage curcuminoid degradation

[a,b,c,d,e,f,g,h] Values with different superscripts differ significantly at $p<0.001$

5.2 Measurement of colour

To determine the effect of time of storage on colour (L^*, a^*, b^*, C, H°) degradation of turmeric oleoresin samples stored either exposed or protected from external environmental conditions, as well as with and without antioxidants and Mg^{2+}- complexation, separate one-way ANOVA was conducted per treatment.

5.2.1 Effect of external environmental conditions on L^*, a^*, b^*, C and H° values of turmeric oleoresin powders

Table 5.3 shows mean L^*, a^*, b^*, C, and H° values of turmeric oleoresin powders exposed to external environmental conditions. After direct exposure to external environmental conditions, turmeric oleoresin powders exhibited higher L^* values (L^* = 87.80) relative to the control, which was stored in a refrigerator at 4 °C (L^* = 83.50; Table 5.3). During storage, a constant increase in L^* values was observed, with L^* values of 83.72 and 87.80 in weeks 1 and 10, respectively. Despite some fluctuations in the a^* values, a general decline in b^* values occurred throughout the time of the storage.

Accordingly, C values exhibited the same trends as b^* values. Since C reflects colour brilliance or purity and is correlated with the degree of curcuminoid pigment content, the C values of turmeric oleoresin samples that were directly exposed to external environmental conditions was expected to be lower than turmeric oleoresin samples that were light-protected and exposed to external environmental conditions due to the lack of a protective physical barrier in the former. This was evidenced, with a 34 degree decline in C from week 1 to week 10 of the study.

Throughout the study, H° values of the turmeric oleoresin powders exposed to external environmental conditions increased, from 92.85° in week 1 to 97.21° in week 10, respectively, which in combination with comparatively decreasing C values, described the faded yellow colour of the samples.

Table 5.3: Mean (±SD) L*, a*, b*, C and H° values of turmeric oleoresin powders exposed to external environmental conditions over a 10 week period

Week	L*	a*	b*	H°	C
0	83.5^{a}	-3.1^{b}	59.3^{j}	93.6^{a}	59.4^{b}
	(±0.1)	(0.0)	(±0.30)	(±0.1)	(±0.3)
1	83.7^{b}	-3.6^{a}	47.4^{i}	92.8^{b}	47.4^{a}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
2	85.3^{c}	-3.5^{a}	32.7^{h}	92.8^{b}	32.9^{a}
	(±0.14)	(±0.3)	(±0.5)	(±0.0)	(±0.5)
3	85.9^{d}	-2.8^{c}	28.9^{g}	93.2^{c}	29.1^{a}
	(±0.0)	(±0.0)	(±0.4)	(±0.2)	(±0.5)
4	86.2^{e}	-3.0^{bc}	25.2^{f}	96.2^{d}	25.4^{c}
	(±0.18)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
5	86.0^{f}	-2.8^{c}	23.0^{e}	96.2^{e}	23.2^{c}
	(±0.2)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
6	86.5^{g}	-2.4^{d}	21.8^{d}	96.6^{f}	22.0^{c}
	(±0.0)	(±0.0)	(±0.5)	(±0.0)	(±0.4)
7	86.1^{f}	-2.3^{d}	20.1^{c}	96.7^{g}	20.2^{c}
	(±0.2)	(±0.0)	(±0.1)	(±0.0)	(±0.1)
8	87.4^{h}	-1.5^{e}	14.7^{b}	96.9^{h}	14.7^{c}
	(±0.0)	(±0.0)	(±0.3)	(±0.4)	(±0.3)
9	87.1^{i}	-0.8^{f}	14.3^{ab}	97.0^{i}	14.3^{c}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
10	87.8^{j}	-0.7^{f}	13.8^{a}	97.2^{j}	13.8^{c}
	(±0.0)	(±0.0)	(±0.0)	(±0.1)	(±0.0)

^{a,b,c,d,e,f,g,h,i,j} Mean values with the same letter superscripts in columns do not differ significantly at $p<0.05$.

5.2.2 Effect of external environmental conditions on L*, a*, b*, C and H° values of foil-protected turmeric oleoresin powders over a 10 week period

Table 5.4 shows mean L*, a*, b*, C and H° values of foil-protected turmeric oleoresin powders exposed to external environmental conditions over a ten week period. Throughout the duration of storage, constant L* values were observed for the foil-protected turmeric oleoresin powders, with L* values ranging from 84.00 in week 1 to 83.26 in week 10, respectively (Table 5.4). An

overall decline in b^* values throughout the time of the storage was observed, with C values following the same trend. C values decreased over the 10 week study, from 59.98 in week 1 to 41.80 in week 10, respectively. No significant differences ($p>0.05$) in C values were observed in turmeric powders that were analysed in weeks 9 and 10 of the experiment. H° values of turmeric oleoresin samples were relatively constant throughout the experiment, with no significant differences ($p>0.05$) in h° values observed between turmeric oleoresin powders in all weeks of analysis.

5.2.3 Effect of external environmental conditions on L^*, a^*, b^*, C and H° values of turmeric oleoresin powders containing ascorbic acid over a 10 week period

Table 5.5 shows mean L^*, a^*, b^*, C and H° values of ascorbic acid-containing turmeric oleoresin powders exposed to external environmental conditions over a ten week period. A steady increase in L^* values was observed, with values ranging between 84.53 in week 1 to 88.30 week 10, respectively. After 10 weeks of exposure to external environmental conditions, turmeric oleoresin samples exhibited comparable L^* values ($L^* = 88.30$) to turmeric oleoresin powders without ascorbic acid ($L^* = 87.80$; Data shown in Table 5.3). Throughout the study, b^* values decreased progressively during storage. C values exhibited the same trends as b^* values, with C decreasing from 40.25 to 11.00 from week 1 to 10, respectively. H° values increased significantly throughout the experiment, from 91.20 to 97.33 in week 1 to 10, respectively.

Table 5.4: Mean (±SD) L*, a*, b*, C and H° values of foil-protected turmeric oleoresin powders exposed to external environmental conditions over a ten week period

Week	L*	a*	b*	H°	C
0	83.3^{a}	-3.7^{b}	57.6^{h}	93.0^{a}	57.7^{h}
	(±0.0)	(0.0)	(±0.0)	(±0.0)	(±0.0)
1	84.0^{a}	-3.0^{a}	57.9^{h}	92.0^{a}	57.9^{gh}
	(±0.0)	(±0.0)	(±0.04)	(±0.0)	(±0.0)
2	83.4^{a}	-2.9^{a}	57.1^{i}	92.4^{a}	57.2^{g}
	(±0.0)	(±0.0)	(±0.04)	(±0.0)	(±0.0)
3	83.2^{a}	-2.8^{a}	56.1^{g}	92.8^{a}	56.2^{g}
	(±0.0)	(±0.0)	(±0.05)	(±0.0)	(±0.0)
4	83.3^{a}	-2.7^{ac}	54.8^{f}	92.8^{a}	54.9^{f}
	(±0.0)	(±0.0)	(±0.04)	(±0.0)	(±0.0)
5	83.1^{a}	-2.6^{acd}	53.1^{e}	92.4^{a}	53.1^{e}
	(±0.0)	(±0.0)	(±0.04)	(±0.0)	(±0.0)
6	83.1^{a}	-2.4^{cd}	51.0^{d}	92.5^{a}	51.1^{d}
	(±0.0)	(±0.0)	(±0.04)	(±0.0)	(±0.0)
7	83.2^{a}	-2.2^{de}	48.6^{c}	92.7^{a}	48.7^{c}
	(±0.0)	(±0.0)	(±0.05)	(±0.0)	(±0.1)
8	83.1^{a}	-1.9^{ef}	43.8^{b}	92.7^{a}	43.8^{b}
	(±0.0)	(±0.0)	(±0.04)	(±0.0)	(±0.0)
9	83.8^{a}	-1.8^{f}	41.8^{a}	92.9^{a}	42.9^{a}
	(±0.0)	(±0.0)	(±0.28)	(±0.0)	(±0.0)
10	83.2^{a}	-1.4^{f}	42.9^{a}	92.9^{a}	41.8^{a}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.3)

^{a,b,c,d,e,f} Mean values with the same letter superscripts in columns do not differ significantly at $p<0.05$.

Table 5.5: Mean (±SD) L*, a*, b*, C and H° values of ascorbic acid-containing turmeric oleoresin powders exposed to external environmental conditions temperature over a ten week period

Week	L*	a*	b*	H°	C
0	83.3^{a}	-3.7^{b}	57.6^{h}	93.0^{a}	57.7^{h}
	(±0.0)	(0.0)	(±0.0)	(±0.0)	(±0.0)
1	84.5^{a}	-3.7^{a}	40.1^{a}	91.2^{h}	40.2^{g}
	(±0.0)	(±0.20)	(±0.0)	(±0.0)	(±0.0)
2	85.6^{b}	-3.6^{a}	31.8^{b}	91.5^{a}	32.0^{f}
	(±0.0)	(±0.1)	(±0.5)	(±0.2)	(±0.5)
3	85.6^{c}	-3.2^{bc}	31.4^{c}	93.3^{b}	31.5^{f}
	(±0.0)	(±0.0)	(±0.6)	(±0.0)	(±0.6)
4	85.2^{d}	-3.2^{b}	29.5^{d}	94.5^{c}	29.7^{e}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
5	86.0^{e}	-3.0^{b}	25.0^{e}	95.8^{d}	25.2^{d}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
6	86.9^{f}	-2.3^{dc}	18.5^{f}	96.4^{de}	18.7^{c}
	(±0.0)	(±0.0)	(±0.0)	(±0.2)	(±0.0)
7	86.9^{e}	-2.2^{d}	18.2^{g}	96.6^{ef}	18.3^{c}
	(±0.0)	(±0.0)	(±0.0)	(±0.3)	(±0.0)
8	86.6^{g}	-1.7^{e}	15.2^{h}	96.8^{efg}	15.2^{b}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
9	88.3^{h}	-0.2^{f}	10.9^{hi}	97.1^{fg}	10.9^{a}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
10	88.3^{i}	-0.3^{f}	11.0^{i}	97.3^{g}	11.0^{a}
	(±0.1)	(±0.0)	(±0.1)	(±0.0)	(±0.1)

^{a,b,c,d,e,f,g,h,i}Mean values with the same letter superscripts in columns do not differ significantly at $p<0.05$

5.2.4 Effect of external environmental conditions on L*, a*, b*, C and H° of turmeric oleoresin powders containing TBHQ over a 10 week period

Table 5.6 shows mean L*, a*, b*, C and H° values of TBHQ-containing turmeric oleoresin powders exposed to external environmental conditions over a ten week period. There was an

increase in L^* values during storage in direct external environmental conditions, from 83.15 in week 1 to 87.00 in week 10, respectively. A general decline in b^* values was evidenced throughout the time of the storage. The a^* values of turmeric oleoresin powder in weeks 9 and 10 were similar ($p>0.05$). C decreased from 41.05 in week 1 to 11.90 in week 10, respectively. C values for weeks 3 and 4 were similar ($p>0.05$). H° values increased significantly throughout the experiment, from 91.80 in week 1 to 94.93 in week 10. H° values for turmeric oleoresin samples analysed between 1 and 3 weeks were not different ($p>0.05$).

Table 5.6: Mean (±SD) L^*, a^*, b^*, C and H° values of TBHQ-containing turmeric oleoresin powders exposed to external environmental conditions over a ten week period

Week	L*	a*	b*	H°	C
0	83.3^{a}	-3.7^{b}	57.6^{h}	93.0^{a}	57.7^{h}
	(±0.0)	(0.0)	(±0.0)	(±0.0)	(±0.0)
1	83.1^{b}	-1.5^{e}	40.9^{i}	91.8^{a}	41.0^{c}
	(±0.0)	(±0.1)	(±1.8)	(±0.0)	(±1.8)
2	82.7^{a}	-2.2^{ab}	38.3^{a}	92.2^{a}	38.4^{d}
	(±0.7)	(±0.2)	(±0.3)	(±0.3)	(±0.3)
3	83.3^{b}	-2.4^{a}	33.6^{b}	92.5^{a}	33.7^{cd}
	(±0.0)	(±0.0)	(±0.0)	(±0.1)	(±0.0)
4	82.9^{ab}	-2.2^{ab}	33.2^{c}	93.0^{b}	33.2^{cd}
	(±0.2)	(±0.0)	(±0.4)	(±0.2)	(±0.4)
5	84.0^{c}	-1.7d^{e}	26.5^{d}	93.6^{bc}	26.5^{c}
	(±0.2)	(±0.0)	(±1.0)	(±0.1)	(±1.0)
6	84.4^{cd}	-1.9^{cd}	23.3^{e}	93.3^{b}	24.2^{bc}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
7	84.8^{d}	-2.0^{bc}	23.6^{e}	93.7^{cd}	23.7^{bc}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
8	84.2^{c}	-1.6^{e}	24.2^{f}	94.0^{cd}	23.4^{cd}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
9	87.5^{e}	-0.3^{f}	10.9^{g}	94.5^{de}	11.0^{a}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
10	87.0^{f}	-0.5^{f}	11.9^{h}	94.9^{e}	11.9^{ab}
	(±0.0)	(±0.1)	(±0.1)	(±0.0)	(±0.1)

^{a,b,c,d,e,f,g,h,}Mean values with the same letter superscripts in columns do not differ significantly at $p<0.05$.

Table 5.7: Mean (±SD) L^*, a^*, b^*, C and H° values of Mg^{2+}- complexed turmeric oleoresin powders exposed to external environmental conditions over a ten week period

Week	L*	a*	b*	H°	C
0	83.3^{a}	-3.7^{b}	57.6^{h}	93.0^{a}	57.7^{h}
	(±0.0)	(0.0)	(±0.0)	(±0.0)	(±0.0)
1	83.8^{h}	-3.7^{g}	44.8^{g}	93.2^{h}	44.6^{i}
	(±0.0)	(±0.0)	(±0.0)	(±0.0)	(±0.0)
2	84.1^{a}	-3.6^{a}	42.7^{a}	94.4^{a}	42.9^{a}
	(±0.2)	(±0.0)	(±0.0)	(±0.1)	(±0.1)
3	84.6^{b}	-3.3^{b}	38.6^{b}	94.6^{b}	38.7^{b}
	(±0.2)	(±0.1)	(±0.0)	(±0.1)	(±0.0)
4	85.0^{c}	-3.0^{c}	36.3^{c}	95.4^{c}	36.5^{c}
	(±0.0)	(±0.0)	(±0.1)	(±0.1)	(±0.1)
6	86.4^{d}	-3.0^{c}	25.6^{d}	95.6^{d}	25.8^{d}
	(±0.2)	(±0.0)	(±0.2)	(±0.0)	(±0.3)
7	86.0^{e}	-2.5^{d}	25.6^{d}	96.6^{e}	25.5^{e}
	(±0.0)	(±0.0)	(±0.2)	(±0.2)	(±0.3)
8	86.0^{e}	-2.4^{e}	19.3^{e}	96.6^{f}	19.5^{f}
	(±0.0)	(±0.0)	(±0.2)	(±0.2)	(±0.2)
9	87.9^{f}	-2.4^{e}	19.1^{e}	97.0^{g}	19.2^{g}
	(±0.1)	(±0.5)	(±1.1)	(±1.1)	(±1.2)
10	87.1^{g}	-1.3^{f}	16.5^{f}	97.0^{g}	16.5^{h}
	(±0.2)	(±0.0)	(±0.0)	(±0.3)	(±0.0)

^{a,b,c,d,e,f,g,h,i}Mean values with the same letter superscripts in columns do not differ significantly at $p<0.05$.

5.2.5 Effect of external environmental conditions on L^*, a^*, b^*, C and H° values of Mg^{2+}-complexed turmeric oleoresin powders over a 10 week period

Table 5.7 shows mean L^*, a^*, b^*, C and H° values of Mg^{2+} - complexed turmeric oleoresin powders exposed to external environmental conditions over a ten week period. A constant increase in L^* values was observed, from 83.80 to 87.10 in week 1 to 10, respectively.

Similarly, H° values were considerably increased throughout the experiment, from 93.20 to 97.03 in weeks 1 and 10, respectively. C values decreased with increasing storage time, with a 28.1 degree decrease between weeks 1 and 10. Correspondingly, b^* values decreased throughout the duration of the study, from 44.82 in week 1 to 16.50 in week 10. The b^* values of turmeric oleoresin samples were not different ($p>0.05$) in weeks 6 and 7. A two ANOVA analysis was conducted in order to test the effect of each treatment and time on L^*, a^*, b^*, H° and C colour values (Table 5.8).

Table 5.8: Means per treatment of L^*, a^*, b^*, C and H° values of turmeric oleoresin powders exposed to external environmental conditions over a 10 week period

Treatment	L^*	a^*	b^*	C	H°
With Mg^{2+} complex	85.49^{b}	-2.82^{c}	31.91^{b}	32.05^{b}	95.48^{a}
With TBHQ	84.31^{c}	-1.75^{a}	29.50^{bc}	29.56^{bc}	93.35^{b}
Control	85.95^{a}	-2.41^{b}	$27.25c^{d}$	27.37^{cd}	95.36^{a}
With ascorbic acid	86.15^{a}	-2.41^{b}	26.31^{d}	26.43^{d}	95.17^{a}
Covered with foil	83.26^{d}	-2.49^{b}	53.26^{a}	53.32^{a}	92.59^{c}

^{a,b,c,d,e,f,g,h,i}Mean values with the same letter superscripts in columns do not differ significantly at $p<0.05$.

H° values for turmeric oleoresin powders exposed to external environmental conditions with and without ascorbic acid and Mg^{2+}- complexation were not different ($p>0.05$) to turmeric oleoresin samples without antioxidants and Mg^{2+} complexes (Table 5.8). L^*, b^* and C values for turmeric oleoresin samples covered with foil and exposed to external environmental conditions were statistically different ($p<0.05$) to all other corresponding values from the other treatments. The a* value for turmeric oleoresin powders with TBHQ was higher than the a^* values of all other treatments.

5.3 Sensory evaluation of turmeric oleoresin powders with and without protection from external environmental conditions over a 10 week period

Table 5.9 shows means of colour ratings for turmeric oleoresin samples stored for 10 weeks exposed to and protected from external environmental conditions. Table 5.10 shows means of colour ratings for turmeric oleoresin samples exposed to and protected from external environmental conditions for all storage time points, as evaluated by a sensory panel.

Results indicated that there was a significant difference between the treatments (with and without aluminium foil protection) over the time of storage, with turmeric oleoresin powders with foil protection being more yellow than samples without foil protection (Table 5.9). There was a difference in the way samples that were treated differently (exposed or protected), behaved over time, with turmeric oleoresin powders becoming less yellow with increasing time of exposure to external environmental conditions.

Table 5.9: Means (± SD) of colour ratings of turmeric oleoresin powders stored for ten weeks exposed to or protected from external environmental conditions over a 10 week period

Treatment	Means
With foil protection	7.0^{a} (±0.1)
Without foil protection	3.0^{b} (±0.2)

[a,b]Means followed by different letters are significantly different at level $p<0.05$. Colour rated on a 9 point scale (1=not yellow, 9=extremely yellow).

For turmeric oleoresin powders that were exposed to external environmental conditions, a steady decline in the sensory ratings were observed, i.e. from 8.9 in week 0 to 1.0 in week 10,

respectively (Table 5.10). These ratings correlated well with the percentage curcuminoid degradation that was observed in these weeks (Figure 5.1). In terms if sensory ratings, turmeric oleoresin powders exposed to external environmental conditions for weeks 3 and 4, 5 to 7 and 8 to 10 were not different ($p>0.05$).

Turmeric oleoresin powders that were protected from external environmental conditions (covered in foil) were statically similar ($p>0.05$) in sensory ratings in weeks 2 and 3. A steady decline in the rating values was also observed, i.e. from 8.6 in week 0 to 5.9 in week 10, respectively. The mean sensory ratings for the different treatments i.e. samples exposed to and protected from external environmental conditions, were statistically different ($p<0.05$) (Table 5.9). This showed that the aluminium foil covering prevented the degradation of curcuminoid pigments in turmeric oleoresin powders, relative to turmeric oleoresin powders without aluminium foil protection.

Table 5.10: Means (±SD) of colour ratings of turmeric oleoresin samples exposed to and protected from external environmental conditions for all storage time points, as evaluated by a sensory panel

	Storage Conditions	
Week of Analysis	**Exposed to external environmental conditions, including sunlight**	**Protected from external environmental conditions, including sunlight**
0	8.9^{a} (±0.1)	8.6^{a} (±0.1)
1	5.6^{gh} (±0.1)	8.0^{b} (±0.0)
2	4.4^{i} (±0.1)	7.0^{cd} (±0.0)
3	3.3^{j} (±0.1)	7.0^{cd} (±0.0)
4	3.0^{j} (±0.0)	7.3^{c} (±0.0)
5	2.4^{k} (±0.1)	6.7^{de} (±0.0)
6	2.3^{k} (±0.1)	6.9^{cde} (±0.1)
7	2.2^{k} (±0.0)	6.4^{ef} (±0.1)
8	1.6^{l} (±0.1)	5.9^{fg} (±0.1)
9	1.2^{l} (±0.0)	5.2^{h} (±0.1)
10	1.0^{l} (±0.0)	5.9^{fg} (±0.1)

^{a,b,c,d,e,f,g,h,i,j,k,l}Means followed by different letters are significantly different per treatment at level $p<0.05$. Colour rated on a 9 point scale (1=not yellow, 9=extremely yellow).

5.4 Correlation between percentage curcuminoid degradation and rating of colour of turmeric oleoresin powders exposed to external environmental conditions

Table 5.12 shows correlation between percentage curcuminoid degradation and rating of colour of turmeric oleoresin powders exposed to and protected from external environmental conditions. There was a strong linear correlation between percentage curcuminoid degradation and rating of colour of turmeric oleoresin powders exposed to external environmental conditions (Figure 5.12). The correlation coefficient, r was -0.93. This negative value denotes a negative linear correlation. This value also quantifies the direction and strength of the linear association between the two variables, which in this study, was a strong negative correlation (i.e., higher levels of one variable are associated with lower levels of the other).

Equation of the model was % curcuminoid degradation = 126.7 – 13.27 x (rating of colour). This equation is important as it could be used to predict the sensory scores of the metal and antioxidant treated turmeric oleoresin samples (Table 5.11). This model could also be used to predict the minimum sensory score values that would be regarded as still acceptable. From this data, shelf life can then be predicted.

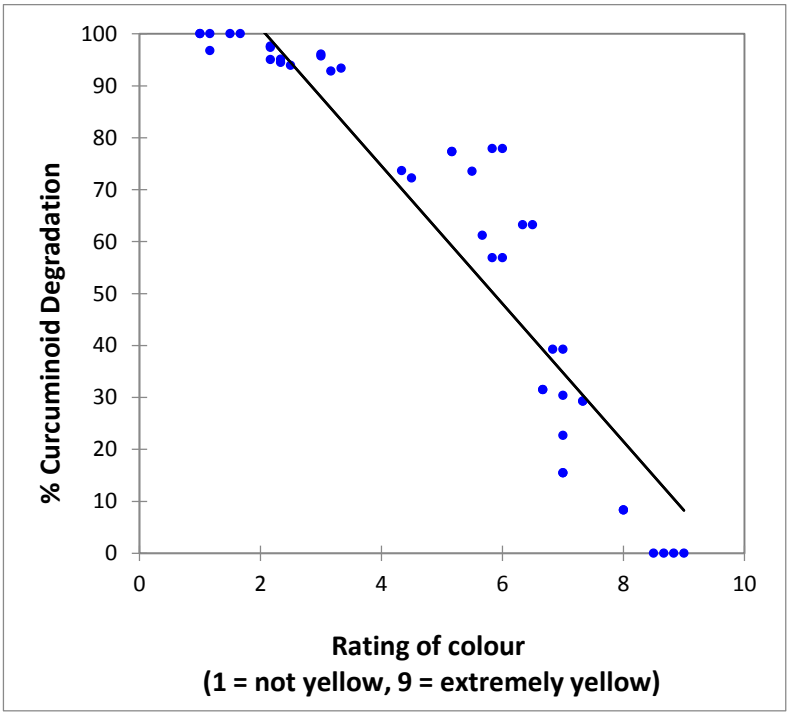


Figure 5.12: Correlation between percentage curcuminoid degradation and rating of colour of turmeric oleoresin powders for samples exposed to and protected from external environmental conditions at $p<0.05$. $R^2 = 0.871$.
% Curcuminoid degradation = 126.7 – 13.27 x (Colour rating)
Correlation coefficient (r) = -0.93.

Table 5.11 shows means of predicted colour rating scores for turmeric oleoresin powders with ascorbic acid, TBHQ and Mg^{2+}- curcuminoid complexes and exposed to external environmental conditions for all storage time points. The colour rating values were predicted using the equation of the model: % curcuminoid degradation = 126.7 – 13.27 x (rating of colour).

Table 5.11: Means (±SD) of predicted colour ratings of turmeric oleoresin powders with ascorbic acid, TBHQ and Mg^{2+}-curcuminoid complexes and exposed to external environmental conditions for all storage time points

	Storage Conditions		
Week of Analysis	**Turmeric powders with ascorbic acid**	**Turmeric powders with TBHQ**	**Turmeric powders with Mg^{2+} complex**
0	9.0^{a} (±0.0)	9.0^{a} (±0.0)	9.0^{a} (±0.0)
1	4.7^{b} (±0.1)	5.7^{b} (±0.0)	5.0^{b} (±0.0)
2	3.2^{c} (±0.1)	4.5^{c} (±0.3)	4.8^{c} (±0.0)
3	3.1^{c} (±0.0)	3.9^{d} (±0.0)	4.0^{d} (±0.1)
4	2.8^{d} (±0.0)	3.7^{d} (±0.0)	3.4^{e} (±0.0)
5	2.3^{d} (±0.2)	3.1^{e} (±0.0)	2.5^{f} (±0.2)
6	2.3^{d} (±0.0)	2.5^{f} (±0.0)	2.4^{f} (±0.0)
7	2.2^{d} (±0.0)	2.6^{f} (±0.1)	2.3^{f} (±0.1)
8	2.2^{d} (±0.0)	2.7^{f} (±0.0)	2.3^{f} (±0.0)
9	2.0^{e} (±0.0)	2.0^{g} (±0.1)	2.3^{f} (±0.0)
10	2.0^{e} (±0.0)	2.0^{g} (±0.0)	2.3^{f} (±0.1)

^{a,b,c,d,e,f,g,h,i,j,k,l}Means followed by different letters are significantly different per treatment at level $p<0.05$. Colour rated on a 9 point scale (1=not yellow, 9=extremely yellow).

For turmeric oleoresin powders that were exposed to external environmental conditions with ascorbic acid, a steady decline in the sensory ratings were observed, i.e. from 9.0 in week 0 to 2.0 in week 10, respectively (Table 5.11). Sensory ratings of turmeric oleoresin powders with TBHQ and Mg^{2+} complexes followed the same trend, with a steady decline in colour ratings from week 1 to 10 for both treatments.

In terms of sensory ratings, turmeric oleoresin powders exposed to external environmental conditions with ascorbic acid for weeks 2 and 3, 4 to 8 and 9 to 10 were not different ($p>0.05$). Turmeric oleoresin powders that were exposed from external environmental conditions with Mg^{2+} complexes were not different ($p>0.05$) in weeks 5 to 10.

In this study, correlation analysis was conducted to determine the relationship between percentage curcuminoid degradation, sensory scores and colour values (L^*, a^*, b^*, C, and H°) of turmeric oleoresin powders that were exposed to external environmental conditions (Tables 5.12 and 5.13).

For turmeric oleoresin samples exposed to external environmental conditions without aluminium foil protection, a perfect positive relationship was observed between C and b^* values. C values also had a strong positive relationship with L^* values (Table 5.12). Percentage curcuminoid degradation showed a weak positive relationship with both a* values and H° values. H° values correlated weakly with all other colour values, sensory scores, as well as percentage curcuminoid degradation values (Table 5.12).

Table 5.12: Correlation coefficients (r) between percentage curcuminoid degradation, colour values and sensory scores of turmeric oleoresin powders exposed to external environmental conditions

Variables	% Curcuminoid Degradation	L*	a*	b*	C	Hue Angle	Rating of colour
% Curcuminoid Degradation	**1**	**0.788**	0.342	**-0.872**	**-0.871**	0.415	**-0.952**
L*	**0.788**	**1**	**0.595**	**-0.970**	**-0.971**	0.237	**-0.829**
a*	0.342	**0.595**	**1**	**-0.547**	**-0.551**	**-0.551**	-0.405
b*	**-0.872**	**-0.970**	**-0.547**	**1**	**1.000**	-0.351	**0.887**
C	**-0.871**	**-0.971**	**-0.551**	**1.000**	**1**	-0.346	**0.886**
H°	0.415	0.237	**-0.551**	-0.351	-0.346	**1**	-0.333
Rating of colour	**-0.952**	**-0.829**	-0.405	**0.887**	**0.886**	-0.333	**1**

Values in bold are significant at level $p<0.05$.

Colour ratings showed a strong positive correlation with C values (Table 5.12). This correlated well with the fact that a higher sensory rating corresponds to a darker yellow colour, and this corresponded to a larger C value. The relationship between colour rating and C values is important as it could also be used to predict colour values of Mg^{2+} - complexed and antioxidant treated powders.

For turmeric oleoresin samples protected from external environmental conditions, a strong negative, linear relationship was observed between percentage curcuminoid degradation and rating of colour (Table 5.13). A perfect positive relationship was observed between C and b^* values. C values had a weak positive relationship with L^* values, whilst percentage curcuminoid degradation showed a positive relationship with a* values. The relationship between H° values and percentage curcuminoid degradation was weak. Colour ratings showed a moderate positive relations with both C and b^* values.

Table 5.13: Correlation coefficients (r) between percentage curcuminoid degradation, colour values and sensory scores of turmeric oleoresin powders protected from external environmental conditions

Variables	% Curcuminoid Degradation	L*	a*	b*	C	Hue Angle	Rating of colour
% Curcuminoid Degradation	**1**	**0.538**	**0.839**	**-0.846**	**-0.846**	**-0.762**	**-0.919**
L*	**0.538**	**1**	**0.525**	**-0.513**	**-0.513**	**-0.559**	**-0.488**
a*	**0.839**	**0.525**	**1**	**-0.975**	**-0.976**	**-0.960**	**-0.797**
b*	**-0.846**	**-0.513**	**-0.975**	**1**	**1.000**	**0.878**	**0.747**
C	**-0.846**	**-0.513**	**-0.976**	**1.000**	**1**	**0.879**	**0.747**
H°	**-0.762**	**-0.559**	**-0.960**	**0.878**	**0.879**	**1**	**0.789**
Rating of colour	**-0.919**	**-0.488**	**-0.797**	**0.747**	**0.747**	**0.789**	**1**

Values in bold are significant at level $p<0.05$.

5.5 Discussion of results

Curcuminoid pigment degradation in turmeric oleoresin powders was evidenced over time, with 100% degradation observed relative to the control that was refrigerated at 4 °C. It was evident that environmental temperature had a strong influence on the degradation of curcuminoid pigments in turmeric oleoresin powders (average daytime temperature of the surroundings was ± 24 °C).

Curcuminoid pigments were less sensitive to degradation when shielded from light (covered with aluminium foil), with a 22% reduction in degradation observed relative to turmeric oleoresin powders without aluminium foil protection after 10 weeks of exposure to external environmental conditions. However, after 9 weeks of exposure to external environmental conditions with aluminium foil protection, 100% degradation of curcuminoid pigments was observed. These results indicate that temperature had a strong influence on the degradation of curcuminoid pigments in turmeric oleoresin and that constant storage of ingredients and products containing turmeric oleoresin at low temperature (<5 °C), together with light protection is vital to retard colour degradation. These results were also consistent with results for turmeric oleoresin powders that were refrigerated at 4 °C in the dark, which showed less degradation compared to samples that were covered with aluminium foil and exposed to direct external environmental conditions, with a four times lower percentage degradation observed after 10 weeks for turmeric oleoresin powders that were refrigerated at 4 °C.

The findings of this investigation was consistent with studies conducted on other natural pigments, where it was found that certain pigments (e.g. anthocyanins) degrade faster as the temperature increases to 25 ˚C and the stability of these pigments is maintained at low temperatures (i.e. 4 ˚C) (Yusoff, Kumara, Lim, Ekanayake, and Tennakoon, 2014; Janna, Khairul, Maziah, and Mohd, 2006). Similar results were reported by Janna and co-workers (2006), who studied the stability of *Melastoma malabathricum* L. and found that the suitable storage condition for anthocyanin pigment is in acidic solution in the dark and at low temperatures (4 ˚C) (Janna, Khairul, Maziah, Mohd, 2006).

According to the L^*, a^* and b^* colour values, the addition of ascorbic acid did not significantly improve the colour stability of the turmeric oleoresin powders. The curcuminoid degradation process was inhibited in the presence of TBHQ in the first eight weeks of exposure to external

environmental conditions, with a 17% reduction in degradation observed after 1 week of exposure to external environmental conditions, relative to turmeric oleoresin powders containing ascorbic acid.

Interestingly, the presence of ascorbic acid had no significant influence on curcuminoid stability, with the degradation trend following that of the control, which did not contain ascorbic acid. This is possibly due to the fact that environmental conditions may alter the antioxidative capacity of ascorbic acid. For example, natural ascorbic acid in foods can be easily destroyed during processing as a result of susceptibility to heat, light, pH, oxygen and water activity. It is thus is often added to foods exogenously.

Allam and Mohamed (2002) reported that using the induction period for the oxidation of sunflower oil as a measure of antioxidant activity after heating (180 °C), ascorbyl palmitate was less thermally stable than mixed tocopherols, propyl gallate, BHT, or BHA. This may be a function of the water solubility of ascorbic acid. Oxidation of ascorbic acid to dehydroascorbic acid increases with exposure to light and correlates to light intensity and exposure time (Kim *et al*., 2009; Allam and Mohamed, 2002).

The effect of complexation of curcuminoids with divalent ion (Mg^{2+}) by mechanical mixing, was investigated. After exposure to external environmental conditions, no significant improvement in curcuminoid stability was observed after the addition of Mg^{2+} to turmeric oleoresin powders when compared to the control, which did not contain Mg^{2+} complexes.

Correlation analysis was conducted to determine the relationship between percentage curcuminoid degradation, sensory rating values and colour values (L^*, a^*, b^*, C, and H°) of turmeric oleoresin powders that were exposed to external environmental conditions. Colour rating values showed a strong positive correlation with C values. This correlated well with the fact that a higher sensory rating corresponds to a darker yellow colour and this corresponds to a larger C value.

For turmeric oleoresin powders that were exposed to external environmental conditions, a steady decline in the colour rating values was observed, i.e. from 8.9 in week 0 to 1.0 in week 10. This correlated well with the percentage curcuminoid pigment degradation that was observed in these weeks, which revealed that exposure to external environmental conditions negatively affected

the colour stability of curcuminoid pigments and that the level of pigment degradation was dependent on time of storage. A steady decline in the colour rating values was also observed, i.e. from 8.6 in week 0 to 5.9 in week 10 for turmeric oleoresin powders that were covered in foil and exposed to external environmental conditions. Significant sensory differences were observed between turmeric oleoresin samples with and without light protection compared to the corresponding control samples.

5.6 Conclusions

Curcuminoid pigments in turmeric oleoresin show degradation when exposed to external environmental conditions and the level of pigment degradation is dependent on time of storage. Constant storage of ingredients and products containing turmeric oleoresin at low temperature (<5 °C), together with light protection is vital to retard colour degradation.

The addition of ascorbic acid to turmeric oleoresin powders does not significantly improve curcuminoid stability. TBHQ enhanced the stability of curcuminoid pigments in turmeric oleoresin to a greater extent compared to ascorbic acid during the earlier weeks of storage when exposed to external environmental conditions over time. This shows the relative stability of TBHQ to oxidation compared to ascorbic acid. Furthermore, Mg^{2+}- curcuminoid complexation does not improve curcuminoid stability.

CHAPTER 6

6 General Discussion

This chapter will present a critical review of the main methods used in this study. It will also include a discussion of the results and the mechanisms involved in curcuminoid pigment degradation.

In this study, forced degradation of curcuminoid pigments was conducted in order to quantify and investigate the effect of external environmental conditions, namely light, temperature and humidity on its stability in turmeric oleoresin powders, as well as to investigate the effect of curcuminoid pigment degradation on the colour of food. This work also explored physical strategies that could stabilize curcuminoid pigments in turmeric oleoresin powders, with the aim of enhancing the application of natural colourants in the food processing industry.

6.1 Critical discussion of experimental methodologies

The analysis of curcuminoids in food products is very important, not only in terms of quality control but also for ensuring the efficacy and effectiveness of curcuminoids as active compounds in functional food preparations. To investigate colour quality in a systematic way, it is necessary to objectively measure colour as well as pigment concentration. In this context, colour represents the visual appearance of the product, whereas pigments or colourants are the chemical compounds that impart the observed colour (Wrolstad, Durst, and Lee, 2005).

Spectroscopic, chromatographic and electrophoretics-based methods are analytical techniques which are continuously developed for quantification of curcuminoids. The use of instruments capable of providing fast, reliable and on-site applications are highly needed (Rohman, 2012).

6.1.1 Optimization of chromatographic conditions

In this study, reversed-phase high performance liquid chromatography (RP-HPLC) was used for curcuminoid pigment quantification. The RP-HPLC optimization procedure was focused on the analytical column, mobile phase composition and separation temperature.

In the present work, a Zorbax Eclipse XDB C_{18} (4.6 x 150 mm, 5 µm) column was used for curcuminoid quantification. This column delivered high efficiency and excellent peak shape. The reason a 150 mm column was used is due to the fact that with shorter columns (50 mm), it is

difficult to separate mixed standards which elute at the same time. With longer columns (250 mm), the analysis time is unnecessarily long. Hence, the 150 mm column was selected to perform this experiment and was a good compromise between the 50 mm and 250 mm columns.

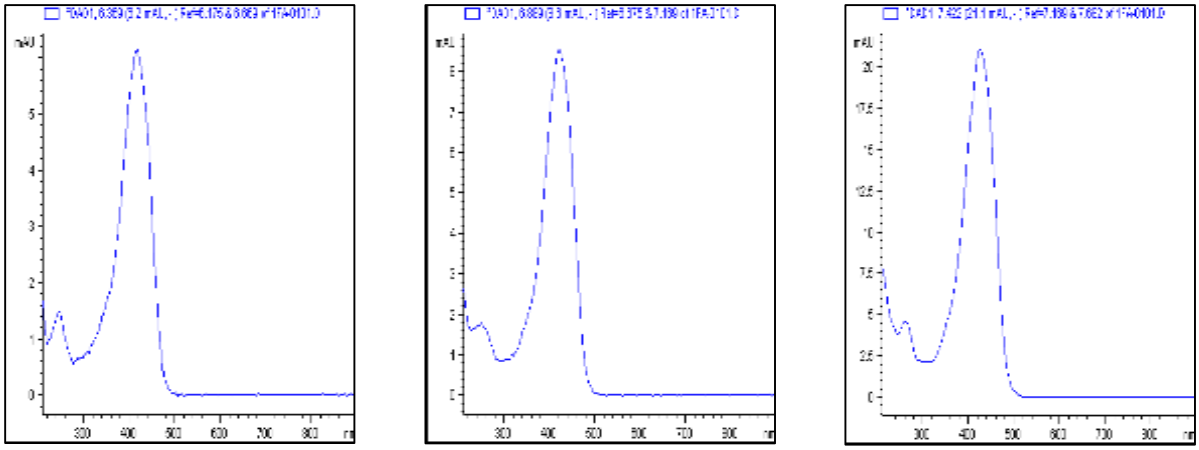
Mobile phase optimization of the HPLC method was conducted using various concentrations of acetonitrile while keeping the pH of the aqueous phase constant. A solvent combination of phosphoric acid and acetonitrile gave a satisfactory separation of the compounds of interest. This optimized mobile phase separated curcumin at 7.4 min. An increase in the concentration of organic phase in the solvent system resulted in faster elution but loss of resolution. On the other hand, increasing the aqueous phase concentration caused peak broadening and an increase in retention time. The flow rate was maintained at 1 ml/min, the injection volume was 1 µl and the column temperature was set at 45 °C. Elution proceeded by gradient elution, whereby the amount of organic solvent was increased over time. Therefore, the solutes eluted in order of increasing molecular hydrophobicity (Aguilar and Hearn, 1996). Using these conditions, all the curcuminoids were separated within 15 minutes and showed good resolution between the analyte peaks. The calibration curve of curcuminoids was linear (R^2 value of 0.999).

In order to understand the influence of temperature on the separation process, a temperature test on the chromatographic column was carried out by varying the temperature between 30 and 45 °C. It was observed that there was a variation in retention time and signal-noise ratio as column temperature changed. As expected, with an increase in temperature of the chromatographic column, the retention time slightly decreased. However, some of the analyte peaks were not separated completely because their retention times were too close. The best results were achieved at 45 °C as the peaks were narrow and the signal to noise ratio was reduced.

The type of detector used is also a very important component of HPLC. Detector selection depends on the chemical nature of analytes, potential interference, limit of detection required, availability and/or cost of detector. A UV-Visible detector, which was used in this study, is a versatile, dual wavelength absorbance detector for HPLC. This detector offers the high sensitivity required for routine UV-based applications to low-level impurity identification and quantitative analysis (Gupta, Kumar, Gill, and Gupta, 2012). In this study, all curcuminoids showed a maximum absorption wavelength near 420 nm (Figure 6.1).

In the past, various solvents with varying polarity such as hexane, chloroform, methanol and acetone have been used for the extraction of curcuminoids from turmeric oleoresin (Revathy, Benny, and Antony, 2011). In this study, methanol was the choice of extracting solvent as it is relatively inexpensive, is able to dissolve curcuminoids, is relatively free of regulation compared to ethanol and is easily evaporated.

Figure 6.1 shows the UV spectra of curcuminoids. All of them showed a maximum absorption wavelength near 420 nm.



A B C

Figure 6.1: UV spectra of curcuminoids (A: *bis*-demethoxycurcumin, B: demethoxycurcumin, C: curcumin)

6.1.2 Measurement of colour

Measurement of visual colour using the CM-700d optical sensor (Konika Minolta Sensing Inc., Japan) proved to be highly useful in monitoring colour changes of turmeric oleoresin powders over time in the presence of external environmental conditions. Poor colour memory, eye fatigue, colour blindness and viewing conditions can all affect the human eye's ability to distinguish colour differences. In addition to these limitations, the eye does not detect differences in hue, C or lightness equally (Ross, 2009). In these cases, the use of analytical methods to measure visual colour is of extreme importance (Piggott, 1995).

Objective quality control of colour by instrumental assessment has proven to be a reliable and affordable technology for any product where colour is an important quality criterion, as in the food industry. In addition, the increasingly global relationship between raw material suppliers,

component manufacturers, assemblers and buyers make precise colour data communication essential.

In this study, colour was measured using a CM-700d optical sensor (Konika Minolta Sensing Inc., Japan) and expressed in terms of "L^*" (lightness), "b^*" (yellowness and blueness), hue angle (H°) and chroma (C).

L^* is a measure of lightness which is the reflectance factor given as a percentage (Weatherall and Coombs, 1992). The lightness index L^* ranges from no reflection ($L^* = 0$, black) to perfect diffuse reflection ($L^* = 100$, white). The coordinates a^* and b^* can take positive and negative values: on the horizontal axis, $+a^*$ and $-a^*$ indicate hues of, respectively, red and green, and, on the vertical axis, +b* and -b* represent hues of, respectively, yellow and blue. As a^* and/or b^* increases, chromaticity increases. Numerical values of a* and b* are converted into the saturation index or C ($C = (a^{*2} + b^{*2})^{1/2}$), a measure of chromaticity and the hue angle ($H° = \arctan b^*/a^*$) (Voss 1992).

The H° indicates the colour of the material surface and is an angle in a colour wheel of 360°, with 0, 90, 180 and 270° representing the hues of red, yellow, green and blue, respectively. Together, L*, H° and C give an accurate description of the colour of a sample (Madeira *et al*., 2003).

While this system did not necessarily give an accurate definition of colour, it was effective for measuring colour differences and tracking colour changes during this shelf life study. Additionally, as the instrument is hand-held and weighs 550 g, it allowed for excellent portability for on-site measurement. In addition, its ergonomic vertical alignment was perfectly suited to position and measure round or even concave shaped parts and samples with single hand operation. The instrument also has a colour LCD screen which helped improve colour data reading and perception. The display screen showed colour readings both in numerical or graphical mode. This greatly improved operability and understanding.

6.1.3 Sensory evaluation of colour

Sensory evaluation of turmeric oleoresin powders was conducted in order to relate degradation of curcuminoid pigments in turmeric oleoresin with colour stability during storage.

A rating test method was chosen to match the project objectives. Trained individuals were used to measure yellow colour intensity. The sensory test was conducted under controlled conditions to reduce bias on how panellists view the samples. The sensory room (Figure 6.2) was free from distractions such as sound and odours in order not to influence a panellist's decisions about the samples. Samples were also presented in a random order and assigned three-digit sample codes, to keep food products anonymous and to further reduce influencing the panellists' decision. The sensory test was designed and conducted to measure if any differences detected were truly significant by analysing the sensory data for statistical significance. After statistical analysis, a meaningful interpretation from the results of the sensory data was made.

Figure 6.2: Sensory room in which evaluation of turmeric oleoresin powders was conducted.

In general, analytical sensory methods of evaluating colour are faster and easier in many ways than instrumental methods. Human measurements are also variable, but can be made more reliable if appropriate methods and procedures are used. As an example, in this study, the sensory evaluation procedure was standardised using a colour chart such as that shown in Fig. 4.2. As with any testing, resources are needed for good measurements. Sensory data can facilitate good decisions on a variety of issues and the improvement in the quality of the information collected will have long-term value for product decisions. However, the disadvantages are that these

methods may vary considerably due to human differences in perception and human error. Inadequate or poor quality available light may also affect accuracy (Mitcham, Cantwell, and Kader, 1996).

Instrumental methods, on the other hand, are less variable and can be used to measure small differences. The advantages of instrumental techniques are the fact that some instruments are portable and others may be adapted for packing lines. Disadvantages of instrumental methods are that many instruments used to measure colour may be expensive and slower than sensory measurements (Mitcham *et al*., 1996).

To summarise, it can be said that in addition to odour, taste and texture, visuals in particular play a crucial role in quality assessment of foods by consumers. Even though humans alone are able to provide a comprehensive, holistic sensory test result due to their linking of sensory perceptions in the brain and their empirical knowledge, optical sensors offer supplementary support in key application areas of quality assurance and product development for assessing product appearance due to their high measuring sensitivity, objectivity and reliability (Jiang, Somogyi, Jacobsen, Timmermann and Gang, 2006).

6.2 Discussion of main trends and mechanisms

As shown in this study, curcuminoid pigments in turmeric oleoresin had poor stability to external environmental conditions, including sunlight and outside temperature during the day. The efficiency of turmeric as a food colourant depends on the content and molecular activity of its curcuminoid constituents which can be altered by many factors such as storage, processing conditions and the physico-chemical properties of end-use products. Hence, it is necessary to study the effect of those factors (Ahmed and Sandu, 2002).

The present study focused on investigating the effect of external environmental conditions on the stability of curcuminoid pigments in turmeric oleoresin powders, as well as the effect of curcuminoid pigment degradation on the colour of food. The content of curcuminoid pigments in turmeric oleoresin powders stored over 10 weeks, with and without protection from external environmental conditions, was assessed.

6.2.1 Curcuminoid degradation profiles

6.2.1.1 Degradation of curcuminoids in turmeric oleoresin powders exposed to external environmental conditions

Curcuminoid pigment degradation was evidenced over time, with 100% degradation observed relative to the control that was refrigerated at 4 °C. This degradation occurred due to the fact that, under the influence of light, curcumin acts as photo-sensitizer of oxygen radicals and undergoes self-sensitized decomposition. In general, the mechanism of degradation includes the physical and chemical changes caused by irradiation of polymers with UV or visible light (Tonnesen *et al.*, 1986).

This degradation process occurs due to the presence of chromophoric groups in turmeric. Chromophores consist of chemical bonds and atom configurations that cause the molecule to absorb light. Light energy must first be absorbed by a chromophore for a photochemical reaction to occur. Hence, the existence of these chromophoric groups in turmeric is a prerequisite for the initiation of any photochemical reaction (Schnabel, 1981).

Curcumin is regarded as the major chromophoric substance in turmeric (Heger, van Golen, Broekgaarden, and Martin, 2014; Tonnesen *et al*., 1986). Curcumin has two ketone carbonyl groups (Figure 6.3), of which only one can convert to an enol form. The double bonds in the enol form alternate with single bonds throughout the complete molecule. These alternating chains of double and single bonds constitute an important characteristic of chromophoric molecules (Kumavat, Chaudhary, Borole, Mishra, Shenghani, and Duvvuri, 2013). Curcumin absorbs light energy in the visible wavelength range, making it susceptible to degradation in daylight and artificial lighting (Tonnesen *et al*., 1986; Schnabel, 1981).

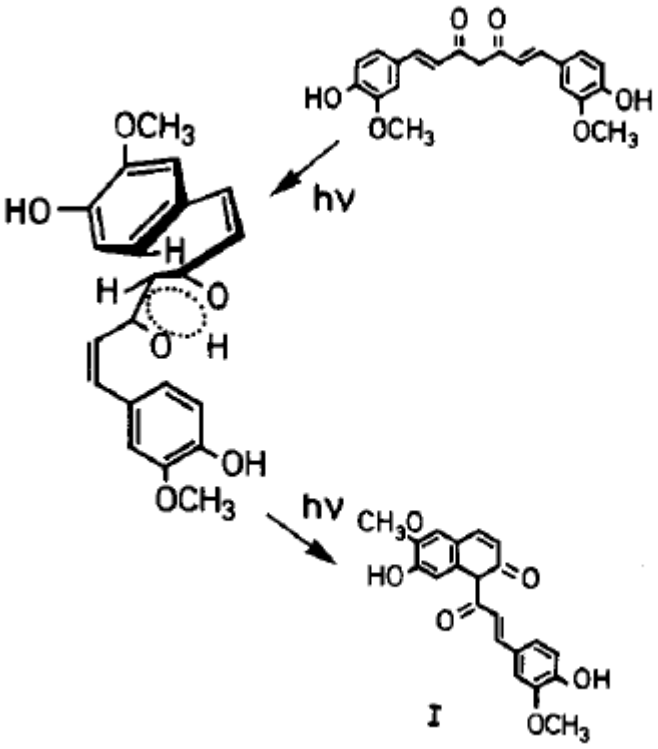


Figure 6.3: Ring-closure of the curcumin molecule after exposure to light (λ>400nm). I represents the postulated structure of the cyclisation degradation product (Tonnesen *et al.*, 1986)

From this study, it was evident that environmental temperature also had a strong influence on the degradation of curcuminoid pigments in turmeric oleoresin (average daytime temperature of the surroundings was ± 24 °C). Results revealed that curcuminoid pigments were less sensitive to degradation when shielded from light (covered with aluminium foil), with a 22% reduction in degradation observed relative to samples without aluminium foil protection after 10 weeks of exposure to external environmental conditions. However, after 9 weeks of exposure to external environmental conditions even with aluminium foil protection, 100% degradation of curcuminoid pigments was observed. This indicates that temperature had a strong influence on the degradation of curcuminoid pigments in turmeric oleoresin and that constant storage of ingredients and products containing turmeric oleoresin at low temperature (<5 °C), together with light protection is vital to retard colour degradation. These results were also consistent with results for samples that were refrigerated at 4 °C in the dark, which showed less degradation compared to samples that were covered with aluminium foil and exposed to direct external environmental conditions, with a four times lower percentage degradation observed after 10 weeks for samples that were refrigerated at 4 °C.

6.2.1.2 Degradation of curcuminoids in turmeric oleoresin covered with aluminium foil and exposed to external environmental conditions

Any kind of spoilage or quality loss can be reduced or even stopped by the right regulation of moisture, oxygen, temperature and light. As demonstrated in this study, food packaging such as aluminium foil should be used as a measure to control or retard colour loss of curcuminoids in turmeric oleoresin in the presence of external environmental conditions (Tang and Norziah, 2007).

In general, food containing light-sensitive ingredients should be stored away from direct sunlight. This is because direct sunlight contains ultraviolet rays that cause rapid deterioration of some foods or specific components of a food such as certain colourings. Electric lights do not have the same effects on foods because they do not contain the UV component (Otles, 2008).

In this study, powdered turmeric oleoresin samples were placed in labelled plastic jars. The jars were covered with aluminium foil and exposed to external environmental conditions for a period of 10 weeks. It should be noted that the degree of protection offered by packaging depends on the absorption characteristics of the material, material thickness, material processing conditions and colour of the package.

The aluminium foil acted as a good barrier to external environmental conditions, with curcuminoid pigments showing less sensitivity to degradation when shielded from light, with a 22% reduction in degradation observed compared to samples without aluminium foil protection after 10 weeks of exposure to external environmental conditions.

The reason aluminium foil was used in this study was because, besides providing an excellent barrier to light, it has good flexibility and surface resilience. Foil protects foods from adverse external environmental conditions such as exposure to ultraviolet rays from sunlight which could cause loss of colour. The ability of aluminium to form any shape as well as its protective qualities, have made it one of the most versatile packaging material in the world. However, the main disadvantage of aluminium is its high cost compared to other metals (Otles, 2008).

6.3 Stabilization strategies

In an attempt to enhance curcuminoid stability in external environmental conditions, the effect of antioxidants (TBHQ and ascorbic acid) on the stability of curcuminoid pigments in turmeric oleoresin, was studied. The effect of Mg^{2+}- curcuminoid complexation on the stability of curcuminoid pigments in turmeric oleoresin when exposed to external environmental conditions, was also investigated.

6.3.1 Effect of antioxidants (TBHQ and ascorbic acid) on the stability of curcuminoids in turmeric oleoresin powders exposed to external environmental conditions

Several antioxidants, such as citric acid and TBHQ are used as preservatives in vegetable oils (Kim, Lee, Choi, and Won, 2009). The present study investigated the effect of TBHQ and ascorbic acid on the stability of curcuminoid pigments in turmeric oleoresin using ambient shelf life tests. The addition of antioxidants may influence the colour of powdered food products, which could be an issue for product quality. In order to assess this, the colour values of the different powdered curcuminoid samples were measured prior to the stability tests.

According to the L^*, a^* and b^* values, the addition of ascorbic acid did not significantly improve the colour stability of the turmeric oleoresin powders. The curcuminoid degradation process was inhibited in the presence of TBHQ in the first eight weeks of external environmental conditions exposure, with a 17% reduction in degradation observed after 1 week of exposure to external environmental conditions, relative to samples containing ascorbic acid. In general, the antioxidant effect of TBHQ is due to its ability to scavenge lipid free radicals, mainly by hydrogen transfer (Allam and Mohamed, 2002).

Interestingly, the presence of ascorbic acid had no significant influence on curcuminoid stability, with the degradation trend following that of the control, which did not contain ascorbic acid. This is possibly due to the fact that environmental conditions may alter the antioxidative capacity of ascorbic acid. For example, endogenous ascorbic acid in foods can be easily destroyed during processing as a result of susceptibility to heat, light, pH, oxygen and water activity. It is thus is often added to foods exogenously.

6.3.2 Effect of Mg^{2+} complexation on the stability of curcuminoids in turmeric oleoresin powders exposed to external environmental conditions

In the past years, many research papers have been published on metal-curcumin complexes (Song, Xu, Ding, Hou, Liu, and Zhu, 2009; Priyadarsini, 2014). Several metallo-complexes of curcumin have been synthesized, characterized and evaluated for various biological activities (Song, Xu, Ding, Hou, Liu, and Zhu, 2009). Curcumin is a monobasic bidentate ligand and forms stable complexes with almost all the metals and non-metals. In general, stable structures with 2:1 (ligand:metal) stoichiometry are observed (Priyadarsini, 2014). Therefore, formation of complexes between metals and pigment ligands could be a potential way of stabilizing the pigments and preventing their degradation.

In this study, the effect of Mg^{2+} complexation of curcumin by mechanical mixing, was investigated. As curcumin is a monobasic bidentate ligand, it would be expected to form stable complexes with almost all the metals and non-metals (Subhan *et al*., 2014). In general, metal coordination of curcumin occurs through the enolic group, where the enolic proton is replaced by the metal ion and the o-methoxy phenolic moiety remains intact in the complexes (Priyadarsini, 2014).

After 10 weeks of exposure to external environmental conditions, no significant improvement in curcuminoid stability was observed after the addition of magnesium to turmeric oleoresin powders when compared to the control, which did not contain Mg^{2+} complexes. The structure (Figure 6.4) and physical properties of these complexes depend on the nature of the metal ion, as well as the stoichiometry of the reaction conditions, which in turn, decides their stability and reactivity (Priyadarsini, 2014).

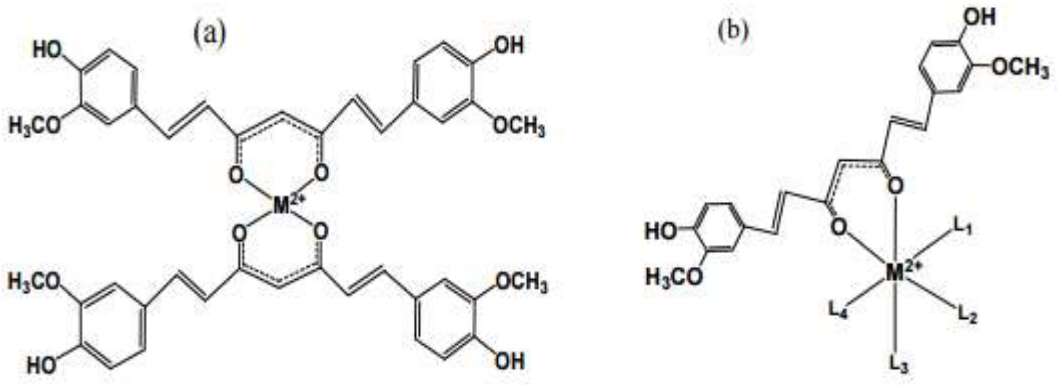


Figure 6.4: (a) Structure of 2:1 curcumin: metal complex; (b) Mixed ligand curcumin: metal complex (Priyadarsini, 2014)

6.4 Colour measurements

Since C reflects colour brilliance or purity and is correlated with the curcuminoid content, the C values of the control samples (stored in a refrigerator at 4 °C) were expected to be superior to those of samples that were directly exposed to external environmental conditions due to the lack of a protective physical barrier in the latter. As C (saturation or vividness) increases, colour becomes more vivid and as it decreases, colour becomes duller. This was evidenced, with a 33.60 degree decline in C from week 1 to week 10 of the experiment.

Colour ratings showed a strong positive correlation with C values. This correlated well with the fact that a higher sensory rating corresponds to a darker yellow colour and this corresponds to a larger C value.

6.5 Limitations of study

A limitation of this study was that environmental conditions i.e. temperature, humidity, light exposure and oxygen concentration were not monitored or controlled. The intention was to simulate the practical situation that occurs in the food industry. Hence, it was not possible to conclude whether the reason for curcuminoid pigment degradation in turmeric oleoresin powders was due to light exposure, temperature, oxygen or humidity, specifically. If these parameters are not measured, then accuracy of experimental results may be compromised. This study also did not take into consideration the combined effects of temperature, light exposure, oxygen or humidity on the stability of curcuminoid pigments in turmeric oleoresin powders. Future studies should include the control and monitoring of experimental and environmental conditions in order to ensure that further research studies can be compared, understood and where necessary, replicated by fellow researchers.

6.6 Future prospects

6.6.1 Packaging

Any kind of spoilage or quality loss in foods can be reduced or even stopped by the right regulation of moisture, oxygen, temperature and light. As demonstrated in this study, food packaging such as aluminium foil may be used as a measure to control or retard colour loss of curcuminoids in turmeric oleoresin in the presence of external environmental conditions (Moller, Jensen, Olsen, Skibsted, and Bertelsen, 2000).

Aluminium foil proved to be an excellent barrier to light and prevented curcuminoid pigment degradation in turmeric oleoresin compared to samples without light protection. However, the main disadvantages of aluminium foil is its high cost compared to other metals and its inability to be welded, which renders it useful only for making seamless containers. To counter such problems, active and intelligent packaging can be used. In recent years, interest in the use of active and intelligent packaging systems for foods like meat and meat products has increased (Otles, 2008).

Active packaging refers to the incorporation of additives into packaging systems with the aim of maintaining or extending meat product quality and shelf-life. Active packaging systems include oxygen scavengers, carbon dioxide scavengers and emitters, moisture control agents and antimicrobial packaging technologies (Gander, 2007). Recognition of the benefits of active and intelligent packaging technologies by the food industry, development of economically viable packaging systems and increased consumer acceptance is necessary for commercial realisation of these packaging technologies (Otles, 2008).

The most common active systems scavenge oxygen from the package or the product and may even be activated by an outside source such as UV light (Gander, 2007). As an example, it is applied in the meat industry. In fresh red meats, myoglobin can exist in one of three chemical forms. Deoxymyoglobin, which is purple, is rapidly oxygenated to cherry red oxymyoglobin on exposure to air. Over time, oxymyoglobin is oxidised to metmyoglobin which results in a brown discolouration associated with a lack of freshness (Faustman and Cassens, 1990). Low oxygen concentrations favour oxidation of oxymyoglobin to metmyoglobin (Ledward, 1970). Therefore, in order to minimise metmyoglobin formation in fresh red meats, oxygen must be excluded from the packaging environment to below 0.05% or present at saturating levels (Faustman and Cassens, 1990).

Exposure to light in combination with oxygen is of critical importance to the colour stability of cooked cured ham as light exposure, even at low oxygen levels, can cause oxidation of nitrosylmyochrome to denatured metmyoglobin, which imposes a dull undesirable greyness to the meat surface (Moller, Jensen, Olsen, Skibsted, and Bertelsen, 2000). In such cases oxygen scavengers are examples of entities described as ‘active packaging components’. This technology can also be used for storage of ingredients and products containing turmeric oleoresin in order to retard colour degradation (Otles, 2008).

Another possible packaging mechanism to avoid colour degradation in turmeric is to incorporate antioxidants into the packaging material. This is because environmental conditions may alter antioxidative capacity (Otles, 2008).

Previous studies have also shown that TBHQ is particularly useful in retarding oxidative rancidity in lard when incorporated into the packaging material and that it is as effective as butylated hydroxyanisole (BHA) when incorporated in waxed liners in packaging materials used for breakfast cereals. TBHQ incorporated into the packaging is an example of how food manufacturers are able to get antioxidant additives into foods without having to declare them on any ingredients list (Cakmakci and Turgut 2005).

However, the disadvantages of the system is the potential migration of the packaging particles into food. The concept of intentional migration of substances, like antioxidants and preservatives from the package into the food are new perspectives for the food packaging which are introduced and these are related with intelligent packaging (Otles, 2008).

CHAPTER 7

7 Conclusions and recommendations

Within the limitations of this study, it can be concluded that curcuminoid pigments in turmeric oleoresin powders show degradation when exposed to external environmental conditions and that the level of pigment degradation is dependent on time of storage. It is also concluded that constant storage of ingredients and products containing turmeric oleoresin at low temperature (<5 °C), together with light protection is vital to retard colour degradation.

The addition of ascorbic acid to turmeric oleoresin powders does not significantly improve curcuminoid stability. This is possibly due to the fact that environmental conditions may alter antioxidative capacity. For example, natural ascorbic acid in foods can be easily destroyed during processing as a result of susceptibility to heat, light, pH, oxygen, and water activity. TBHQ enhanced the stability of curcuminoid pigments in turmeric oleoresin to a greater extent compared to ascorbic acid during the earlier weeks of storage when exposed to external environmental conditions over time. This shows the relative stability of TBHQ to oxidation compared to ascorbic acid.

Furthermore, Mg^{2+}-curcuminoid complexation does not improve curcuminoid stability. Future work on binding of curcuminoids and metals should examine other metals, and explore methods to measure metal affinity to curcuminoid pigments.

Knowledge about the stability of curcuminoids in external environmental conditions, as well as the factors that affect curcuminoid pigment stability may enhance the application of natural colourants in the food industry and may also be used as a tool to evaluate product quality.

However, future research is necessary to elucidate the kinetics and mechanism of curcuminoid pigment degradation. Further studies on the use of antioxidants to improve natural pigment stability should be investigated, such as a potential synergistic effect between TBHQ and ascorbic acid, as well as other antioxidants.

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