

*STRATEGIES FOR STABILIZATION OF
NATURAL COLORANTS*

An Investigation

By

RAHUL SHARMA

Submitted in partial fulfillment for the Degree of
Master of Science in Food Technology

**University of Mysore
Mysore, Karnataka**

Human Resource Development Department
Central Food Technological Research Institute
Mysore-570020

JUNE 2005

Acknowledgement

It gives me great pleasure to express my heartfelt thanks and gratitude to my guide **Dr. P. Srinivas**, Scientist, Plantation Products, Spices and Flavor Technology Department, Central Food Technological Research Institute, Mysore for his valuable guidance, worthy suggestions and continuous encouragement throughout the investigation work.

I am extremely grateful to **Dr. V. Prakash**, Director, CFTRI, Mysore, **Mr. B. Raghvan**, Head, PPSFT Department and **Dr. M. C. Varadaraj**, Head, Human Resource Development for providing me an opportunity to undertake this work.

I wish to extend my thanks to **Mr. S. R. Sampathu**, Scientist, PPSFT, **Ms. H. B. Sowbhagya**, Scientist, **Ms. K. Ramalakshmi**, Scientist PPSFT and **Mr. S. G. Jayaprakash**, FEPD for guidance and help during the product preparation and color assay.

I would like to extend my thanks to **Dr. B. S. Jena**, **Dr. R. P. Singh**, **Dr. P. S. Negi** & **Dr. G. K. Jayaprakash** of HRD for their constant encouragement and all other staff members of HRD for their cooperation.

I am also thankful to **Dr. H. H. Pattekhan**, Scientist, TTBD and **Mr. J. R. Manjunath** and **Ms. Divya Parvati**, research students in PPSFT for their assistance during the experimental work in the laboratory. I convey my sincere thanks to faculty members of the PPSFT department for their help during the course of this work.

I am highly gratified to my beloved parents, brothers and sisters for their constant enlightenment, encouragement and love.

My thanks are also due to all my friends and classmates, who are associated with the successful completion of this work.

CFTRI, Mysore

Date: 21 June 2005

Rahul Sharma

**Dr. P. Srinivas,
Scientist,
Plantation Products, Spices and Flavor Technology,
Central Food Technological Research Institute,
Mysore.**

21 June 2005

CERTIFICATE

I certify that this investigation report on '*Strategies for Stabilization of Natural Colorants*' submitted by Mr. Rahul Sharma in partial fulfilment of the award of Degree of Master of Science in Food Technology of the University of Mysore, is written by him based on the investigation work carried out under my guidance in the Department of Plantation Products, Spices and Flavor Technology, Central Food Technological Research Institute, Mysore.

(Dr. P. Srinivas)

INDEX

SR. No.	CHAPTER	PAGE NO.
1.	INTRODUCTION	1
2.	MATERIALS AND METHODS	10
3.	RESULT AND DISCUSSIONS	14
4.	SUMMARY AND CONCLUSIONS	21
	BIBLIOGRAPHY	23

CHAPTER 1

INTRODUCTION

General

The color of foodstuff is an important attribute, which is considered as one of the primary characteristics perceived by the senses that helps in determining the acceptability, judging the quality and appreciating the aesthetic value of a foodstuff (Sampathu *et al.*, 1981; Santhanakrishna *et al.*, 1981; and Pattnaik *et al.*, 1999). Color helps in recognition of food and also makes it appealing to the consumer. The consumer tends to judge the quality of a food product by its color. Also, appetite is stimulated or dampened in almost direct relation to the observer's reaction to color. The color, therefore, has a direct bearing on the flavor of a food product. In terms of food manufacturing a good strawberry color, for example, can indicate to the consumer a high quality product, whilst a washed out or artificially bright product can indicate poor quality or an inferior product.

The practice of adding colors to processed foodstuffs to make them attractive dates back to very ancient times. Our ancestors used saffron, turmeric and vegetable dyes. In 1856 the first synthetic color (mauvine), was developed by Sir William Henry Perkin (Walford, 1980) and by the turn of century, unmonitored color additives had spread through USA and Europe in many popular foods, including ketchup, mustard, jellies and wine. Sellers at that time offered more than 80 artificial coloring agents, as these were found more technically suitable for use in food than natural ones in efficiency, shades, range and cost. Thus for many years synthetic colorants were used in majority of formulations without having been tested for their safety. From 1907, certification requirements saw many of them gradually withdrawn, as they were found harmful. The list of certified synthetic colorants has been continuously shrinking since then. In India, a

blanket ban on use of synthetic color additives has been suggested in 1981 (Sankarnarayan, 1981).

In recent years, therefore, manufacturers have shown a greater interest in the natural colorants. Identification of alternative safe colorants from natural sources and development of a few synthetic routes to then have partly met the color need of food industry. Also due to public prejudice against synthetic additives, manufacturers want to label their product as having, a natural ingredient or no additives and hence, the preference to use the natural colorants.

Why is Color added:

The colors are added to the foodstuff on the basis of some specific reasons (Sampathu *et al*, 1981), which are further governed by individual requirements. These reasons are as follows:

1. *Original appearance:*

Due to the effect of various processing conditions, the natural color of foodstuff gets destroyed e.g. color of canned pear, beans, strawberries, etc. is lost during storage. To restore the original appearance of these foods, it is necessary to add color in the foodstuffs.

2. *Uniformity of Product:*

Product obtained may vary in their appearance from season to season and even batch-to-batch e.g. addition of a specific quantity of color becomes necessary e.g. in case of butter, to make the product uniform throughout the year.

3. *Intensifying Natural Color:*

In some cases, the colors of the natural ingredients are too weak and it is well known that the consumer prefers stronger colors. So colors are used to intensify colors of those naturally occurring in foods e.g. fruit yogurts.

4. ***Product Identification:***

There are some products where there is little or no color of its own e.g. liquid beverages, sherbets, jams, and jellies. So color addition helps to preserve the identity or character of the food.

5. ***Protection:***

Coloring helps to protect flavor and light sensitive vitamins during shelf storage by a sunscreen effect.

6. ***Attractive Appearance:***

To give an attractive appearance to food, which would otherwise look unattractive or unappetizing, e.g. colorless gelatin base jelly and thus enhance enjoyment.

7. ***Antioxidant and nutroceutical action***

The carotenoids and other pigments like curcumin have been shown to possess strong antioxidant properties and a host of other health promoting and disease alleviation effects which further enhance the importance of incorporation and use of natural colours in foods and other products.

Some other benefits of addition of colorants to food products are as follows (Pattnaik *et al*, 1999):

1. Some indication for how much to use and how long to store.
2. Improved stability and reduced wastage in storage.
3. Reduced cost and improved quality through manufacturer benefits.

Market Trends:

There are no reliable published statistics on the size of the indigenous color market. However, on a global scale a reasonable estimate would be \$940 million, which can be segmented as follows (Downham, 2000):

- a) Synthetic colors - \$400 million
- b) Natural colors - \$250 million (of which \$100 million is in the USA)
- c) Nature identical colors - \$189 million
- d) Caramel colors - \$100 million

Consumer pressure, sociological changes and technological advances leading to more advances in the food processing industry have increased the overall color market. The most significant growth has been in naturally derived colors owing to the improvements in stability as well as the food industries aim to meet the increasing consumer perception that '*natural is the best*'. Future growth is thought to be going to be greatest for naturally derived colors with a predicted annual growth rate of 5-10%. Synthetic colors are still forecast to grow but a lower rate of between 3 and 5%.

Natural Colors:

Natural colors are the colors extracted from natural sources or manufactured by biosynthetic routes. Natural colour is spread widely throughout nature in fruits, vegetable, seeds and roots. In our daily diets we consume large quantity of many pigments especially anthocyanins, carotenoids and chlorophylls. These are also known as naturally derived colors. The shades and key characteristics of most commonly used permitted naturally derive colour are shown in table 1.0 (Downham and Collins, 2000).

Table – 1

Pigment	Sources	Color Shade	Comments
Curcumin	Turmeric Rhizome roots	Bright lemon Yellow	A non-soluble pigment that is light sensitive once solubilized and in the presence of water. Used widely in desserts and confectionery.
Lutein	Marigold Alfalfa	Golden Yellow	Oil soluble carotenoid pigment naturally present to use water-soluble forms with increased oxidative stability has extended its use to applications such as desserts, soft drinks and bakery products. Only permitted in the USA in chicken feed.
Natural mixed carotenes	Palm oil, <i>D. Salina</i> (algae)	Golden yellow to orange	Another oil soluble mixture of carotenoids (principally β -carotene), which is naturally sensitive to oxidation. The USA permits only algal source. Has pro vitamin A activity
Bixin/ Norbixin	<i>Bixa orellana</i> bush seeds	Orange	Bixin is oil soluble and norbixin water dispersible. Widely used in ice cream, cheese, yellow fats and smoked fish
Capsanthin/ Capsorubin	Paprika <i>Capsicum annum L.</i>	Reddish orange	Natural oil soluble carotenoids. Used in savory products such as sauces and coatings as well as desserts, confectionery and bakery products.
Lycopene	Tomatoes	Orange red	Natural oil soluble carotenoid pigment. Has found little commercialization as a color owing to high cost, poor stability and orange rather than red shade.
Anthocyanin	Black grapeskin, elderberries, black carrots, red cabbage	Pink/red to mauve/blue depending on pH	Natural pigments of many red fruits, flowers and vegetables.
Chlorophyll	Grass, lucerne and nettle	Olive green	Chlorophylls are oil dispersible and chlorophyllins water-soluble. Limited use owing to poor stability and dull color shade.

However, these pigments from nature vary widely in their physical and chemical properties. Many are sensitive to oxidation, pH change and light. Due to less stability of natural colorant, this poses many problems in their use in food systems.

Strategies for Stabilization:

From last few past decades much emphasis has been given to colour formulation, which offer stability to natural and natural identical pigments as they have much variation in their solubility and stability to various conditions. Colour formulation development has focus on improvement in three following areas.

1. Making water dispersible from natural oil soluble pigments by forming a dispersion of oil soluble pigment throughout in aqueous disperse phase using emulsifier and stabilizer.
2. Enhancing stability to oxidation via the synergistic use of antioxidant such as tocopherol and ascorbyl palmitate.
3. Improved stability and clarity for application such as soft drink, jellies and conserves.

Another approach to stability and solubility of natural colours is through processing technology such as pigment encapsulation into insoluble carrier to produce ranges of suspension with following benefits.

1. Improvement to light stability (e.g. Curcumin) and extending its area of applications to product like sauces, dressing and soft drink.
2. Conversion of oil dispersible form of natural colours to water dispersible pigments
3. A reduced level of certain additives

4. Production of different colour shades from those normally exhibited by the soluble pigments
5. Less migration of colors during application such as in desserts.

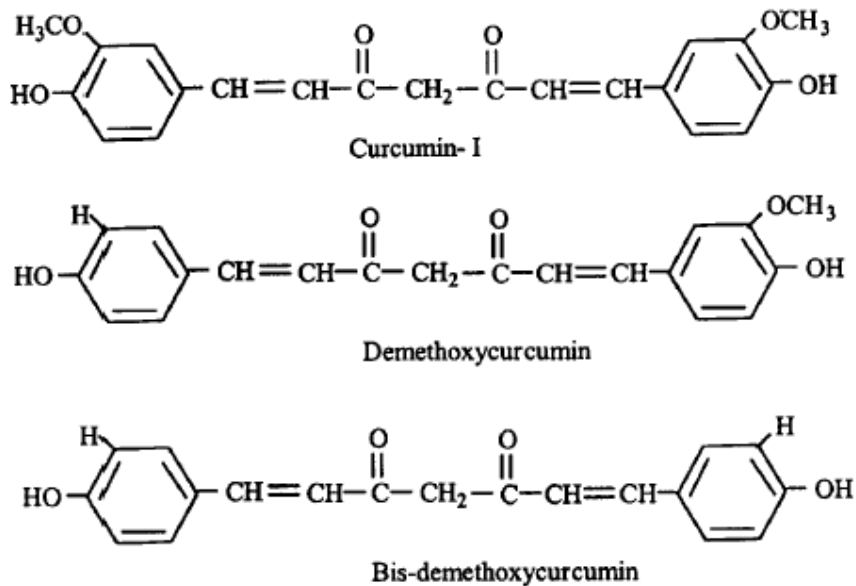
In addition to this, protection provided is dependent on encapsulating matrix, efficiency of encapsulation or coating and point at which the pigment is released. The ranges of encapsulating material and methods suitable for colours are carbohydrates, gums, phospholipids and proteins and spray drying (SD), spray coating, spinning disc technology, freeze-drying (FD) respectively.

Curcumin

Curcumin (1-7-bis (4-hydroxy-3-methoxy phenyl) –1,6- heptadiene-3, 5-dione) is a natural yellow orange dye extracted from the rhizome of plant *Curcuma longa* L. (Zingiberaceae). It is a natural compound primarily used for food coloring. Further, the substance has pharmacological properties such as antitumor and anticancer activities. It is also a photodynamic agent used for the destruction of bacterial and tumor cells. Curcumin is non-toxic even at high dosages. Other than food colourant, it is used as antioxidant additives for lubricants and motor oil and in sunscreen compounds (Hoon Bong, P, 2000).

Curcumin and curcuminoids are practically insoluble in water at neutral and medium acid condition. On the other hand, it is soluble in alkali or in glacial acetic acid. However, its colour is not constant in aqueous medium or in organic solvents due to degradation or conversion to its dissociation forms (Tonnesen and Karlsen, 1985). Alkaline degradation of curcumin (CC), demethoxy curcumin (DMC) and Bis-demethoxy curcumin (BDMC) in aqueous solution follows first order kinetics. This response is also similar for pigments combined or alone in solution. Degradation rate constant rapidly increases from pH 7.45 to a maximum at approximately at pH 10.2 and

then tends to decline slightly at higher pH. The rate of alkaline degradation follows CC>DMC>BDMC in increasing order (Prince and Buescher, 1997)



Prince, 1996 had studied the light induced degradation of CC, DMC, and BDMC by exposing purified pigments to light of 1450-lux intensity. On analytical separation and molar quantification on reverse-phase HPLC, they had reported that stability of curcumin depends on the solvent system and presence of oxygen that in turns follows first order kinetics. In the same study curcumin solution in methanol sparged with air. The stability of curcuminoids against photo-oxidation was in the following increasing order CC>DMC>BDMC. The degradation products were later identified to be a cyclization product, vanillin, vanillic acid, ferulic acid, ferulic aldehyde and 4- vinylguaiacol.

Thus, use of curcumin in foods as coloring agent, pharmaceuticals and as a drug is limited due to its low solubility under acid and neutral condition, high decomposition in alkaline condition, oxidation in presence of oxygen and photodegradation in organic and aqueous solutions.

Strategies for Stabilization of Curcumin

Few attempts have been made for achieving stability of curcumin. Manabe *et al.*, 1974, enhanced the stability of curcumin by adding ascorbic acid with it showing optimum residual ratio and permeation into peeled chestnuts as well as in syrup. Solution of curcumin in propylene glycol has been reported to be more stable than its powder counterpart (Sowbhagya *et al.*, 1998)

Curcumin is also stabilized against colour change by maintaining it at pH range of from about 3.5 to 4.5 which comprises a spray dried intimate mixture of curcumin, an organic acid, a buffer, a dispersant for curcumin and a film forming encapsulate. (Richard, 1980).

Curcuminoids are not affected by water activity. It was shown that there was no influence of water activity on the stability in curcumin and turmeric oleoresin-microcrystalline cellulose model system. (Souza *et al.*, 1997). A patent involves the formation of metal-curcumin coloring complexes, which are water soluble and more stable than curcumin. (Maing and Miller, 1981).

In one study, cyclodextrin complex of curcumin was found to improve the water solubility, hydrolytic and photochemical stability of compound (Tonnesen *et al.*, 2002). However, photodecomposition rate increases compared to curcumin solution in organic solvent as the cavity size as well as charge and bulkiness of cyclodextrin side chain influence the stability of complexion and degradation rate of curcumin. Therefore, it is of interest to study the use of other polysaccharides like gum acacia as wall material for micro-encapsulation of curcumin with additives.

Gum Acacia (GA) is a hydrocolloid obtained as natural exudates of acacia tree. GA is composed of a highly branch arrangement of simple sugar galactose, arabinose, rhamnose and glucuronic acid (Aderson and stodard, 1966; Street and Aderson, 1983). It

also contains proteinaceous material covalently attached to the polysaccharide component with an enhanced ability to absorb at oil-water interface and stabilizing effect on emulsion. This is due to the hydrophobic nature of proteinaceous chain, which absorbs at the surface of the oil droplets, while the hydrophilic carbohydrate blocks attached to protein chain protrude into aqueous solution preventing droplet aggregation and coalescence (Islam *et al.*, 1997). GA is also good encapsulant due to its high water solubility, low viscosity of concentrated solution relative to other hydrocolloids gums and ability to act as oil in water emulsion (Gliksman, 1983).

Literature survey showed that the approach of stabilization of curcumin by use of GA as encapsulant with antioxidants like TBHQ, Ascorbyl palmitate and their combination by SD as well as by FD has not been explored. In present study, preparation of Spray Dried Encapsulated Curcumin (SDEC) and Freeze Dried Encapsulated Curcumin (FDEC) with and with out additives and study of their stability against exposure to severe conditions like UV (365nm) and heat (45 °C) has been envisaged. It was conceptualized that encapsulation with GA could impart physical protection and act as a barrier to light as well as O₂. Also, it was thought that antioxidant additives could further stabilize the pigment against photo-oxidation.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS REQUIRED:

<i>Curcumin</i>	Natural flavours and essences, Mysore
<i>Gum Acacia</i>	Gum acacia-71, Alok Chemicals Corporation, India
<i>Ethyl Alcohol</i>	95 percent
<i>Tertiary Butyl Hydroxy Quinone (TBHQ)</i>	Sigma-Aldrich, USA
Ascorbyl Palmite (AP)	Sigma-Aldrich, USA
<i>Distilled Water</i>	Double distilled
Refine Sunflower oil	
Tween 60	Sigma-Aldrich, USA

EQUIPMENTS USED:

UV Lamp Allen 425 model LCF-750-Q

UV-VIS Spectrophotometer GBC Cintra 10

Ultra-Sonication Branson Model 1510

LSD-48 Mini Spray drier JISL

Freeze Drier

Lyophilization Model-503, USA

Homogenizer

Ultra-Turrax, T-25 Basic, Ira-Werke,
Germany

METHODS:

Product preparation

a) Preparation of Emulsion

Emulsion was prepared at room temperature as described by Mc Nanee *et al.*, 1998, with some modification. Emulsion dispersed aqueous phase consisted of GA (10%) and Tween 60. In addition to this oil phase consisted of refined sunflower oil (2.7% w/v), Curcumin (0.5% w/v) and additives in equal ratio to curcumin, were mixed with aqueous phase using high shear and subsequently homogenized at 16000 rpm in homogenizer (Ultra-Turrax T-25 Basic) for 10 minutes. Then emulsion was kept at low temperature in dark condition until it was subjected to drying.

b) Spray drying of Emulsion

For preparation of micro-encapsulated curcumin, prepared emulsion with or without additives was fed to LSD-48 Mini Spray drier at rate of 2 ml/ minutes, 2 kg/cm² with inlet temperature of 142± 5 °C and outlet temperature at 80± 3 °C. Spray dried product was collected in sealed plastic bags and kept at dry cool and dark place.

c) Freeze drying of Emulsion

Emulsion was frozen to -30°C . The freeze drier Lyophilization Model-503, USA operated at pressure of 250 mtorr. The temperature was increasing stepwise in following sequence i.e. -30°C for 2 hours the -20°C to 15°C within 18 hours. Dried sheets of microencapsulated curcumin were obtained at ambient temperature, collected in sealed plastic bags, which were subsequently powdered by hand pressing to uniform size particle size. The product was stored at a dry cool and dark place.

Sample Preparation

Determination of total colour value of Freeze Dried Encapsulated Curcumin (FDEC) control and FDEC with AP + TBHQ in equal amounts was done in duplicate as per American Spice Trade Association (ASTA) method with some modification. In that 0.2 g of sample was taken, to which 80 ml of 95% ethanol was added. Subsequently contents were refluxed for $2\frac{1}{2}$ hours. After refluxing, the contents were cooled to room temperature and volume was made to 100 ml in volumetric flask that was kept overnight in dark condition to have a clear supernatant. Sample was taken from it and was diluted ten times then absorbance (OD) was taken at 425 nm in spectrophotometer (UV-VIS Spectrophotometer GBC Cintra 10).

In an alternative method, determination of total colour value for all encapsulated formulation of curcumin with and without additive was done by Sonication method that was developed for the faster estimation of colour value. A known weight (0.2 g) of FDEC or SDEC was taken in a 250 ml conical flask and 30 ml of 95% alcohol added, which in turn was sonicated in water bath at room temperature for 5 and 10 minutes respectively. Subsequently contents were transferred to a 100 ml volumetric flask with 2-3 washings. The volume was made up to 100 ml and content is kept for overnight in dark. Then sample was taken from it that was diluted to 1:10 times and absorbance was taken at 425nm in Spectrophotometer.

$$\% \text{ Curcumin} = \frac{(\text{Volume}) \times (\text{Dilution}) \times (\text{Absorbance at 425nm}) \times (0.0025 \times 100)}{(\text{Weight of sample}) \times (1000 \times 0.42)}$$

Quantification of % Retention of curcumin on exposure to UV light and Heat

FDEC and SDEC with and without additives were exposed to UV light at 365nm (*UV Lamp Allen 425 model LCF-750-Q*) and 45°C temperature in incubator. Samples were mixed thoroughly and known weight (0.2 g) was drawn at different time interval then colour value was determined by ultra-sonication method at different period of exposure. In addition to this colour value of different formulation of FDEC and SDEC without exposure (0 hours) was also be determine for calculation for percentage retention. The whole study was done in duplicates and average value is reported.

$$\% \text{ Degradation} = \frac{(\text{CV of sample without exposure}) - (\text{CV of sample with exposure}) \times 100}{(\text{CV of sample without exposure})}$$

$$\% \text{ Retention} = 100 - (\% \text{ Degradation})$$

CHAPTER 3

RESULTS AND DISCUSSION

The yield of microencapsulated curcumin for different formulations for FD and SD method is shown in table 3.1. From the table it is evident that the yield of FD method of encapsulation is lesser than SD method of encapsulation. However, practically the yield of product from freeze-drying is always more than products obtained from spray drying. The reason for lesser yield in the present case was the losses during the scrapping of freeze dried material from the tray. Because of the atmospheric moisture, condensation occurred on the thin bottom layer during the course of scrapping, which became sticky mass and that resulted in incomplete product recovery.

Table 3.1

		CONTROL	TBHQ	AP	AP + TBHQ	AVERAGE
YIELD %	FD	41.21	36.49	35.03	45.54	35.57
	SD	37.20	30.71	43.06	40.89	37.97

Sonication using ultrasound is an efficient non-thermal method for extraction of compounds from matrices as it creates intense agitation, which causes the total dispersion of solids and improves the material transfer. Ultrasound was used for disruption of cells and tissues in migration of proteins, minerals and other substance into solutions (Huges, D. E. and Nyborg, W. L., 1962, Kim, S. M and Zayas, J. F., 1989). Rozzi *et al.*, 2002 had used sonication for extraction of lycopene from tomato peel for preparation of standard solution. Thus, this method is extensively used for extraction of compounds from solid material or matrices into solvent.

The comparison between ASTA and sonication method for estimation of colour value was made for control and formulation with both the additives (Table 3.2). It was found that there was little difference between the colour values obtained from both the methods. Therefore, sonication method, which was simpler and consumed lesser time, was employed further for estimation of curcumin in all formulations.

Method	Control			AP + T		
	I	II	Average	I	II	Average
ASTA	1.9621	2.088	2.025	2.288	2.580	2.434
Ultra-Sonication	1.984	2.054	2.019	2.344	2.45	2.401

While comparing the stability offered by both FDEC and SDEC control against UV light at 365nm and 45⁰C incubation, it can be inferred from Table 3.3 and figure1 that stability of FDEC and SDEC against UV light is almost same. However, in case of stability against thermal exposure SDEC exhibited more stability than FDEC (Table 3.4 and figure 2). This difference in stability of freeze and spray dried products is probably due to morphological difference that exists in the constitution of both products. In case of spray dried product, case hardening might have occurred during at high temperature of drying which would allow lesser penetration of heat within the matrix. However, freeze dried product that is more porous in nature, offered lesser resistance to heat transfer within the particles, which probably could be the cause for higher degradation of curcumin in this product.

In case of UV light exposure it was evident that negligible difference exists between the stability offered to curcumin by spray as well as freeze dried encapsulation. This is apparently due to the UV light exposure being more severe than thermal exposure. In addition to this, UV light can penetrate within the particle of spray and freeze dried product to the similar extent leading to decomposition of curcumin to the same level. However, these have to be substantiated further by investigations using SEM studies of

encapsulated curcumin. The stability of encapsulated curcumin against UV light at 365 nm is more than that in solutions, both organic and aqueous systems, reported by Kumawat, A. and Srinivas P., 2002.

Table 3.3

% RETENTION OF ON EXPOSURE TO UV light @ 365nm

HOURS OF EXPOSURE	FREED DRIED ENCAPSULATED CURCUMIN (FDECuv)	FREED DRIED ENCAPSULATED CURCUMIN (SDECuv)
0	100	100
96	96.13	92.96
168	94.7	92.3
240	88.51	87.93
336	86.73	85.48
556	79.31	82.11
724	75.78	75.71

Figure 1: EFFECT OF UV LIGHT EXPOSURE ON CONTROLS

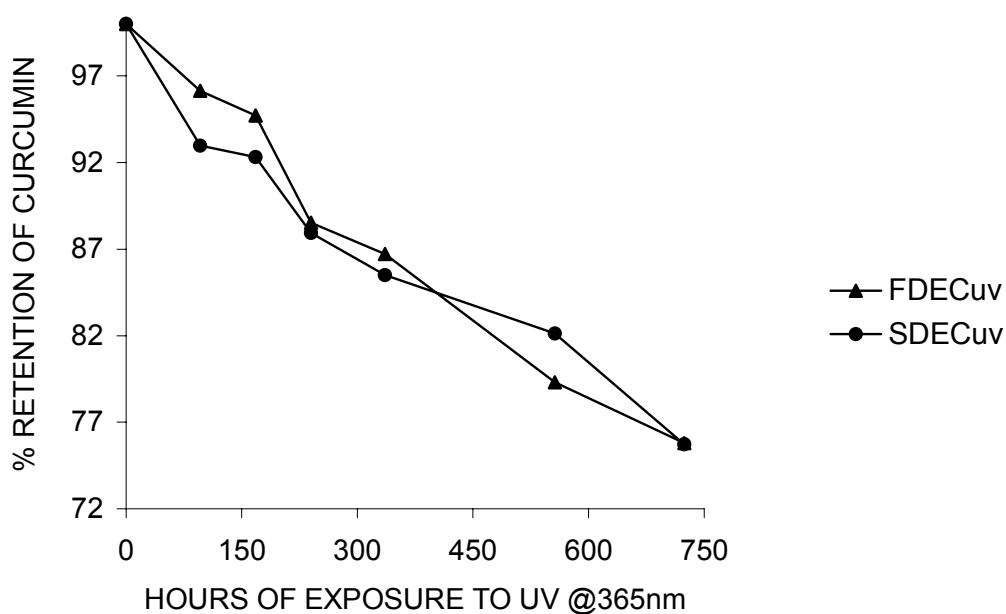
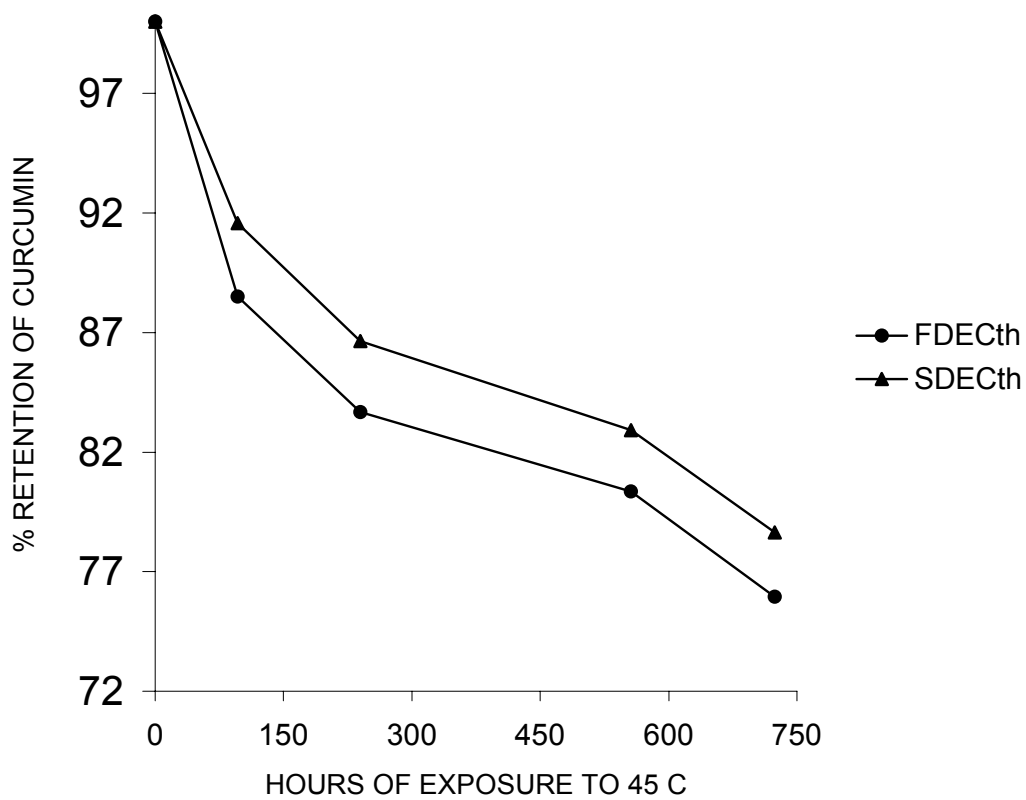


Table 3.4

% RETENTION OF ON EXPOSURE TO HEAT AT 45°C

HOURS OF EXPOSURE	FREED DRIED ENCAPSULATED CURCUMIN (FDEC _{uv})	SPRAY DRIED ENCAPSULATED CURCUMIN (SDEC _{uv})
0	100	100
96	88.5	91.58
240	83.67	86.65
556	80.36	82.91
724	75.95	78.63

Figure 2 : EFFECT OF HEAT EXPOSURE ON CONTROLS



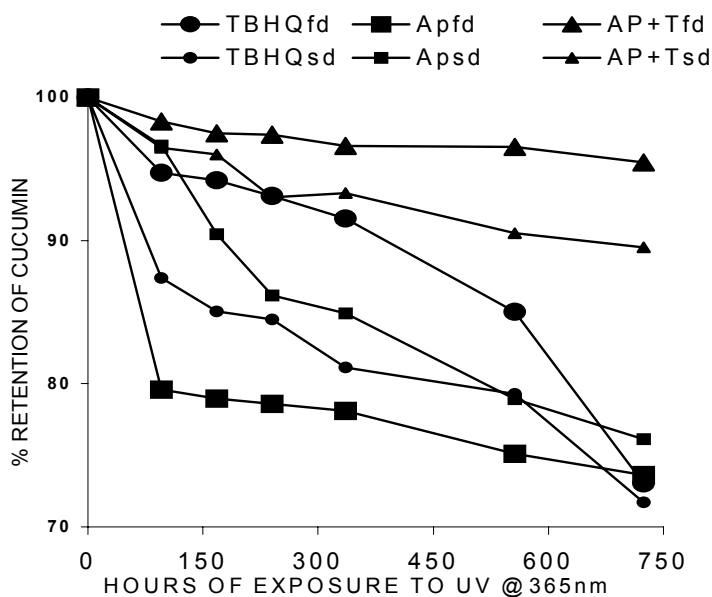
From Table 3.5 and figure 3, it can be concluded that stability offered by different formulations with additives of FDEC and SDEC against UV light at 365nm exposure is in decreasing order as follows AP+T > AP>TBHQ respectively.

Table 3.5

% RETENTION OF ON EXPOSURE TO UV LIGHT AT 365nm

HOURS OF EXPOSURE	FREED DRIED ENCAPSULATED CURCUMIN			SPRAY DRIED ENCAPSULATED CURCUMIN		
	TBHQ	AP	AP+T	TBHQ	AP	AP+T
	0	100	100	100	100	100
96	94.74	79.59	98.31	87.4	96.64	96.48
168	94.22	78.95	97.5	85.06	90.44	96
240	93.12	78.6	97.4	84.48	86.18	93.01
336	91.55	78.1	96.62	81.13	84.91	93.33
556	85.03	75.1	96.54	79.29	78.94	90.53
724	73.017	73.622	95.46	71.7	76.14	89.54

Figure 3 : EFFECT OF UV LIGHT EXPOSURE ON FORMULATIONS



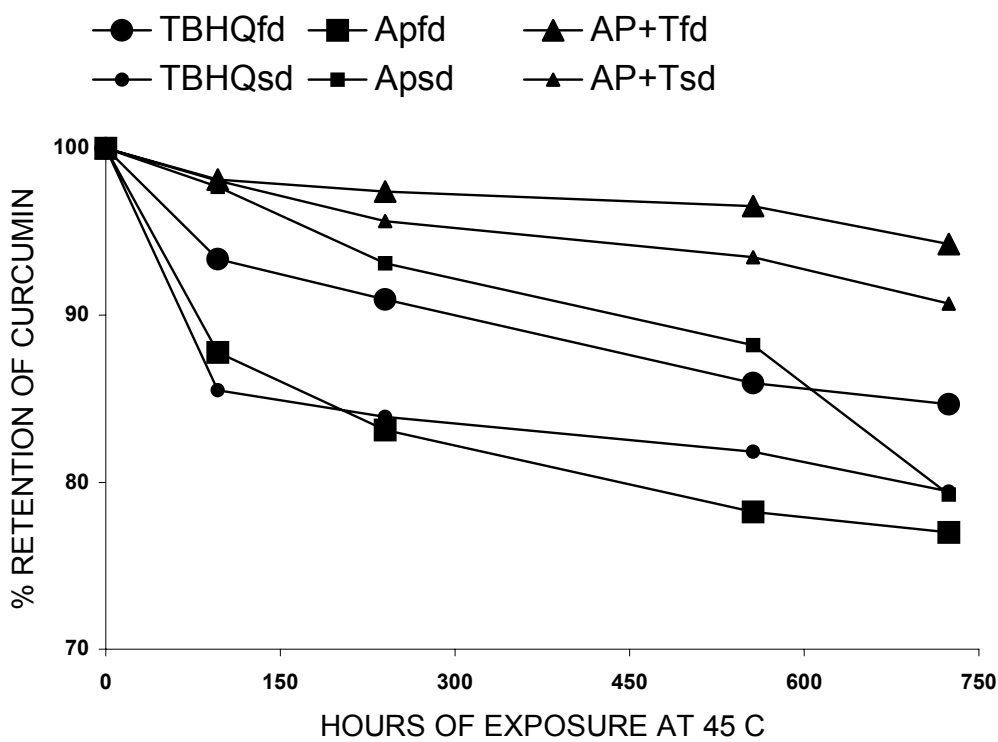
Stability offered by different formulations of FDEC against 45⁰C exposure is also in the decreasing order of AP+T > TBHQ > AP. However, for different SDEC formulations, AP offers more stability than TBHQ (Table 3.6 and figure 4).

Table 3.6

% RETENTION OF ON EXPOSURE TO 45⁰C

HOURS OF EXPOSURE	FREED DRIED ENCAPSULATED CURCUMIN			SPRAY DRIED ENCAPSULATED CURCUMIN		
	TBHQ	AP	AP+T	TBHQ	AP	AP+T
	0	100	100	100	100	100
96	93.36	87.79	98.11	85.49	97.68	98.04
240	90.95	83.14	97.39	83.93	93.1	95.64
556	85.94	78.22	96.54	81.81	88.21	93.49
724	84.68	76.98	94.24	74.46	79.25	90.71

Figure 4: EFFECT OF HEAT EXPOSURE ON FORMULATIONS



Thus, by comparing the data of stability of encapsulated curcumin with and without additives it can be inferred that stability of curcumin in matrix is further enhanced by the presence of different additives, TBHQ (T), Ascorbyl palmitate (AP) and their combination. In addition to this, the combination of TBHQ and Ascorbyl palmitate is more effective than either of additive when used alone.

In a similar study by Kumawat, A and Srinivas, P., 2002, it was shown that use of antioxidant stabilized the curcumin in acetone system exposed to UV light at 365 nm and their NMR spectral studies had indicated the association of protons of the colorant with that of the additive and indication of photo-catalyzed transformations. It was concluded that color instability arises from the free access of the radicals to the chromophore and related transformation reactions and presence of additives, such a process is inhibited, which lends better stability to the colorant.

Present experimental results also indicate that the color degradation by light and heat is probably a radical mediated reaction, which causes the formation of products that alter the structure of the chromophore and hence affect the color of the colorant. The mechanism of degradation of curcumin under the influence of heat and UV light, also its stabilization by additives is to be studied in depth by spectroscopic studies like NMR and MS for a better understanding of the chemical processes involved both in degradation of the pigment and its stabilization by anti-oxidants.

SUMMARY AND CONCLUSIONS

For the use of natural colors in food products, it is important to have a complete understanding of the chemical and physical environment that exist in the product(s) to be colored during and after processing. Also the capabilities and limitations of natural colors as they apply to the product(s) need to be taken into consideration. Instability of natural colors is one of the major limitations in the application of these colors.

A good number and variety of natural color pigments are now available for coloring foods. The global market for natural colors is estimated at more than \$939 million. Despite processing limitations and higher costs, the natural food colorant market is growing and this growth is largely influenced by the consumer perception that 'natural is superior'.

Curcumin colour pigment is highly unstable to light and less stable to heat. In the present study, effect of encapsulation of curcumin along with different additives viz., TBHQ, Ascorbyl palmitate and their combination on the color stability of curcumin has been investigated.

Following conclusions can be drawn from this study:

- Encapsulation of curcumin offers stability against exposure to UV light and heat
- Stability of curcumin is further enhanced by the presence of different additives, TBHQ (T), ascorbyl palmitate (AP) and their combination.

- Combination of AP and T is much more effective than either of the additives showing synergistic action.
- This methodology for stabilization of curcumin pigment is novel
- Results indicate that free radical mediated oxidative reactions occur during photo and thermal degradation
- The mechanism of degradation of curcumin and its stabilization by additives has to be studied in depth by NMR Spectral studies
- Further studies are necessary for arriving at the optimum concentration, economics and regulatory levels for these additives.

BIBLIOGRAPHY

- Anderson, D. M. W and Stoddart, J. F, 1966, The use of Molecular sieve chromatography on *Acacia Senegal* gum, *Carbohydrate Research*, 2, 104 –114.
- ASTA, 1985, *Official Methods American Spice Trade Association*, Engelwood Cliff, New Jersey
- Downham, A and Collins, P., 2000, Coloring our food in the last and new millennium; *Internal Journal of Food Science and Technology*, 35(1), 5-22.
- Downham, A., 2000, Coloring our foods for the next millennium, *Food Ingredients and Analysis International*, 35(4), 18-22.
- Gliksman, A. K., 1983, Gum Arabic, In *food hydrocolloids*, Glicksmam, M., Ed., CRC Press, Boca Raton, FL, 7-36.
- Hoon Bong, P., 2000, Spectral and Phytophysical Behaviors of Curcumin and Curcuminoids, *Bulletin of Korean Chemical Society*, 21(1), 81-86.
- Hughes, D. E. and Nyborg, W. L., 1962, Cell disruption by Ultrasound, *Science*, 138, 108-144.
- Islam, A. M., Phillips, G. O., Sljivo, A., Snowden, M. J. and William, P. A., 1997, A review of recent development on the regulatory, structural and function aspect of Gum Arabic, *Food Hydrocolloids*, 11, 493-505.
- Kim, S. M and Zayas, J. F., 1989, Processing parameter of Chymosin Extraction by Ultrasound, *Journal of food science*, 54(3), 700-704.
- Kumawat A and Srinivas P., 2002, Studies on Photodegradation and Stabilization of Chlorophyll & Curcumin Colorants: *An M. Sc. (Food Tech.)*

Investigation report submitted to Univ. of Mysore; Submitted by Anil Kumawat under the guidance of Dr. P. Srinivas.

- Maing, I. Y. and Miller, I., 1981, Curcumin-metal color complexes, *U S Patent 426333*, General Foods Corp.
- Pattnaik, P., Roy, U. and Jain, P., 1999, Biocolors: New generation additives for food, *Beverage and Food World*, 26(2), 36-40.
- Price, L. C., 1996, Decomposition of turmeric Curcuminoids as effected by light, solvent and oxygen, *Journal of food Biochemistry*, 20(2), 125-133.
- Price, L. C. and Buescher, R. W., 1997, Kinetic of alkaline degradation of food pigments Curcumin and curcuminoids, *Journal of Food Science*, 62(2), 267-269.
- Richard L R., 1980, Stabilized curcumin complex, *U S Patent – 134610*, 1980.
- Rozzi, N. L., Singh, R. K., Vierling, R. A and Watkins, B. A., 2002, Supercritical fluid extraction of lycopene from tomato processing By-product, *Journal of Agricultural chemistry food Chemistry*, 50, 2638-2643.
- Sampathu S. R., Krishnamurthy, N., Shivasankar, S., Shankarnarayan, R., Srinivas Rao, P. N. & Lewis, Y. S., 1981, Natural Food Colours; *Indian Food Packer*, 35, 97-105.
- Sankaranarayana, R., 1981, Food Colors, *Indian Food Packer*, 35, 25-29.
- Santhanakrishnan, T. S., 1981, Food Colors and their future, *Indian Food Packer*, 35, 79-96
- Souza, C. R. A., Osme, S. F. and Gloria, M. B., 1997, Stability of curcuminoid pigments in model systems, *Journal of Food processing and Preservation*, 21(5), 353-363.

-
- Sowbhagya, H. B., Sampathu, S. R., Vatsala, C. N and Krishnamurthy, N., 1998, Stability of curcumin, A natural yellow colorant, during processing and storage of fruit bread, *Beverage and Food World*, 26(7), 40-43.
 - Street, C. A. and Anderson, D. M. W, 1983, Refinement of structure previously proposed for Gum Acacia and Acacia Gum exudates, *Talanta*, 30,878-893.
 - Tonnesen, H. H and Karlsen, J., 1985, Studies on curcumin and curcuminoids. VI. Kinetics of curcumin degradation in aqueous solution, *Zeitschrift-fuer-Lebensmittel-untersuchung-und-Forschung*, 180(5), 402-404.
 - Tonnesen H. H., Masson, M and Loftsson, T., 2002, Studies of Curcumin and C Curcuminoids. XXVII. Cyclodextrin complexation: Solubility, Chemical and Photochemical Stability, 244,127-135.
 - Walford J (1980), Historical development of food coloration, In: *Developments in Food Colors*; Applied Science Publishers, London, UK, Vol. 1: 1-25.