STUDIES IN OLEORESINS

AN INVESTIGATION INTO FOOD COLOUR FROM INDIAN CHILLI OLEORESIN

A Thesis submitted to the
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by

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CERTIFICATE

This is to certify that the thesis entitled "STUDIES IN OLEORESINS (AN INVESTIGATION INTO FOOD COLOUR FROM INDIAN CHILLI OLEORESIN)" being submitted by Shri. K.V.BALAKRISHNAN in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy is a record of the bonafide research work carried out by him in the Department of Polymer Science and Rubber Technology, Cochin University of Science and Technology under our supervision and guidance. No part of the work presented in this thesis has formed the basis of the award of any other degree, diploma or other similar title from any other institution.

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DECLARATION

I hereby declare that the work presented in this thesis is based on the original work done by me under the supervision of Dr.K.E. George, Reader and Dr.D. Joseph Francis, Emeritus Professor, Department of Polymer Science and Rubber Technology, Cochin University of Science and Technology No part of this thesis has been presented for any other degree or diploma from any other institution.

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CONTENTS

CHAPTER I

INTRODUCTION

1.1 GENERAL

If we were to look into the chequered history of spices, it is seen that, though spices were known for centuries, it was only by 1930 food technology flowered into a serious field of study, thus paving the way for the utilisation of spice oleoresins (FARRELL, 1985)

A spice oleoresin may be defined as the total soluble extract of the spice in a specific solvent and embraces all the active components that contribute to aroma, taste and related sensory factors associated with the spice, together with varying amounts of pigments, plant waxes, resins and fixed oils. Whereas, in general, oleoresins are coveted for their flavour qualities, in some cases, the pigments present therein play a vital role in food technology Of these, capsicum oleoresin is the most outstanding, since it contributes both colour and flavour principles.

Carotenoids which donate colour to capsicums have exceptionally high degree of tinctorial potency Prolifically available in nature, capsicum spice is perhaps the best source for this group of pigments. Before we enter into the domain of capsicum carotenoids, let us touch upon the current status of food colourants.

Colour is one of the factors -"perceived attributes"- on which hinges the acceptance of a food product. The art of artificially colouring food is more than 2000 years old (CHIKKASUBBANNA et al., 1994) Since then, there has been a spurt in the use of synthetic dyestuffs. Till recently, the food, pharmaceutical and cosmetic manufacturers could draw from the pool of artificial dyes and pigments to colour their products. However, with increasing awareness of the carcinogenic properties of synthetic colourants, a number of these pigments were delisted. After rigorous screening FAO/WHO cleared only 31 such dyestuffs (CHIKKASUBBANNA et al., 1994) This list is flexible from country to country depending on the toxicity level of the pigments. For example, about 31 dyes were allowed in U.K. in 1966, but this has now dwindled to 22. In Japan, 11 colours were in circulation of which 3 have since been blackballed. Only 10 colours are allowed in India for food colouring. Norway has withdrawn all artificial food dyes from the permitted list. Even for chicken feed formulations, only natural pigments are allowed in Switzerland (APARNATHI & BINDAL, 1995) These stringent legislations, coupled with hostile anti-chemical press and mounting consumer activitism precipitated a colour crisis. The net outcome of all these is to actively welcome pigments from natural sources as food colourants (HORNERO-MENDEZ et al., 1993)

Nature is a recurring source for natural pigments through leaves, flowers, seeds, bark and rhizomes of plants. The most important botanicals of this group and their active pigment components are given below:

Botanical	Pigment
Annatto seeds	Bixin
Beet root	Betalin
Capsicum	Carotenoids
Carrot	ß-Carotene
Grape skin	Anthocyanins
Green leaves	Chlorophylls
Marigold	Lutein
Red Sandalwood	Santalins
Saffron	Crocetin
Tomato	Lycopene
Turmeric	Curcuminoids

Of these, capsicum pigments are among the most commonly used natural food colourants (SHUSTER & LOCKHART, 1954; GREGORY et al., 1987; REEVES, 1987)

1.2 CAPSICUM

The term 'Capsicum' generally refers to fruits of numerous species of the solanaceous genus Capsicum. Among the spices, capsicum is the most colourful in appearance and unique

in terms of history, antiquity and influence on the culinary art (GOVINDARAJAN, 1985) In tropical America, this spice was cultivated by native Indians centuries before the arrival of Spaniards and were used both as condiment and for medicinal applications. By the late 16th and early 17th century, the cultivation of capsicum spread throughout the Mediterranean and Central European regions, shortly followed by Asian and African continents. Due to its long period of cultivation in many different areas of the world under diverse soil and climatic conditions, a great deal of hybridisation has taken place resulting in vastly modified forms (SUZUKI et al.,1957; MAGA, 1975; GOVINDARAJAN, 1985; PURSEGLOVE et al., 1987)

Though precise classification of the capsicum genus is difficult, according to American Spice Trade Association (ASTA, 1992), capsicum now includes five species and more than 300 varieties, making this one of the largest clans of the vegetable kingdom. The five species recognized are Capsicum annuum, Capsicum frutescens, Capsicum chinese, Capsicum pendulum and Capsicum pubescens (MAGA, 1975) The industry uses two species: Capsicum annuum and Capsicum frutescens. Capsicum annuum is the most widely cultivated species. Most of the sweet capsicums belong to this group (MAGA, 1975) Fruits of Capsicum frutescens are smaller

and more pungent than Capsicum annuum. The spice industry knows the whole pungent varieties as 'chilli' (ASTA, 1992) Capsicums generally contain both colour and pungency constituents in varying concentrations. Agro-climatic conditions of growth, maturity at harvest, mode of drying and storage conditions determine the relative concentration of these functional components in each type.

Before we deal with capsicum pigments, let us consider paprika, which is celebrated for its tinctorial excellence.

1.3 PAPRIKA

Capsicums that spread to different geographical regions from tropical America gradually developed new charactristics with respect to size, shape, colour and pungency (MAGA, 1975; PURSEGLOVE et al., 1987) Though originally reputed for high degree of pungency (PAUL, 1940; KRISHNAMURTHY et al., 1970), the fruits later evolved in many European countries almost lacked this character; they also developed brilliant red colour and mild flavour. This variety came to be known as Paprika. Paprika was originally grown in Hungary and Spain; the cultivation now extends to Bulgaria, Yugoslavia, Morocco, Rumania, Czechoslovakia, Turkey, Greece, Portugal, Chile, Canada and USA (ASTA, 1966; PURSEGLOVE et al., 1987) Spanish paprika is sweet whereas

its Hungarian cousins retain a mild, but distinctive nip characteristic to the family. Strictly, the term paprika refers to the ground spice, but now is generic to both whole and ground spice (ASTA, 1966) The most conspicous property of paprika is its colour varying in all shades from dark to bright red. This property tinged with negligible pungency level qualifies paprika as an ideal food colourant (GOVINDARAJAN, 1986; ALMELA et al., 1991; HORNERO-MENDEZ et al., 1993) Most of the pioneering work on capsicum pigments were with paprika and its oleoresin substrates.

1.4 CAPSICUM PIGMENTS

Capsicum pigments, mainly carotenoids, are polyene compounds built from isoprenes; they are alicyclic and their chromophore system is characterised by double bonds which predispose for cis-trans isomerism taking place in the central part of the molecule. According to recent investigations, many carotenoids are in trans-configuration. Their colour may be of any shade from yellow to deep red (VINKLER & RICHTER, 1972)

Capsicum carotenoids fall into two groups

1. The carotenes, that are unsaturated hydrocarbons
2. The xanthophylls, that are oxygen containing carotenoid derivatives (alcohols, aldehydes, esters)

Of these, the xanthophylls dominate the pigment pool.

Zechmeister and Cholnoky (ZECHMEISTER & CHOLNOKY, 1927a, 1927b) who pioneered investigations into the colouring matter of capsicum isolated and identified the red components as capsanthin and capsorubin and yellow components as ß-carotene, cryptoxanthin and zeaxanthin. Later, depending on the variety, presence of violaxanthin, lutein, neoxanthin, antheraxanthin, cryptocapsin, mutatoxanthin, capsolutein, phytoene and small amounts of numerous other carotenoids were also reported (CHOLNOKY, 1937,1939; CHOLNOKY et al., 1955; CURL, 1962; VINKLER & RICHTER, 1972; PURSEGLOVE et al., 1987) Lutein is a major colourant in the green spice; the pigment, however, almost disappears on ripening and a lutein-like pigment replaces lutein in the ripe fruit (CURL, 1964) Though the exact composition is unspecific, the major colour constituents generally encountered in capsicum are ß-carotene (1), cryptoxanthin (2), zeaxanthin (3), violaxanthin (4), capsanthin (5) and capsorubin (6) (HORNERO-MENDEZ et al., 1993) (Fig 1) The xanthophylls largely occur in the form of esters of fatty acids which can be regarded as pigment waxes.

The distribution of colour components in the pigment pool varies considerably with the cultivar and agro-climatic conditions. For C. annuum species, the concentrations

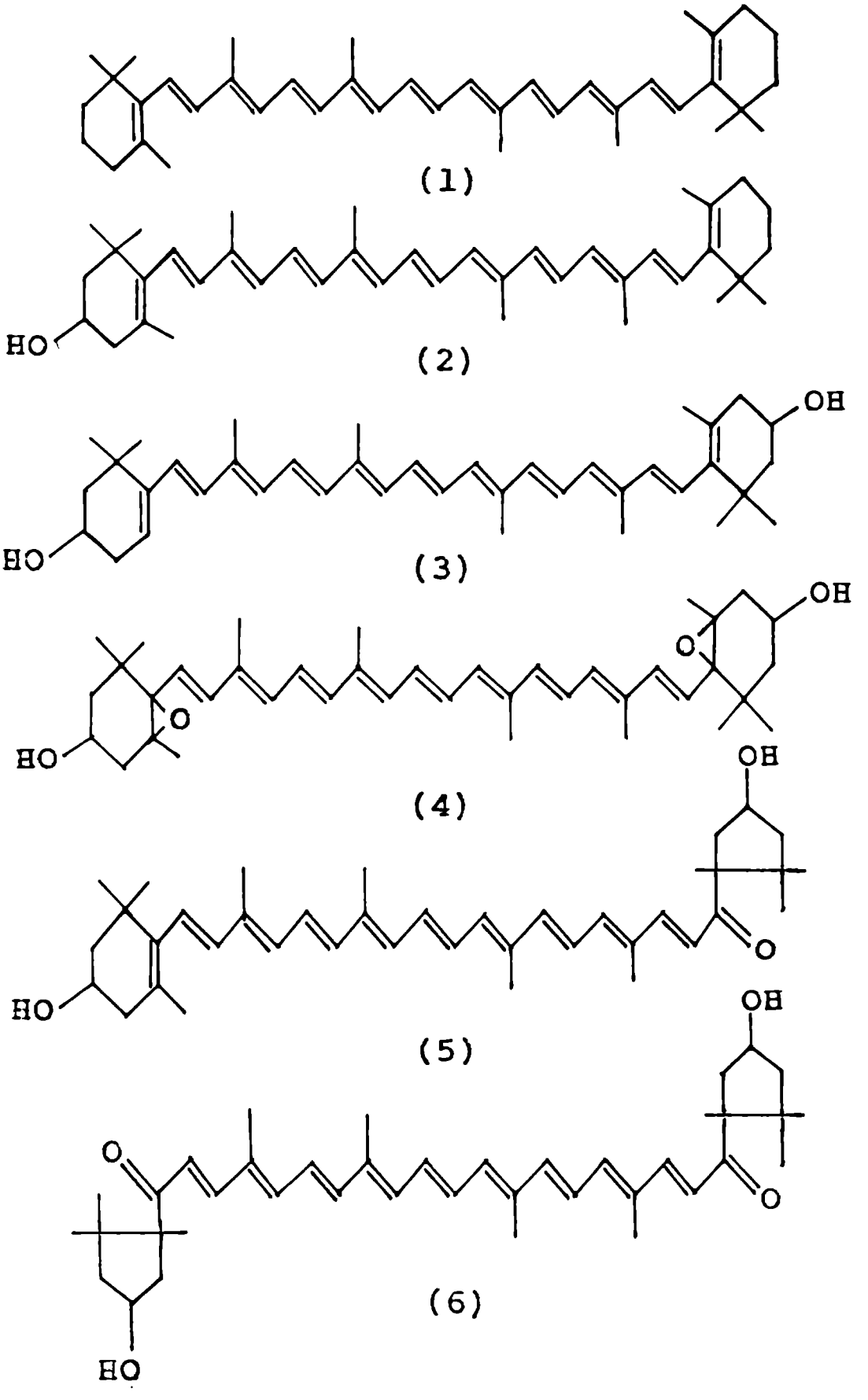


Fig 1. Capsicum pigments

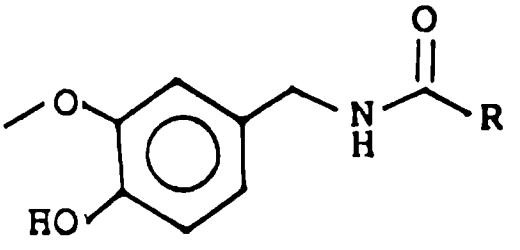
reported are in the range ß-carotene 5-12%, zeaxanthin 2-24%, cryptoxanthin 3-6%, violaxanthin 3-10%, capsanthin 32-68% and capsorubin 4-8% (CURL, 1962; DAVIES et al., 1970; SALMERON & GARRIDO, 1976; BARANYAI et al., 1982; PURSEGLOVE et al., 1987) The colour constituents are concentrated in the skin of the fruit; the seeds and stalks are virtually devoid of colour (HEISER & SMITH, 1953; TANDON et al. 1964; PURSEGLOVE et al., 1987; ANDREWS, 1984; GOVINDARAJAN, 1986)

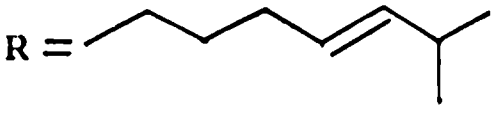
The most popular quality determinant of capsicum and its oleoresin, viz. colour value, is determined by EOA (ESSENTIAL OIL ASSOCIATION OF USA) (EOA, 1965), ASTA (AMERICAN SPICE TRADE ASSOCIATION) (ASTA, 1985a) or MSD-10 (MSD-10, 1959) method.

In the following section, we shall deal with the pungent principles of capsicum.

1.5 PUNGENT PRINCIPLES OF CAPSICUM

Capsaicinoids are the pungent principles of capsicum fruit and comprise of capsaicin (7) and its analogues such as dihydrocapsaicin (8), nordihydrocapsaicin (9), homodihydrocapsaicins (10 and 11), caprylic acid vanillyl amide (12), nonylic acid vanillyl amide (13) and decylic acid vanillyl amide (14) (JURENITSCH et al., 1979) (Fig 2) Presence of homocapsaicin is also reported (WOOD, 1987)





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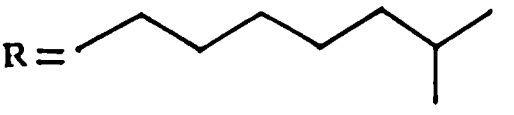

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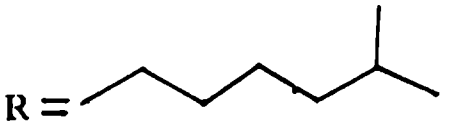

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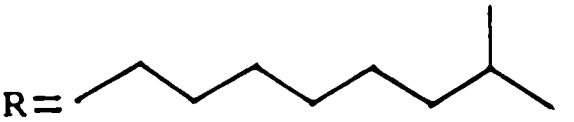

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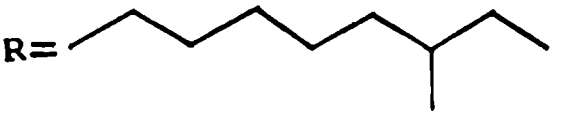

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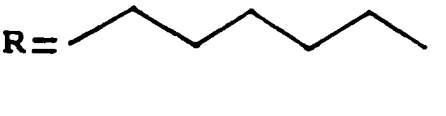

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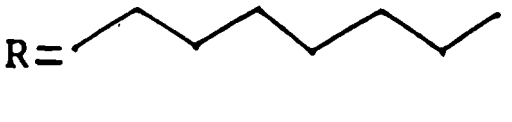

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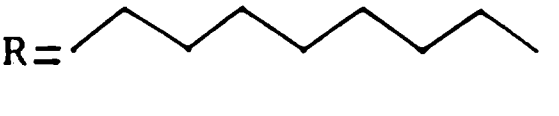

(14)

Fig 2. Pungent principles of capsicum

Usually, the pungency level in capsicum is declared in percentage capsaicin. Scoville heat ratings based on taste tests are also designed to reflect capsaicin content (SCOVILLE, 1912; IS, 1976; GOVINDARAJAN et al., 1977; FCC, 1981; GILLETTE et al., 1984; ASTA, 1985b) Human taste perceptions, integral to the Scoville system are subject to variability which may even run to as high as 50 percent (SUZUKI et al., 1957; ASTA, 1992) Instrumentation methods are substantially more accurate than the Scoville, especially in the lower pungency ranges. Another simple method is based on the chemical reaction between the pungency components and vanadium oxytrichloride solution in carbon tetrachloride (TING & BARRONS, 1942) The extent of colour change in the reaction mixture on visual examination is correlated to the pungency level. Tracking the colour reaction by colorimetry or spectrophotometry is an improvement over the above method (NORTH, 1949; TIRIMANNA, 1972) Column, paper and thin layer chromatographic methods, frequently in conjunction with spectrophotometry, have also been reported (SUZUKI et al., 1957; HOLLA et al., 1957; KARAWYA et al., 1967; SPANYAR & BLAZOVICH, 1969; MATHEW et al., 1971; GOVINDARAJAN & ANANTHAKRISHNA, 1973; GONZALEZ & TAMIRANG, 1973; DiCECCO, 1979; RAJPOOT & GOVINDARAJAN, 1981), but are too long and cumbersome to warrant routine use. Gas liquid chromatography (GLC)

(TODD, 1961; MORRISON, 1967; HOLLO et al., 1969; MULLER-STOCK et al., 1971; TODD et al., 1977), GLC combined with mass spectrometry (MS) (MASADA et al., 1971), high pressure liquid chromatography (HPLC) (STICHER et al., 1978; JURENITSCH et al., 1979; WOODBURY, 1980, KAWADA et al., 1985; ASTA, 1985c; CHIANG, 1986; WOOD, 1987), combination of HPLC-MS (HERESCH & JURENITSCH, 1979) and GLC-MS-HPLC (IWAI et al., 1979) have also been developed for the estimation. Spectrophotometric determination is generally employed for routine analysis (JT. COMMITTEE OF PHARM. SOC. & SOC. FOR ANAL. CHEM., 1964)

What are the other constituents in capsicum, in addition to colour and pungent principles ?

1.6 OTHER CONSTITUENTS IN CAPSICUM

Apart from the colour and pungent principles which are present in less than 1%, capsicum contains carbohydrate, protein, fat and fibre as the major components. The fibre content varies greatly between the sources. Water soluble ash forms the larger part of the fairly high total ash, showing capsicums as a source of minerals. Generally, capsicums are poor in volatiles, though they have appreciable sensory impact in some varieties. Presence of fructose, glucose, galactose and sucrose in capsicum have been recorded. Free sugars in the seeds are higher than in

the pericarp or placenta. Of the acids, citric acid is the major one, others being succinic, fumaric, malic and quinic. Fat is mostly confined to the seed of the capsicum. The total fat content varies from 9 to 16% depending on the seed content of the variety (GOVINDARAJAN, 1985)

Vitamin C is the most valuable nutritional component in the spice, a discovery due to Szent Gyorgi. This is present in the dried spice to the extent of 30 to 60 mg/100 g. Substantial loss of vitamin C occurs during the processing of capsicum (GOVINDARAJAN, 1985)

Also, capsicums contain vitamins A, B complex and E and small amounts of α-tocopherol (GOVINDARAJAN, 1985)

A total of 14-16 amino acids have been identified in capsicum, dominant being asparagine and proline (GOVINDARAJAN, 1985)

Now we will concentrate our attention on capsicum oleoresin.

1.7 CAPSICUM OLEORESIN

Spices in the raw form suffer from certain inherent drawbacks. The variety, soil, agroclimatic conditions of growth, maturity at harvest and post-harvest storage conditions influence the aroma, taste and pigment level.

This leads to lack of flavour and colour constancy in food products from batch to batch. Raw spices have high percentage of inert matter and hence require large storage space and heavy transportation expenses. In spice powders, the active principles are locked within the tissues. These components are not fully released during cooking and are unavailable for perception. Moreover, raw spices are prone to microbial attack which reduce their shelf life.

Oleoresin recovered from the spice by solvent extraction encompasses the aroma, taste and colour components of the spice in the most concentrated form. Oleoresin, thus, represents the true essence of the spice and serves as the standardised, hygienic and convenient substitute for raw spice in food applications. Moreover, the quality parameters of the oleoresin could be standardised to any level to meet specific customer requirements.

The earliest study on capsicum extraction dates back to 1816 when Bucholtz realised that the pungent principles of capsicum could be isolated by maceration of the fruits with organic solvents (WALKER, 1968) Since then, there have been extensive investigations and substantial technological improvements on the optimisation of the process steps.

Capsicum oleoresin is essentially the integral of pungent priciples, pigments, fixed oils, resins and waxes. The level of pungency and colour constituents in a straight extracted oleoresin is usually low due to the dilution by the other components, especially the fixed oil. Beneficiation of these functional components by fractionation of the oleoresin is employed by industry for quality improvement.

1.8 INDIAN CHILLI

From the above review we find that capsicum can be broadly grouped into two types paprika, exceptionally rich in colour with nil/faint pungency, and others with varying degrees of these quality parameters. As it stands, paprika alone cannot meet the growing demand for carotenoid pigments. World's production of non-paprika varieties far outweighs paprika (ASTA, 1995; GOVINDARAJAN, 1986) Non-paprika varieties, therefore, represent a rich source for carotenoid pigments.

Capsicums grown in India chillies - are pungent and constitute a principal article of commerce. The spice is cultivated extensively in the country Important growing areas are Andhra Pradesh, Orissa, Maharashtra, West Bengal, Karnataka, Rajasthan and Tamilnadu. Andhra Pradesh alone commands 46% of the chilli production in India. As per the latest statistics, India

produces around 779 000 tons of the dry spice from an area of 917 600 hectares (SPICES BOARD, 1995) A large number of capsicum varieties are cultivated in different parts of the country The Spices Board (SPICES BOARD, 1995) has identified 16 major Indian varieties. No country in the world has so many varieties, area and production of this spice as India. Oleoresins with high colour strength and low pungency level can be recovered from selected varieties of the spice.

1.9 CHILLI COLOUR

Whereas traditional paprika oleoresin can be directly incorporated in food by virtue of its nil/negligible pungency level, oleoresin derived from Indian chilli contains fair amount of capsaicinoids which restricts its direct application as food colour. Chilli colour devoid of pungency can be conveniently retrieved from the oleoresins by selective elimination of the pungent principles by fractionation using appropriate solvent systems. Chilli colour with optimum pigment concentration has now gained wide acceptance as substitute for paprika oleoresin.

Furthermore, the pungency phase, concentrated separately, is an excellent food flavouring ingredient.

1.10 OBJECTIVES AND SCOPE OF THE WORK

Earlier, spotlight was played on the significance of natural colourants for food and the emergence of Indian chilli as an important source of natural pigments. Considering, on one hand, the large availability of Indian chilli and on the other hand, the paucity of reliable data on the retrieval of colour matter from this spice, a commanding problem was to study comprehensively the various parameters for extraction process and to standardise analytical procedures which will open up a new dimension in capsicum technology

In addition to the Introduction (Chapter I), the thesis comprises of four more chapters.

Chapter II deals with (a) the distribution of colour and pungent principles in different varieties of Indian chillies available for extraction, (b) relative efficiency of different solvents for capturing the colour and pungency factors and (c) influence of spice particle size on extraction process.

The harvesting of colour principles from the oleoresin by selective removal of pungent principles using different solvent systems is described in Chapter III.

Evaluation of colour strength and pigment concentration

is a crucial step in the quality control of the redeemed colour concentrate. The inadequacy of popular analytical methods to accurately quantify the colour power of capsicum pigments, which demands solution, forms the subject matter of Chapter IV

Finally, Chapter V gives the summary and conclusions of the study

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CHAPTER II

ON THE EXTRACTION OF OLEORESIN FROM INDIAN CHILLIES

PREAMBLE

The extraction of oleoresin from chilli is the first step in the production of pungency-free colour concentrate. With a view to gathering data for the optimum retrieval of colour matter from Indian chilli, the following investigations were undertaken:

1. Screening of different chilli varieties to determine the yield, colour strength and capsaicin content of the oleoresins obtained therefrom.

2. Examining the relative efficiency of popular extraction media in capturing the colour and pungency factors from the spice.

3. Determining the influence of particle size of the spice on the relative recovery of colour and pungent principles.

The results obtained from the above investigations are presented in Sections (a), (b) and (c) respectively of this chapter.

SECTION (a)

DISTRIBUTION OF COLOUR AND CAPSAICIN IN DIFFERENT VARIETIES OF INDIAN CHILLIES

2(a).1 INTRODUCTION

India cultivates a number of chilli varieties in different parts of the country (SPICES BOARD, 1995) All these varieties do not serve as substrates for oleoresin extraction. Availability, price and oleoresin yield with requisite amount of active principles form the basis for the selection of spice for extraction.

The following are the types exploited by oleoresin industry:

1. Bird's eye
2. Byadgi
3. Guntur
4. Jwala
5. Jwala Green
6. Tomato
7 White

Capsaicin and pigment level are the pivotal factors desired in the oleoresin. The distribution of these active principles in the oleoresin, in turn, depends on their concentration in the spice and the yield of oleoresin obtained therefrom. Quantification of these factors in different varieties of the spice are, therefore, necessary for the selection of raw material for extraction in relation to the quality requirements of the redeemed oleoresin.

The capsaicin content of some varieties of Indian chillies have been examined earlier (DEB et al., 1963; GORDE, 1967; LUHADIYA & KULKARNI, 1977; SANKARIKUTTY et al., 1978; TEWARI, 1979; DAMAYANTHY et al., 1980; RAJPOOT & GOVINDARAJAN, 1981; GOVINDARAJAN, 1986), but the results obtained do not disclose the pigment level. In publications where colour strengths are also reported (TANDON et al., 1964; MATHEW et al., 1971; MURTHY & BAVAJI, 1978; NARAYANAN et al., 1980), the investigations are either confined to a few hybrid varieties of academic interest or to some selected types with a view to comparing only the capsaicin level. A fairly detailed information on the colour value and capsaicin content of different Indian chilli varieties has been recently published (SPICES BOARD, 1995); the data on the yield and concentration of the functional components in the oleoresin are, however, missing.

Unavailability of simultaneous data on the yield, colour strength and capsaicin content of the oleoresin obtained from Indian chillies prompted us to screen them to quantify these quality parameters.

2(a).2 MATERIALS AND METHODS

The following chilli cultivars were collected from growing areas: Bird's eye, Byadgi, Guntur, Jwala, Jwala Green, Tomato and White chilli.

Ethylene dichloride (EDC) (technical grade, purity > 99% GLC) was used as model extraction medium.

The chilli (4% moisture) was powdered to pass through 1 mm screen (100g) Extraction runs (static bed) were carried out in a 40 mm dia. x 500 mm long glass column by gravity percolation of solvent (500 ml) at room temperature. A contact time of 1 h was given for first wash and 15 min for subsequent washes. Combined extracts were filtered and desolventised under reduced pressure on boiling water bath.

Colour values were determined by MSD-10 (MSD-10, 1959) and capsaicin content by spectrophotometric difference (JT COMMITTEE OF PHARM. SOC. & SOC. FOR ANAL. CHEM., 1964) methods in a Hitachi U - 2000 Spectrophotometer.

Total colour and total capsaicin, calculated as yield x colour value and yield x capsaicin content of the oleoresin are used to represent the colour strength and pungency level of the spice respectively

2(a).3 RESULTS AND DISCUSSION

Table 1 gives data on the yield, colour value and capsaicin content of oleoresin derived by EDC extraction of different varieties of Indian chilli.

The yield, colour value and capsaicin content of the oleoresin follow the order:

Table 1

Yield, Colour value and Capsaicin content of oleoresins derived from different varieties of Indian chillies

Variety	Oleoresin yield (%)		Colour value (CU)		Capsaicin content (%)		Total colour	Total capsaicin
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		
Bird's eye	10.00	0.50	6 204	255	5.20	0.27	62 040	52.00
Byadgi	7 75	0.35	46 530	1240	1.42	0.20	360 607	11.00
Guntur	9.50	0.55	16 170	478	2.63	0.25	153 615	24.98
Jwala	8.41	0.44	22 176	772	4.15	0.38	186 500	34.90
Jwala Green	5.50	0.32	4 158	220	5.90	0.40	22 869	32.45
Tomato	6.25	0.28	54 648	1375	1.48	0.15	341 550	9.25
White	6.83	0.40	4 488	284	2.97	0.33	30 653	20.28

$\bar{x}$ Mean of 3 extractions (2 measurements for each extraction)

$\pm s$ Standard deviation of 3 extractions (2 measurements for each extraction)

Yield	Bird's eye > Guntur > Jwala > Byadgi > White > Tomato > Jwala Green.
Colour value	Tomato > Byadgi > Jwala > Guntur > Bird's eye > White > Jwala Green.
Capsaicin	Jwala Green > Bird's eye > Jwala > White > Guntur > Tomato > Byadgi.

The total capsaicin and total colour derived from the yield, capsaicin content and colour value of the oleoresin follow the order:

Total colour	Byadgi > Tomato > Jwala > Guntur > Bird's eye > White > Jwala Green.
Total capsaicin	Bird's eye > Jwala > Jwala Green > Guntur > White > Byadgi > Tomato.

The yield of oleoresin is optimum in the case of Bird's eye, followed by Guntur and Jwala. Bird's eye chilli contains exceptionally high amount of capsaicinoids as disclosed by the total capsaicin, followed by the Jwala varieties. However, the capsaicin concentration in the oleoresin is much lower than Jwala Green due to comparatively high yield of extractives.

Although rich in pungent principles, the availability of

Bird's eye chilli is limited. Jwala varieties represent the right raw material for the retrieval of pungent principles.

Byadgi chilli exhibits maximum total colour; however, colour value is highest for Tomato chilli oleoresin. Low pungency level and exceptionally high colour make these two varieties the ideal substrates for the extraction of chilli colour.

Based on the above results, extraction quality chillies can be conveniently classified into:

1. High colour, low pungent

Tomato and Byadgi chilli containing high concentration of pigments and low pungency level fall in this category. Chilli colour can be recovered from the oleoresins of these varieties by selectively removing the pungency components.

2. Medium colour, high pungent

These varieties are the best for the production of oleoresin with high level of pungent principles and medium colour strength. Jwala variety of chilli is most popular in this class.

3. Low colour, high pungent

Bird's eye chilli with low colour strength and high pungency level belongs to this group.

Another raw material with this qualification is the Jwala Green, the faded and immature pods obtained while grading Jwala chilli.

4. Medium colour, medium pungent

This variety represents the most widely cultivated type which are primarily consumed for culinary purpose. Andhra Pradesh and Tamilnadu are the main growing areas of this variety Popular Guntur chilli belongs to this category.

5. Low colour, medium pungent

This group is best represented by White chilli which is obtained as seconds while grading item 4. White chilli composed of faded, immature and damaged pods is widely exploited in oleoresin industry because of its attractive price level.

2(a).4 CONCLUSIONS

Results on the yield, colour value and capsaicin content of oleoresin obtained from different varieties of Indian chilli are presented. These data form the framework for the selection of raw material for the production of oleoresin meeting the required quality parameters. For convenience in selection, the chilli varieties preferred by oleoresin industry have been classified into five groups based on the concentration of colour and pungent principles.

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SECTION (b)

RELATIVE EFFICIENCY OF DIFFERENT SOLVENTS FOR THE RECOVERY OF COLOUR AND CAPSAICIN FROM CHILLIES

2(b).1 INTRODUCTION

Solvents generally used for producing oleoresins are unspecific and varying amounts of fixed oils, resins, sugars, phosphatides, mucilages etc. as well as the functional components contributing colour and flavour are extracted (GOVINDARAJAN, 1986) The quality of the oleoresin is influenced by non-functional constituents which dilute, alter the physical structure, restrict the solubility and dispersibility characteristics or affect the shelf life adversely by phase separation and oxidative rancidity development. In essence, the best solvent is one which extracts all active principles and least amount of other constituents that alter the quality attributes.

Unlike other spices which are valued mainly for their flavour properties, chilli contains both flavour and colour as active principles. The best chilli oleoresin is the one which concentrates both these factors and recreates the colour and sensory qualities of the original spice when added to food formulations after sufficient dilution.

Common extraction solvents exhibit varying degrees of solubility for the functional components of capsicum. The selection of solvent, therefore, plays a vital role in determining the quality characteristics of the oleoresin. In 1816, Bucholtz reported that the pungent principles

of capsicum could be isolated by maceration of the fruits with organic solvents (WALKER, 1968) Since then a number of solvents such as hexane, methanol, ethanol, isopropanol, ethylene dichloride (dichloroethane), trichloroethane, dichloroethylene, chloroform, diethyl ether, petroleum ether, acetone, ethyl acetate, benzene, polysorbate and water, either alone or in combination, and alkali have been used for capsicum extraction (SCOVILLE, 1912; DOTT, 1922; DICKEY & NITARDY, 1933; BERRY, 1935; BERRY & SAMWAYS, 1937; MOSTER & PRATER, 1957; BENEDEK, 1958; TODD, 1958; POHLE & GREGORY, 1960; CHEN & GUTMANIS, 1968; BALBAA et al., 1968; DE LA MAR & FRANCIS, 1969; SZABO, 1970; MATHEW et al., 1971; PHILIP et al., 1971; TIRIMANNA, 1972; ANDRE, 1973; GONZALEZ & TAMIRANG, 1973; RAMAKRISHNAN & FRANCIS, 1973; MAGA, 1975; TANDON et al., 1964; IS, 1976; SASS et al., 1977; WOODBURY, 1977; HUFFMAN et al., 1978; IWAI et al., 1979; DiCECCO, 1979; DAMAYANTHY et al., 1980; VARGA et al., 1984; MALCHEV et al., 1982; GILLETTE et al., 1984; GOVINDARAJAN, 1986; GREGORY et al., 1987) These investigations were carried out on a large assortment of chillies under diverse extraction conditions targetted to quantify different parameters and hence provide no comparative results on the extraction efficiency of the solvents employed.

As discussed in the last chapter, the yield, colour strength and capsaicin content collectively determine the quality of the oleoresin and the economy of the extraction process. Since earlier investigations were not aimed at deriving simultaneous data on these parameters, they fail to provide vital guidelines required for the industry This demanded further investigation with a view to ascertaining yield, capsaicin content and colour strength of oleoresins derived from a typical Indian chilli using different extraction media. Model chilli chosen for the investigation is the Jwala variety which contains fairly high concentration of pigments and capsaicinoids.

2(b).2 MATERIALS AND METHODS

Jwala chilli for the investigation was obtained from growing area. Technical grade hexane (n-hexane), methanol, ethanol, isopropanol (IPA), ethylene dichloride (EDC), chloroform, acetone, methyl ethyl ketone (MEK), ethyl acetate (EA) and benzene were used as extraction media.

Jwala chilli (100 g), dried to 4% moisture, was pulverized to pass through 1 mm screen. Extraction runs (static bed) were carried out in a 40 mm dia. x 500 mm long glass column by gravity percolation of solvent (500 ml) at room temperature. For the first wash, 1 h contact time was given and for subsequent washes, 15 min. Combined extracts

were filtered and solvent removed under reduced pressure on boiling water bath.

Colour values were determined by MSD-10 (MSD-10, 1959) and capsaicin content by spectrophotometric difference (JT. COMMITTEE OF PHARM. SOC. & SOC. FOR ANAL. CHEM., 1964) methods in a Hitachi U 2000 Spectrophotometer.

Recoveries are based on total colour and total capsaicin, calculated as yield x colour value and yield x capsaicin content respectively

2(b).3 RESULTS AND DISCUSSION

Yield, colour value, capsaicin content, total colour and total capsaicin of oleoresin obtained by extraction of Jwala chilli by 10 different solvents are recorded in Table 1. These factors follow the order:

Yield — Methanol > acetone > ethylene dichloride > benzene > ethyl acetate > hexane > chloroform > isopropanol > methyl ethyl ketone > ethanol.

Colour value — Hexane > ethyl acetate > benzene > chloroform > ethylene dichloride > isopropanol > acetone > methyl ethyl ketone > ethanol > methanol.

Table 1

Yield, Colour Value and Capsaicin Content of oleoresins derived from Jwala chilli using different solvents

Solvent	Yield (%)		Colour value (CU)		Capsaicin content (%)		Total colour	Total capsaicin
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		
Hexane	7.51	0.37	24 535	815	2.08	0.21	184 257	15.62
Methanol	10.48	0.62	4 934	446	3.64	0.24	51 708	38.14
Ethanol	6.84	0.33	18 792	527	5.39	0.30	128 537	36.86
IPA	7.29	0.29	22 342	378	3.65	0.28	162 873	26.60
EDC	8.25	0.28	22 821	397	4.32	0.16	188 273	35.64
Chloroform	7.43	0.30	23 345	795	4.10	0.32	173 453	30.46
Acetone	9.05	0.43	21 953	722	3.76	0.22	198 674	34.02
MEK	6.95	0.18	21 738	594	2.81	0.14	151 079	19.52
EA	7.64	0.27	23 657	635	4.30	0.27	178 478	32.85
Benzene	7.94	0.25	23 361	530	4.21	0.25	185 486	33.42

$\bar{x}$ Mean of 3 extractions (2 measurements for each extraction)

$\pm s$ Standard deviation of 3 extractions (2 measuements for each extraction)

Capsaicin	Ethanol > ethylene dichloride > ethyl acetate > benzene > chloroform > acetone > isopropanol > methanol > methyl ethyl ketone > hexane.
Total colour	Acetone > ethylene dichloride > benzene > hexane > ethyl acetate > chloroform > isopropanol > methyl ethyl ketone > ethanol > methanol.
Total capsaicin	Methanol > ethanol > ethylene dichloride > acetone > benzene > ethyl acetate > chloroform > isopropanol > methyl ethyl ketone > hexane.

It may be noted that hexane yields an oleoresin with the highest colour value, but with least capsaicin content. Oleoresin derived using ethanol exhibits the highest capsaicin content, but the lowest colour value next to methanol. The concentration of active principles in other oleoresins fall within these extremes. Whereas colour value and capsaicin content represent the quality characteristics of the oleoresin, total colour and total capsaicin are measures of the extent of recovery of these components from the spice. Acetone retrieves the highest total colour followed by ethylene dichloride. Next to methanol and

ethanol which display poor recovery of colour matter, ethylene dichloride extracts the highest amount of pungent principles, followed by acetone. Benzene is a good solvent for both colour and pungency, but this solvent is not used commercially due to toxicity Hexane is efficient in retrieving the colour and hence finds application in the extraction of capsicum varieties like paprika, which are practically devoid of pungency

Acetone and ethylene dichloride are, thus, effective in extracting both the colour and pungency factors. However, being water miscible, acetone tends to get diluted with water during processing necessitating frequent rectification. Ethylene dichloride is, therefore, preferred for the extraction. In view of the suspected carcinogenicity of chlorinated hydrocarbons, some countries have exercised restriction on the use of ethylene dichloride as extraction medium. In such cases, ethyl acetate with fairly good extraction efficiency for both colour and pungency, coupled with comparatively low water miscibility qualifies as a satisfactory alternative.

2(b).4 CONCLUSIONS

Solvent plays an important role in the extraction of chilli, both from the point of view of product quality and process economics. We have now evaluated the performance of a series

of extraction solvents for the retrival of oleoresin, colour matter and capsaicinoids from chilli. Ethylene dichloride and acetone have been found to be the best extraction media; ethylene dichloride has an edge over acetone by virtue of its water immiscibility Wherever ethylene dichloride, being a chlorinated hydrocarbon, is not preferred for extraction, ethyl acetate is found to be a satisfactory alternative.

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SECTION (c)

EFFECT OF PARTICLE SIZE ON THE RECOVERY OF COLOUR MATTER FROM CHILLIES

2(c).1 INTRODUCTION

Earlier, we established the excellence of Byadgi and Tomato cultivars as sources for colour concentrate since they possess high pigment and low pungency level and that ethylene dichloride and ethyl acetate are best extraction media. Logically then, the next step is to standardise parameters for the isolation of the oleoresin which is an essential prerequisite for capturing the colour matter.

Using paprika as substrate and acetone as solvent, the effect of three parameters viz. particle size, time and temperature on the extraction rate have been examined earlier (SHUSTER & LOCKHART, 1954) This work disclosed that there is a gradual increase in the absorbance of a dilute acetone extract of the spice at 464 nm with decrease in particle size from 2.0 mm to 0.5 mm; the difference, however, narrows down with increase in contact time. Later it was shown that fineness of the raw material augmented the yield of oleoresin (MATHEW et al., 1971; DAMAYANTHY et al., 1980; GOVINDARAJAN, 1986), but no data was presented on particle size versus pigment recovery In the case of paprika extraction, 300 to 400 micron particle size seems the best (SALMERON & ROMOJARO, 1975) Also, a rate equation has been established (HOUSER et al., 1975) for the extraction of paprika with organic solvents, correlating

concentration of the extract to other process parameters at a fixed particle size.

Commercial Indian chillies contain ca. 6% stalks, 40% pericarp and 54% seeds (TANDON et al., 1964); their relative percentages, however, vary with the cultivar. Whereas the pigments and pungent principles are concentrated in the pericarp (TANDON et al., 1964; NAGLE et al., 1979), stalks and seeds are devoid of these factors. Bulk of the fixed oil which is located in the seeds not only dilutes the colour and pungency of the oleoresin, but also causes its rancidity (PURSEGLOVE et al., 1987; GOVINDARAJAN, 1986) Hence, extraction of chilli after depletion of seeds, though not totally, but with retention of ca. 5% to facilitate grinding (GOVINDARAJAN, 1986), furnishes an oleoresin with improved quality characteristics.

In addition to what has been mentioned above, there are other factors of industrial importance which revolve on the particle size. For example, the bulk density which represents weight per unit volume of the powder depends on the particle size and this, in turn, is related to the capacity of the extractor. Solvent required to fill the charge is linked to the voids in the bed. Fine particles give a compact bed with minimum voids and thus reduce the solvent requirement. In static bed batch extraction, the

solvent percolates down the raw material bed under gravity As the bed becomes more and more compact, the percolation rate decreases; this makes the extraction uneconomical when very fine particles are used. The solvent front, as it moves through the charge, gets enriched in extractives with concurrent increase in viscosity and this also slows down the percolation rate.

Carotenoid pigments dictate the tinctorial power of capsicum concentrate (HORNERO-MENDEZ et al., 1993) As discussed in Chapter I, chilli oleoresin contains several constituents in addition to colour matter. Hence, increase in yield need not necessarily certify higher pigment recovery Total colour measured as the product of yield and colour value is a more reliable measure of this factor.

In summary, for optimum economic recovery of pigments from high colour Indian chilli, we must have a knowledge of the influence of particle size on the (1) extractor capacity, (2) solvent requirement, (3) rate of solvent percolation, (4) oleoresin yield and (5) pigment recovery From the above appraisal, it is clear that much more work remains to be done to evaluate these factors.

2(c).2 MATERIALS AND METHODS

Tomato chilli for the investigation was obtained from

growing area (vide supra) Technical grade ethylene dichloride and ethyl acetate used were > 99% pure (GLC)

Chilli, sun dried to 8% moisture level, was coarsely ground and seeds separated from pericarp mechanically, leaving residual seed content of ca. 5%. The pericarp, after drying to 4% moisture level in the hot air oven, was communited to pass through a series of sieves from 6 mm to 0.5 mm providing grinds of different dimensions. Grind size was designated by the corresponding screen size. Extractions were carried out at ambient temperature using 100 g sample in 40 mm dia. x 500 mm long glass column by simple gravity percolation. Two sets of experiments were done using 500 ml and 1000 ml of each solvent giving 1 h contact time for the first wash and 15 min for subsequent washes. Combined percolates were filtered and desolventised.

Colour values were determined by MSD-10 (MSD-10, 1959) method.

2(c).3 RESULTS DISCUSSION

In Table 1, columns 1, 2, 3 and 4 give the particle size and the corresponding bulk density, solvent quantity and percolation time. From the above work, the following conclusions are deduced:

1. With decrease in particle size from 6 mm to 0.5 mm, the

Table 1

Variation in Bulk density, Solvent quantity and Percolation time with particle size

Particle size (mm) 1	Bulk density (g/lit) 2		Solvent quantity (ml) 3		Percolation time (sec) 4	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
6	158	8	500	--	30	4
5	178	10	480	14	37	6
4	195	12	410	11	45	8
3	225	9	355	10	57	8
2	250	7	320	9	78	10
1	270	12	270	10	140	11
0.5	282	10	245	8	185	9

$\bar{x}$ Mean of 3 measurements

$\pm s$ Standard deviation of 3 measurements

bulk density of the powder increases from 158 g/l to 282 g/l. The same extractor can, thus, accomodate 1.8 times 0.5 mm powder than that of 6 mm powder, thereby considerably increasing the loading capacity Variation in bulk density of powder with particle size is presented in Fig 1.

2. Quantity of solvent required to fill the charge decreases with reduction in particle size. Thus, 0.5 mm powder requires only half the quantity of solvent compared to an equal weight of 6 mm powder.

3. Percolation time, which represents the time required for the solvent front to traverse through the charge and reach the base of the bed, increases with decrease in particle size. Thus, for 0.5 mm powder the percolation time is more than 6 times that required for an equivalent charge of 6 mm powder. Fig 2 shows the influence of particle size on percolation time.

To decide the acceptable level of powder size, we also screened the effect of particle size on the oleoresin yield and pigment recovery, using ethylene dichloride and ethyl acetate as extraction media. The results obtained are assembled in Tables 2 and 3. From this one can conclude that with both the solvents, as the powder size is reduced, there is an increase in yield and a concomitant decrease in

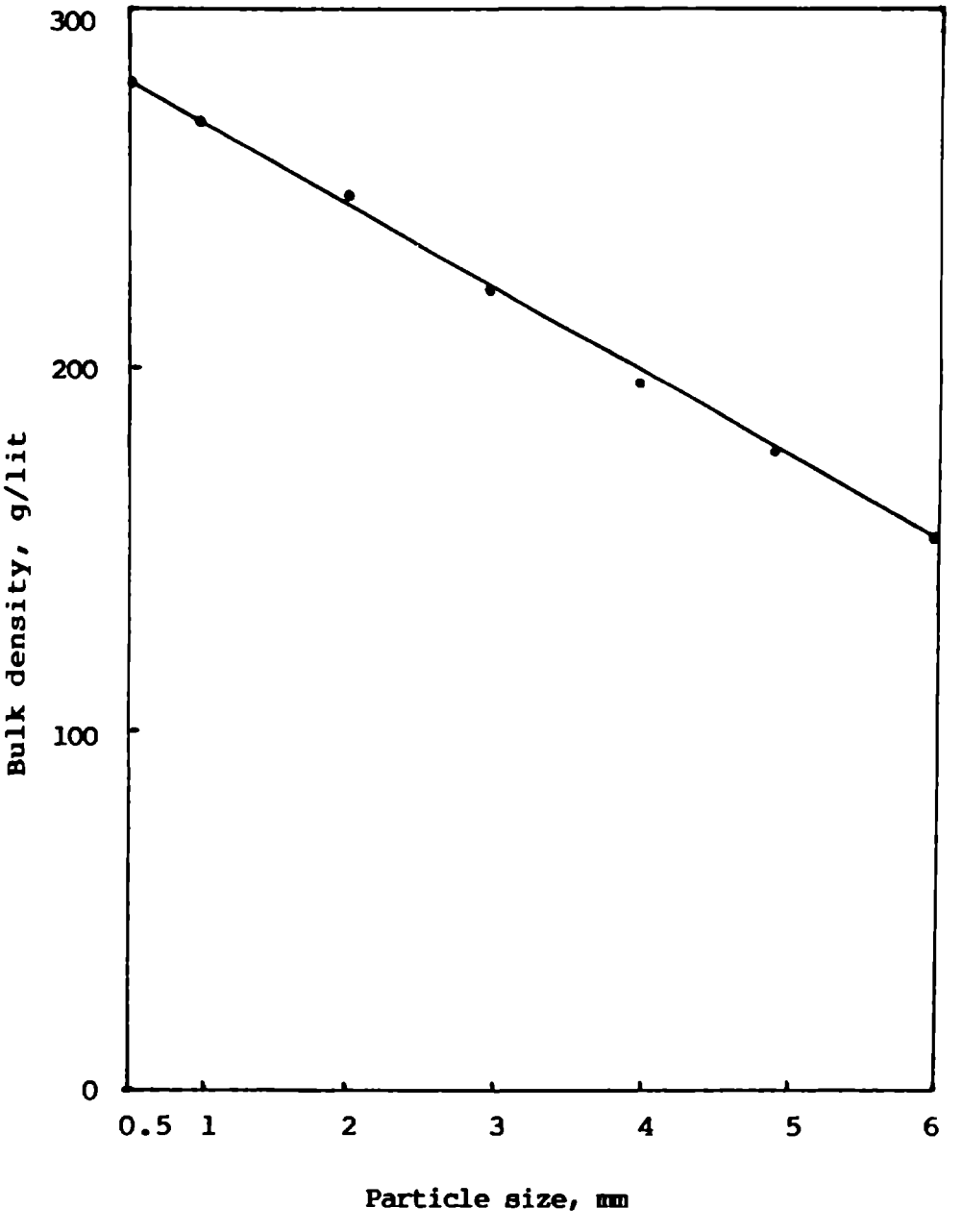

Fig 1. Variation in bulk density with particle size.

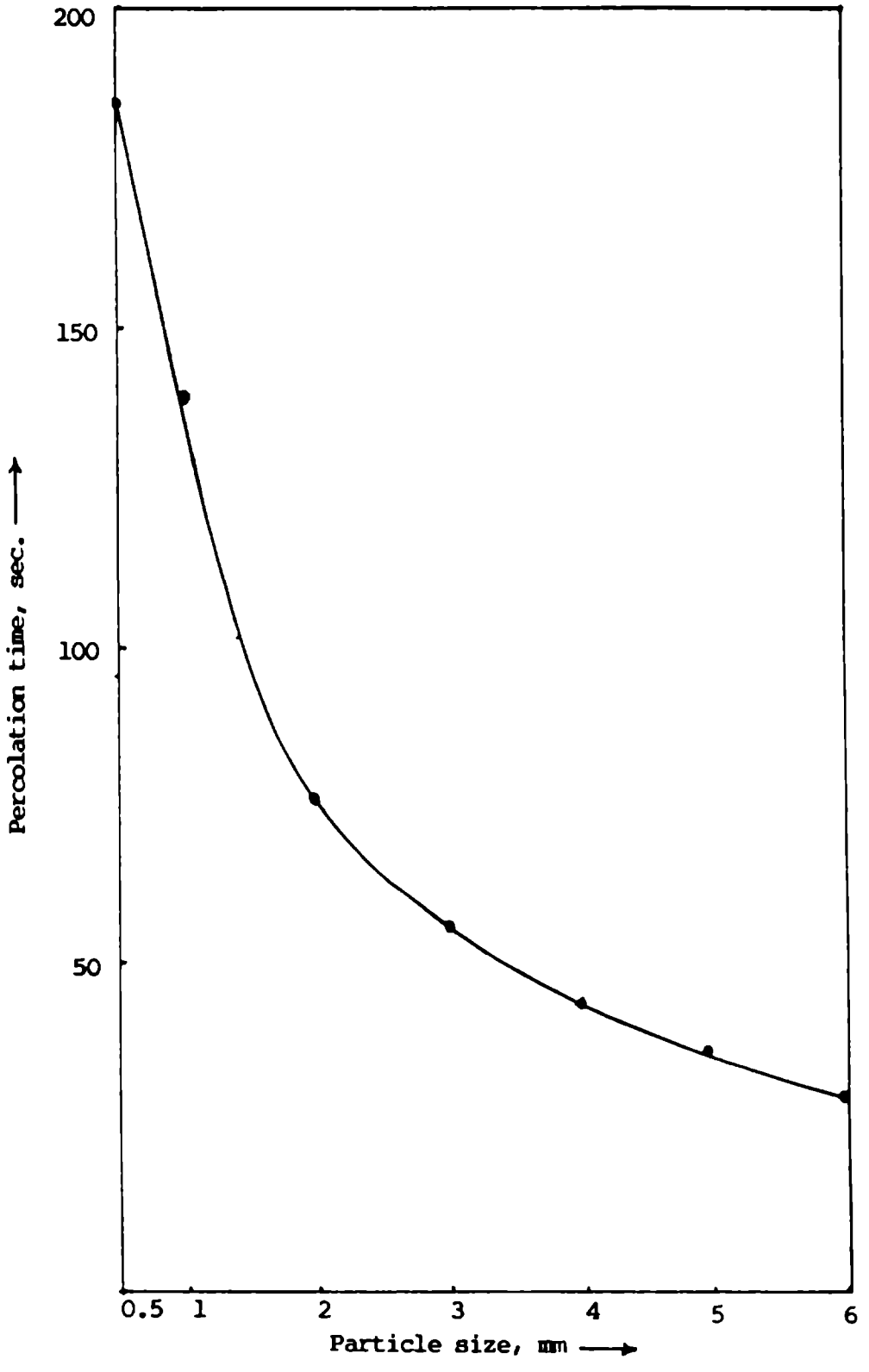

Fig 2. Influence of particle size on percolation time

Table 2

Effect of particle on the extraction efficiency

(Solvent - Ethylene dichloride)

Particle size (mm)	5 times solvent					10 times solvent				
	Yield (%)		Colour value (CU)		Total colour	Yield (%)		Colour value (CU)		Total colour
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	
6	3.44	0.24	72 651	1 215	249 919	5.15	0.20	72 285	1 147	372 267
5	4.05	0.26	71 614	815	290 036	5.40	0.17	69 601	1 335	375 845
4	4.66	0.19	68 198	1 064	317 862	5.79	0.22	65 630	733	380 032
3	5.27	0.22	64 843	785	341 722	6.15	0.16	63 989	1 042	393 532
2	5.85	0.15	62 769	947	367 198	6.93	0.20	60 695	790	420 616
1	6.74	0.21	61 244	1 120	412 784	7 76	0.12	58 987	853	457 739
0.5	7.04	0.14	60 939	894	429 010	7.98	0.09	58 865	620	469 742

$\bar{x}$ Mean of 3 extractions (2 measurements for each extraction)

$\pm s$ Standard deviation of 3 extractions (2 measuements for each extraction)

Table 3

Effect of particle on the extraction efficiency

(Solvent - Ethyl acetate)

Particle size (mm)	5 times solvent					10 times solvent				
	Yield (%)		Colour value (CU)		Total colour	Yield (%)		Colour value (CU)		Total colour
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	
6	3.12	0.21	75 884	1 520	236 758	5.02	0.17	76 433	1 126	383 693
5	3.72	0.19	74 786	1 068	278 203	5.24	0.21	74 176	875	388 682
4	4.34	0.30	72 468	1 245	314 511	5.49	0.19	71 980	1 178	395 170
3	4.78	0.27b	70 089	722	335 025	5.85	0.25	69 296	771	405 381
2	5.52	0.24	65 209	860	359 953	6.78	0.18	62 403	927	423 092
1	6.62	0.16	62 342	594	412 704	7.56	0.14	60 695	560	458 854
0.5	6.95	0.34	61 183	780	425 221	7.75	0.12	60 146	655	466 131

$\bar{x}$ Mean of 3 extractions (2 measurements for each extraction)

$\pm s$ Standard deviation of 3 extractions (2 measuements for each extraction)

colour value. However, the net pigment recovery as indicated by the total colour increases with decrease in particle size.

Two significant observations may be made at this stage. With ethylene dichloride, a combination of 3 mm powder and 5 times solvent gives the same oleoresin yield as 6 mm powder and 10 times solvent; 2 mm powder and 5 times solvent recovers almost the same total colour as 6 mm powder and 10 times solvent. Runs with ethyl acetate also exhibit similar trend. The other observation is that the difference in total colour using 5 times versus 10 times solvent decreases with the fineness of the powder. Thus, with ethylene dichloride, 5 times solvent recovers only 67% of the total colour as compared to 10 times solvent from 6 mm particles, whereas the recovery is 91% from 0.5 mm powder. For ethyl acetate, corresponding figures are 61% and 91%. Thus, the material gets exhausted faster and there is considerable saving in solvent consumption if the spice is reduced to finer size.

Results from Tables 2 and 3 are epitomised in Fig 3 and 4. Yield and total colour increase as particle size reduces from 6 mm to 0.5 mm; the rate of increase steeply declines when the particle size is below 1 mm. A particle size of 1 mm is, therefore, judged as optimum for the extraction of pigments.

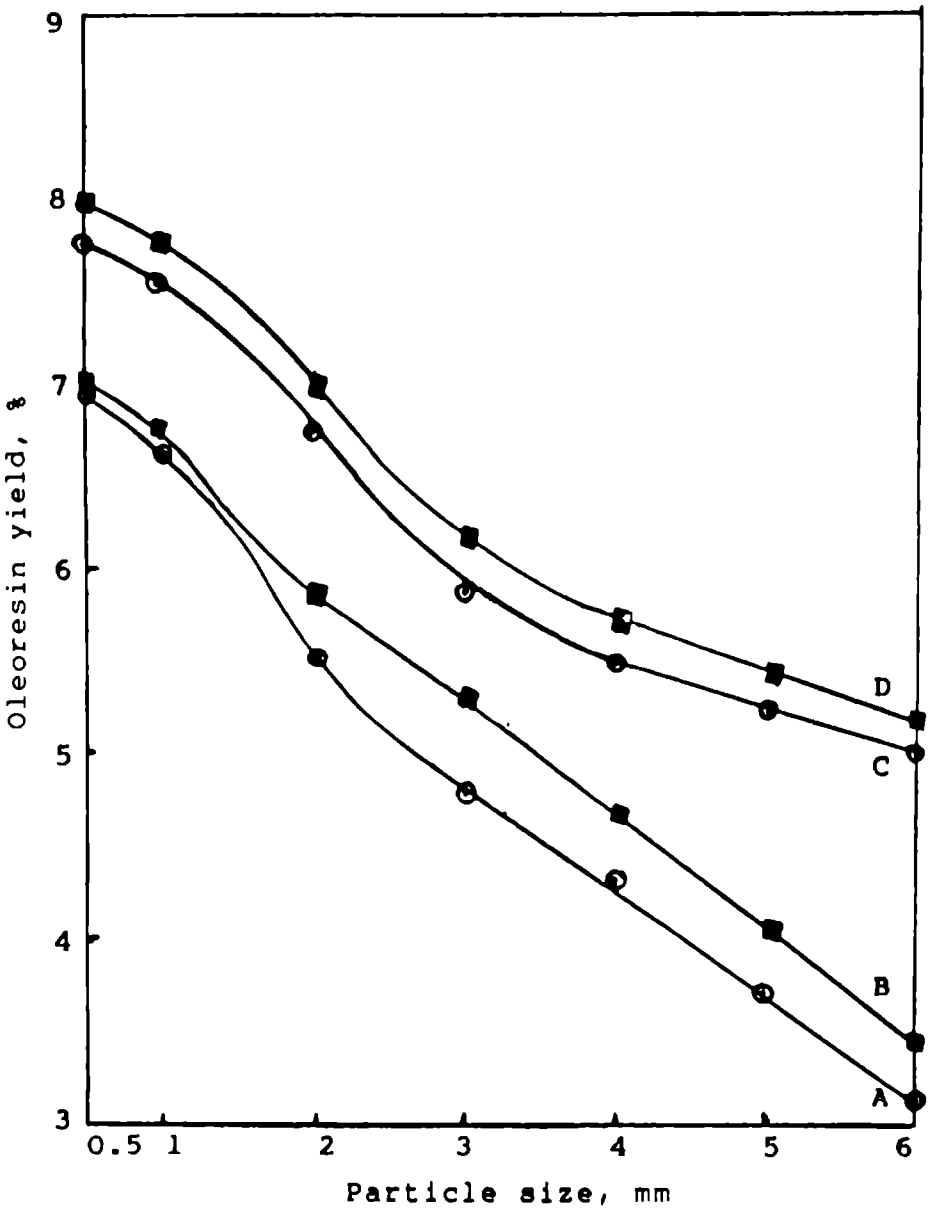

Fig 3. Effect of particle size on oleoresin yield

A: 5 times Ethyl acetate
B: 5 times Ethylene dichloride
C: 10 times Ethyl acetate
D: 10 times Ethylene dichloride

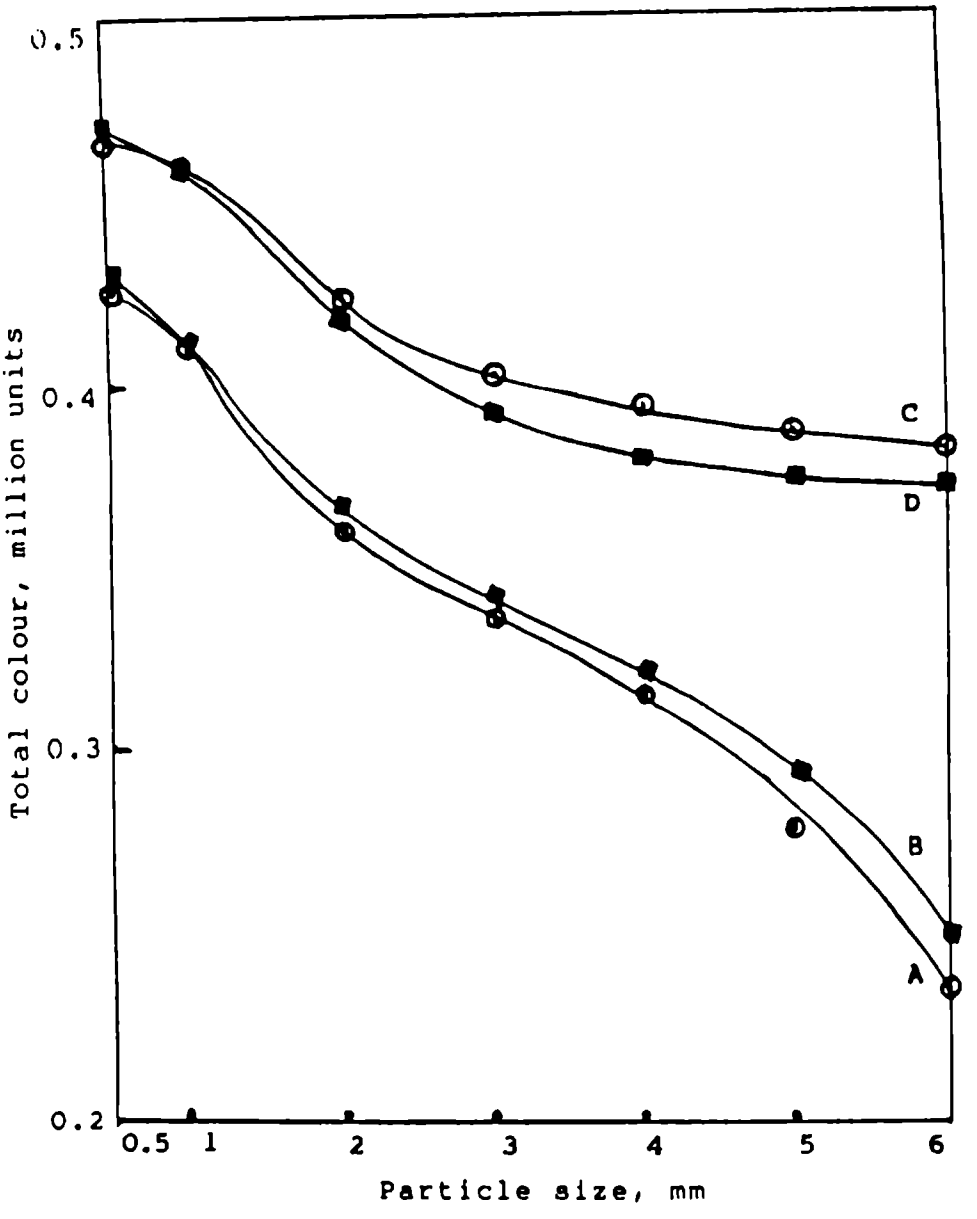

Fig 4. Effect of particle size on pigment recovery

A: 5 times Ethyl acetate
B: 5 times Ethylene dichloride
C: 10 times Ethyl acetate
D: 10 times Ethylene dichloride

2(c).4 CONCLUSIONS

In the production of oleoresin from chilli, particle size is the key factor influencing the extractor capacity, percolation time, oleoresin yield, pigment recovery and solvent requirement. Furthermore, it is established that the first four factors increase with reduction in particle size in contrast to the decrease in solvent requirement. Except for the higher percolation time, all other factors are beneficial for the economy of the process. In conclusion, we recommend a particle size of 1 mm for the extraction.

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CHAPTER III

ON THE FRACTIONATION OF INDIAN CHILLI OLEORESIN

3.1 INTRODUCTION

In Chapter II, we focussed on the selection of spice raw material, extraction media and particle size with a view to optimising the recovery of oleoresin. Spotlight is now played on the retrieval of pungency free colour matter by fractionation of the oleoresin using appropriate solvent systems.

Depending on the capsicum cultivar, the oleoresin derived therefrom encompasses different levels of colour and pungent principles plus fixed oil, resins and waxes (GOVINDARAJAN, 1986) Resolution of oleoresin into colour and pungency parts has sparked numerous investigations. Earlier approaches were concerned with the enrichment of pungent principles through (1) re-extraction of the oleoresin with aqueous ethanol, methanol, acetone, acetic acid or alkali, (2) partitioning between these solvents and hydrocarbon and (3) chromatography (SCOVILLE, 1912; DOTT, 1922; TICE, 1933; DICKEY & NITARDY, 1933; BERRY, 1935; BERRY & SAMWAYS, 1937; DODGE, 1941; BPC, 1949, 1968; LEASE & LEASE, 1956; HOLLA et al., 1957; SUZUKI et al., 1957; TODD, 1958; TANDON et al., 1964; NIPPON SHINYAKU CO. LTD, 1968; MATHEW et al., 1971a, 1971b; TIRIMANNA, 1972; GOVINDARAJAN & ANANTHAKRISHNA, 1973; DAMAYANTHY et al., 1980; NARAYANAN et al., 1980; PURSEGLOVE etal., 1987; GOVINDARAJAN, 1986) The chromatography route

is only of academic interest. More recently, extraction with supercritical carbon dioxide has also been reported (MAYER, 1989) Currently, the upgradation of the oleoresin in industry is largely engineered with aqueous solvent system. Here, the colour and pungent principles are partitioned between the fixed oils of the oleoresin and the solvent system; the colour principles find a berth in the fixed oils and the pungency components in the solvent. Repeated extraction of the oleoresin with the chosen solvent system culminates in colour extract devoid of pungency Thus, if the initial oleoresin is sufficiently rich in colour, this route assures the generation exclusively of the colour concentrate, the spin off being a concentrate containing pungent principles and residual pigments.

As presented in Chapter II, oleoresins isolated from Byadgi and Tomato varieties of Indian chilli possess exceptional tinctorial power and thus can serve as ideal subtrates for chilli colour.

In the resolution of chilli oleoresin, the solvent system dictates the yield, colour and relative distribution of red and yellow pigment matter of the harvested colour concentrate. Eventhough the splitting with aqueous methanol or acetone or these solvents-cum-hydrocarbon media are frequently recommended, very little information is available

on the relative colour recovery and the red/yellow pigment distribution in the two fractions. This commanding problem invited our attention.

The objectives of the present study were to evaluate the (1) yield, (2) colour strength, (3) pungency level and (4) relative distribution of red, yellow and total pigments in the colour and pungency fractions obtained from a typical capsicum extract through liquid-liquid fractionation.

3.2 MATERIALS AND METHODS

Capsicum oleoresin ex tomato chilli for the investigation was Synthite's product. Pure capsanthin and lutein were gifts from Laboratorios Bioquimex S.A. DE C.V Mexico. Fractionation was carried out using laboratory reagent grade solvents. Analytical grade reagents were used for spectrophotometric estimations and chromatography grade for HPLC.

Capsicum oleoresin, 100 g, was stirred (magnet bar) with 400 ml solvent for 1h. The contents were transferred to a separating funnel and allowed to stand undisturbed. The pigment-rich layer was separated off and treated further with 400 ml solvent. The process was repeated until the pigment phase was practically free from pungency The colour and pungency fractions were concentrated separately

on water bath under reduced pressure. Solvent-free pungency fraction was further taken up in ethylene dichloride to remove water and desolventised.

Solvent systems consisting of methanol and acetone respectively with (1) 0, 10, 20, and 30% water content and (2) 0, 10, 20, 30, 40 and 50% water content doped with 10% hexane (n-hexane) were used for the fractionation.

Partitioning between hexane and aqueous acetic acid/ethanol was carried out as per procedure earlier reported (NIPPON SHINYAKU CO. LTD, 1968)

Colour values (CV) were determined by MSD-10 (MSD-10, 1959) and capsaicin content by spectrophotometric difference (JT. COMMITTEE OF PHARM. SOC. & SOC. FOR ANAL. CHEM., 1964) methods using a Hitachi U-2000 Spectrophotometer. HPLC analyses of saponified samples (AOAC, 1990) were conducted in a Hewlett-Packard Series 1050 High Pressure Liquid Chromatograph using C-18 Reversed Phase Column (Shimadzu LC Column, Shim-pack, CLC-SIL (M), 150 mm long x 4.6 mm ID); n-hexane - ethyl acetate - acetone (65:28:7) was used as mobile phase.

Colour and capsaicin recoveries were calculated from total colour and total capsaicin, obtained as quantity x colour value and quantity x capsaicin content respectively Pigment

concentration was derived by dividing colour value by 1600 (BALAKRISHNAN et al., 1996)

3.3 RESULTS AND DISCUSSION

Quality characteristics of the oleoresin used for fractionation experiments are listed in Table 1. Extraction with aqueous methanol or acetone splits the oleoresin into colour and pungency-rich fractions. The colour and capsaicin levels of these fractions with variation in the water content (Wc) of solvents are presented in Table 2.

As the Wc in methanol increases from 0 to 30%, the yield of colour fraction correspondingly uplifts from 57.3 to 79.5%, with synchronised decrease of colour value (CV) from 100 320 to 87 054. The net recovery of colour calculated from total colour increases from 72.10% to 86.80%. Increase in water content, therefore, favours the retrieval of pigments in the colour fraction. In this concentration range, the yield of pungency fraction drops from 36.5 to 11.4%, with a progressive hike in the capsaicin content from 5.68 to 16.06%. However, the net capsaicin recovery based on total capsaicin falls from 94.23% to 83.21%. Therefore, higher water content in the solvent system leads to lower recovery of pungent principles.

Results of similar runs using acetone are also listed in

Table 1

Oleoresin used for fractionation experiments

Property		$\bar{x}$	$\pm s$
Colour value (Colour units, CU)		79 728	162
Capsaicin content (%)		2.20	0.06
Pigment concentration (CV/1600)	(g/kg)	49.83	0.10
Red pigments (HPLC) (%)		57.02	0.37
Yellow pigments (HPLC) (%)		42.98	0.37

$\bar{x}$ mean of 3 measurements.

$\pm s$ standard deviation of 3 measurements.

Table 2

Fractionation of capsicum oleoresin with aqueous methanol and acetone

Solvent	Colour fraction						Pungency fraction								Overall		
	Quantity (g)		Colour value (CU)		Total colour (Units)	Colour recovery	Quantity (g)		Colour value (CU)		Total colour (Units)	Capsaicin content (%)		Total capsaicin (Units)	Colour recovery	Quantity recovery	Colour recovery	Capsaicin recovery
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		%	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		$\bar{x}$	$\pm s$		%	%	%	%
Methanol (%)																		
100	57.3	1.77	100 320	1 971	5 748 336	72.10	36.5	1.36	39 336	1 145	1 435 764	5.68	0.39	207.32	18.00	93.8	90.10	94.23
90	67.2	1.68	96 360	2 045	6 475 392	81.21	25.5	1.15	24 948	1 434	636 174	7.95	0.47	203.49	7.98	92.7	89.19	92.49
80	75.1	2.03	90 948	1 763	6 830 194	85.66	15.2	0.79	22 440	1 075	341 088	12.61	0.78	191.67	4.27	90.3	89.93	87.12
70	79.5	2.25	87 054	2 334	6 920 793	86.80	11.4	0.83	21 054	946	240 015	16.06	0.94	183.08	3.01	90.9	89.81	83.21
Acetone (%)																		
100	Fully Soluble																	
90	51.4	2.18	98 868	1 824	5 081 815	63.74	40.3	1.76	50 292	1 585	2 026 767	5.10	0.45	205.53	25.42	91.7	89.16	93.42
80	67.4	1.89	95 964	1 268	6 467 973	81.12	23.8	1.02	25 014	1 282	595 333	8.07	0.72	192.06	7.46	91.2	88.58	87.30
70	77.9	2.41	87 648	2 261	6 827 779	85.63	11.9	0.75	21 450	1 004	255 255	15.84	1.12	188.49	3.20	89.80	88.83	85.67

$\bar{x}$ mean of 3 extractions (2 measurements for each extraction)

$\pm s$ standard deviation of 3 extractions (2 measurements for each extraction)

Table 2. The oleoresin is fully soluble in pure acetone, but in presence of water it could be partitioned into the colour and pungency-rich fractions. With increase in the Wc of acetone from 10 to 30%, the yield of colour fraction elevates from 51.4 to 77.9% and the recovery of total colour from 63.74 to 85.63%.

At low Wcs', the yield and colour recovery with acetone are less compared to methanol, but the difference narrows down with increase in Wc. The performance of both the solvents are almost comparable at 70% concentration. As with methanol, the CV of the colour fraction progressively falls with increase in the Wc. The recovery of capsaicin is also comparable.

With increase in water content, the density difference between the solvent system and oleoresin decreases, leading to difficulty in layer separation. Below 70% concentration, clear separation cannot be achieved. Better partitioning is effected by the addition of n-hexane to the system. In Table 3 are assembled the results obtained on fractionation of oleoresin capsicum incorporating 10% n-hexane to acetone and methanol at varying Wcs'

When methanol with 0% and 10% Wcs' are doped with hexane, separation of the layers is not satisfactory; a

Table 3

Fractionation of capsicum oleoresin with aqueous methanol and acetone in presence of hexane

Solvent	Colour fraction						Pungency Fraction								Overall		
	Quantity (g)		Colour value (CU)		Total colour (Units)	Colour recovery	Quantity (g)		Colour value (CU)		Total colour (Units)	Capsaicin content (%)		Total capsaicin (Units)	Colour recovery	Quantity recovery	Colour recovery	Capsaicin recovery
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		%	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$		$\bar{x}$	$\pm s$		%	%	%	%
Methanol (%)																		
100	No clear separation																	
90	No clear separation																	
80	75.2	1.54	91 212	1 582	6 859 142	86.03	17.3	1.10	20 460	1 312	353 958	11.10	0.55	192.03	4.44	92.5	90.47	87.28
70	79.1	1.79	88 044	1 834	6 964 280	87.35	11.7	0.75	19 536	1 520	228 571	15.83	0.86	185.21	2.86	90.8	90.21	84.18
60	84.1	2.27	84 480	2 248	7 104 768	89.11	8.2	0.57	17 424	1 126	142 876	22.09	0.81	181.13	1.79	92.3	90.90	82.33
50	86.7	2.54	82 764	2 013	7 175 639	90.00	4.8	0.34	9 570	820	45 936	36.21	1.31	173.80	0.57	91.5	90.57	79.00
Acetone (%)																		
100	Fully Soluble																	
90	No clear separation																	
80	67.6	1.82	96 756	1 682	6 540 705	82.03	24.3	1.25	26 862	2 224	652 746	7.98	0.42	193.91	8.18	91.7	90.21	88.14
70	77.1	2.20	88 242	1 454	6 803 458	85.33	13.5	0.91	24 024	1 745	324 324	14.13	0.84	190.75	4.06	90.6	89.39	86.70
60	81.0	2.57	85 932	2 112	6 960 492	87.30	10.8	0.72	21 714	1 222	234 511	17.13	0.77	185.00	2.94	91.8	90.24	84.09
50	84.2	2.18	83 952	2 342	7 068 758	88.66	6.7	0.57	18 480	1 019	123 816	26.76	1.22	179.29	1.55	90.9	90.21	81.49

$\bar{x}$ mean of 3 extractions (2 measurements for each extraction)

$\pm s$ standard deviation of 3 extractions (2 measurements for each extraction)

minimum of 20% water is the key to clear demarcation. With increase in Wc from 20 to 50%, the yield of colour fraction increases and the CV correspondingly reduces, with a net hike in colour recovery from 86.03 to 90.00%. It may be noted that upto 70% methanol, the addition of hexane has little influence on the colour recovery The presence of hexane, however, facilitated the use of still lower methanol concentrations by effecting better separation between colour and pungency phases. Yield of colour fraction and recovery of total colour improve as the solvent concentration reduces from 70 to 50%; on the other hand, further decrease in the recovery of capsaicin is noticed.

With hexane in acetone, the oleoresin is completely soluble in the absence of water. The minimum requirement is 20% water for adequate partitioning. As the Wc increases from 20 to 50%, the yield of colour fraction rises from 67.6 to 84.2% and the colour recovery from 82.03 to 88.66%.

The HPLC concentration of red and yellow pigments in the colour and pungency fractions are presented in Table 4. An immediate observation is that within a solvent system, distribution of these pigment groups in the colour fraction remains more or less steady irrespective of the water content. The pungency fraction derived in the presence of hexane also follows the same pattern. On the other hand, in

Table 4

HPLC concentration of red and yellow pigment groups in the colour and pungency fractions from capsicum oleoresin

Solvent	Conc (%)	Colour fraction, (%)			Pungency fraction, (%)		
		Red, $\bar{x}$	Yellow, $\bar{x}$	$\pm s$	Red, $\bar{x}$	Yellow, x	$\pm s$
Methanol	100	58.83	41.17	1.21	40.17	59.83	0.95
	90	58.20	41.80	0.90	29.40	70.60	1.11
	80	57.28	42.72	1.46	26.79	73.21	0.81
	70	58.25	41.75	0.75	22.69	77.31	1.97
Acetone	90	59.15	40.85	2.14	46.79	53.21	1.23
	80	58.77	41.23	1.04	43.62	56.38	0.84
	70	58.03	41.97	0.89	36.50	63.50	1.56
Methanol + Hexane	80	58.06	41.94	0.85	40.65	59.35	0.76
	70	58.45	41.55	1.32	38.82	61.18	1.08
	60	58.26	41.74	0.73	39.05	60.95	1.75
	50	58.59	41.41	1.68	39.45	60.55	2.19
Acetone + Hexane	80	61.40	38.60	1.06	36.06	63.94	1.66
	70	61.28	38.72	1.25	35.24	64.76	0.92
	60	61.43	38.57	0.94	33.91	66.09	1.77
	50	62.15	37.85	1.44	34.12	65.88	2.38

$\bar{x}$ mean of 3 extractions (2 measurements for each extraction)

$\pm s$ standard deviation of 3 extractions (2 measurements for each extraction)

the absence of hexane, the red pigment concentration in the pungency fraction drops with increase in Wc and that of yellow pigments elevates correspondingly Aqueous acetone in presence of hexane yields colour concentrate with the highest level of red pigments.

Typical HPLC profile of chilli oleoresin under the specified run conditions is displayed in Fig 1.

In Table 5 are assembled the recovery of red, yellow and total pigments in the colour and pungency fractions. The values are derived from data presented in Tables 1-4. Bulk of the pigments, both red and yellow, are concentrated in the colour fraction. For all the four solvent systems, the recovery of red pigments in the colour fraction progressively increases with Wc. Aqueous acetone-hexane system ranks first in the recovery of red pigments from the oleoresin.

The results on the fractination of chilli oleoresin by partitioning between hexane and 50% acetic acid are presented in Table 6. A striking observation is that in this case the yield of colour matter is maximum and pungency fraction is minimum. However, the total recovery of colour from the oleoresin is 4-5% less than the previous experiments; almost all the total colour is contributed by

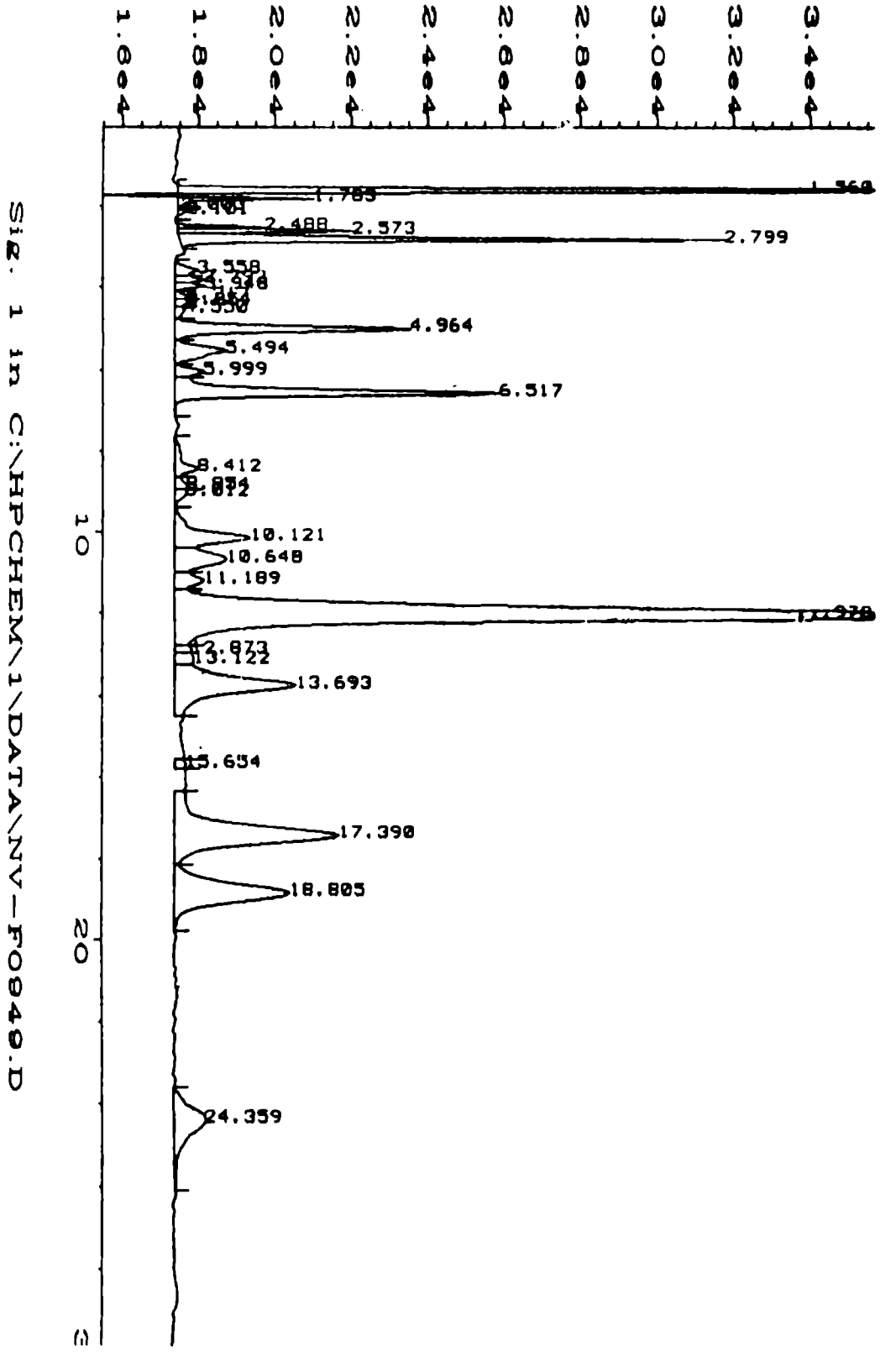

Fig 1. Typical HPLC profile of chilli oleoresin

Table 5

Recovery and distribution of red and yellow pigment groups in the colour and pungency fractions from capsicum oleoresin

(Calculated from the mean values of the results presented in Tables 1, 2, 3 and 4)

		Red pigments (mg) in			Recovery of red pigments (%) in			Yellow pigments (mg) in			Recovery of yellow pigments (%) in		
Solvent	Conc (%)	Colour fraction	Pungency fraction	Total	Colour fraction	Pungency fraction	Total	Colour fraction	Pungency fraction	Total	Colour fraction	Pungency fraction	Total
Methanol	100	2 113	360	2 473	74.37	12.67	87.04	1 479	537	2 016	69.04	25.07	94.11
	90	2 355	117	2 472	82.89	4.12	87.01	1 691	281	1 972	78.94	13.12	92.06
	80	2 445	57	2 502	86.06	2.00	88.06	1 823	156	1 979	85.10	7.28	92.38
	70	2 519	34	2 553	88.66	1.19	89.85	1 805	116	1 921	84.26	5.41	89.67
Acetone	90	1 878	592	2 470	66.10	20.83	86.93	1 297	674	1 971	60.55	31.46	92.01
	80	2 375	162	2 537	83.59	5.70	89.29	1 666	210	1 876	77.77	9.80	87.57
	70	2 476	58	2 534	87.15	2.04	89.19	1 791	101	1 892	83.61	4.71	88.32
Methanol + Hexane	80	2 488	90	2 578	87.57	3.16	90.73	1 798	131	1 929	83.94	6.11	90.05
	70	2 543	55	2 598	89.51	1.93	91.44	1 808	87	1 895	84.40	4.06	88.46
	60	2 587	35	2 622	91.05	1.23	92.28	1 853	54	1 907	86.50	2.52	89.02
	50	2 627	11	2 638	92.46	0.38	92.84	1 856	17	1 873	86.65	0.79	87.44
Acetone + Hexane	80	2 509	147	2 656	88.31	5.17	97.46	1 578	261	1 839	73.66	12.18	85.84
	70	2 606	71	2 677	91.72	2.50	96.33	1 646	131	1 777	76.84	6.11	82.95
	60	2 672	49	2 721	94.05	1.72	97.43	1 677	97	1 774	78.29	4.52	82.82
	50	2 745	26	2 771	96.62	0.91	98.41	1 672	51	1 723	78.05	2.38	80.43

Table 6

Fractionation of capsicum oleoresin using aqueous acetic acid and ethanol in the presence of hexane

	Aq. acetic acid				Aq. ethanol			
	Colour fraction		Pungency fraction		Colour fraction		Pungency fraction	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Quantity (g)	88.2	2.31	3.6	0.54	78.5	1.95	12.7	1.02
Colour value (Colour units)	76 560	1 720	4 620	330	87 912	1 314	23 100	1 022
Red pigment conc. (HPLC) (%)	55.73	1.86	31.42	2.11	57.68	1.27	34.01	1.44
Yellow pigment conc. (HPLC) (%)	44.27	1.86	68.58	2.11	42.32	1.27	65.99	1.44
Capsaicin content (%)	--	--	38.70	1.76	--	--	15.60	0.61
Total colour (Units)	6 752 592		16 632		6 901 092		293 370	
Colour recovery (%)	84.69		0.21		86.55		3.68	
Total pigment conc. (g/kg)	47.85		2.88		53.87		14.43	
Red pigments (mg)	2 352		3		2 439		62	
Yellow pigments (mg)	1 868		7		1 789		121	
Recovery of red pigments (%)	82.78		0.10		85.85		2.18	
Recovery of yellow pigments (%)	87.20		0.32		83.52		5.65	
Total capsaicin (Units)	--		139.32		--		198.12	
Recovery of capsaicin (%)	--		63.32		--		90.05	

$\bar{x}$ mean of 3 extractions (2 measurements for each run)

$\pm s$ standard deviation of 3 extractions (2 measurements for each run)

the pigment fraction. The pigment phase retains traces of acetic acid and its complete removal requires higher temperature and longer desolventisation time or additional process steps, leading to colour loss. Recovery of red pigment pool and its concentration in the colour fraction are also lower. The acetic acid layer contains all the capsaicinoids, the recovery of which from acid is difficult. About two-third of the capsaicinoids present in the starting material only could be recovered.

Table 6 also displays the results of similar runs using hexane and 80% ethanol. The data are fairly comparable with runs using aqueous methanol.

3.4 CONCLUSIONS

Fractionation with methanol or acetone upto 30% water content can be conveniently used for the retrieval of pungency-free colour concentrate from Indian chilli oleoresin. Lower concentrations can be employed in conjunction with n-hexane for better recovery of colour matter and red pigments. Yield of colour matter and pigment recovery increases with the water content of the solvent system. However, the recovery of pungent principles exhibit a gradual fall as the water content is increased.

Concentration of red pigments in colour fraction is an

important quality criterion of capsicum concentrates. Fractionation with aqueous acetone in presence of hexane yields a colour concentrate rich in red pigments.

In the solvent systems considered above, variation in water content has little influence on the distribution pattern of red and yellow pigments in the colour fraction. As regards to pungency fraction, gradual fall in the concentration of red pigments with increase in water content is noticed when derived in the absence of hexane.

Yield of colour fraction is highest when the oleoresin is partitioned between hexane and aqueous acetic acid. However, recovery of total colour and red pigments in the colour fraction are lower.

Partitioning between hexane and aqueous ethanol yields results almost in line with the runs incorporating aqueous methanol.

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CHAPTER IV

EVALUATION OF COLOUR VALUE AND PIGMENT CONCENTRATION OF CAPSICUM EXTRACTS

4.1 INTRODUCTION

Earlier, we examined the different parameters related to the optimisation of the recovery of colour matter from Indian chilli. The quality evaluation of the redeemed colour concentrate constitutes the subject matter of the present chapter.

Colour, the prized property of carotenoid pigments from capsicum, is essentially the cumulative contribution of the red and yellow pigments in the spice (SHUSTER & LOCKHART, 1954; SZABO, 1970; SALZER, 1977) In the pigment pool, the red components constitute about 70 to 85% and yellow, about 15 to 23% (SZABO, 1970; SALZER, 1977; GOVINDARAJAN et al., 1987) Interest lies in the red components and others play only a subordinate role (MOSTER & PRATER, 1952; SHUSTER & LOCKHART, 1954; PURSEGLOVE et al., 1987)

For food colouring, the most coveted quality of capsicum extract is its colour value (CV) (HORNERO-MENDEZ et al., 1993) Measurement of this factor by EOA (ESSENTIAL OIL ASSOCIATION OF U.S.A) (EOA, 1965), ASTA (AMERICAN SPICE TRADE ASSOCIATION) (ASTA, 1985) or MSD-10 (MSD-10, 1959) method exploits the absorbance of a dilute acetone solution of the colour matter at 458, 460 and 462 nm respectively; the optical density obtained is multiplied by appropriate

constant to give the CV. But how the individual colour components in capsicum concentrates influence the colour value is not known.

Further, for evaluating the capsicum extracts, a knowledge of red, yellow and total pigment concentration in them is of importance. Utilising open column (CURL, 1962; DE LA MAR & FRANCIS, 1969), thin layer (TLC) (VINKLER & RICHTER, 1972) and high pressure liquid (HPLC) (BARANYAI et al., 1982; GREGORY et al., 1987; PHILIP & CHEN, 1988; MEJIA et al., 1988; BIACS et al., 1989) chromatography and spectrophotometric techniques (MOSTER & PRATER, 1952,1957; BENEDEK, 1958; POHLE & GREGORY,1960; ANDRE,1973; FEKETE et al., 1976a, 1976b; HASPEL & HORICKOVA, 1976; WOODBURY, 1977; MALCHEV et al., 1982; VARGA et al., 1984), this problem has been examined in depth. Of these, the simple and rapid methods of BENEDEK, FEKETE & co-workers and HASPEL & HORICKOVA do not give agreeable results.

It is reported (VINKLER & RICHTER, 1972) that there exists an equilibrium between the red, yellow and total pigment content in the spice, ie., the percentage of red components increases with increase in total pigment content and that of yellow components decreases correspondingly

A simple correlation suggested (SZABO, 1970) to arrive at

pigment concentration is that pigment content in g/kg obtained according to BENEDEK's method is equivalent to EOA colour value divided by 1600. Another approach is that 40 ASTA colour units approximates a pigment concentration of 1 g/kg; however, this conversion factor has not been backed by published data (ASTA, 1995)

For quick assessment of capsicum extract, the ratio of absorbance values in acetone at 472 and 454 nm is also recommended as a yardstick (MAYER, 1989), but no framework is available as to the limits of this ratio to compositional variation of red-yellow pigment system of the concentrates.

Colour strength of capsicum extract has also been evaluated in terms of micrograms ß-carotene per gram of the sample (POHLE & GREGORY, 1960; TANDON et al., 1964; DE LA MAR & FRANCIS, 1969; MATHEW et al., 1971; RAMAKRISHNAN & FRANCIS, 1973; GOPALAKRISHNAN et al., 1987) This determination is based on the assumption that ß-carotene has colour characteristics very similar to the colouring matter of capsicum. How ß-carotene can represent the complex mixture of pigment components in capsicum extract is not quite clear.

From the foregoing appraisal it may be concluded that the

current analytical methods of capsicum colour are not dependable and this demands scrutiny The present study designed to (1) evaluate the response of the major red and yellow pigments to the colour value, (2) investigate the accuracy of spectrophotometric methods for the estimation of the red, yellow and total pigments, (3) confirm the trustworthiness of commonly adopted conversions of colour value to pigment concentration and (4) re-examine the existence of the alleged equilibrium between the red, yellow and total pigment pigment content in capsicum extracts.

4.1 MATERIALS AND METHODS

ß-Carotene, purity > 97% (Fluka) and pure capsanthin and lutein (gifts from Laboratorios Bioquimex, S.A. DE C.V Mexico) were used. Other carotenoid components for co-HPLC were isolates from capsicum extract by TLC. Analytical grade reagents were used for spectrophotometry and TLC, and chromatography grade for HPLC. Capsicum extracts of varying colour strengths were Synthite's products. Capsicum extract of approx. 40,000 colour units is designated as C-40.

CVs were determined by EOA, ASTA and MSD-10 and quantitative estimation of pigments by spectrophotometric methods (BENEDEK, 1958; FEKETE et al., 1976a, 1976b; HASPEL & HORICKOVA, 1976) in a Hitachi U-2000 Spectrophotometer.

TLC runs were carried out as per procedure earlier reported (VINKLER & RICHTER, 1972) HPLC analyses were carried out on saponified samples in a Hewlett-Packard Series 1050 High Pressure Liquid Chromatograph using C-18 Reversed phase column (Supelcosil LC-SI (3 micron) columns, 15.0 cm x 4.6 mm ID and 7.5 cm x 4.6 mm ID connected in series); n-hexane - ethyl acetate (65 35) was used as mobile phase.

4.2 RESULTS AND DISCUSSION

CVs of ß-carotene, C-40 and C-40 fortified with 1, 2 and 3% ß-carotene obtained by EOA, ASTA and MSD-10 methods are recorded in runs 1, 2, 3, 4 and 5 respectively of Table 1. Results of similar runs by addition of capsanthin are listed in runs 6-9.

ß-Carotene and capsanthin display high CV of 1 513 380 and 1 557 600 MSD-10 colour units respectively, approximately 35-36 times that of C-40 concentrate. With increments in these pigment constituents, the CV of capsicum extract increases; C-40 doped with 3% ß-carotene or capsanthin experiences doubling of CV of the original. Clearly then, CV of capsicum extract leans on the colour value of the pigment components. Thus, a high CV of capsicum extract is no guarantee to richness in red pigments.

Table 1

Colour value of C-40 dosed with varying percentages of β-carotene and capsanthin

Run No	Substrate	Method					
		EOA		ASTA		MSD-10	
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
1	2	3	4	5	6	7	8
1	C-40	39 995	122	1 069	7	42 768	132
2	β-Carotene	1 480 470	5 060	38 950	259	1 513 380	4 620
3	99% C-40 + 1% β-carotene	55 144	183	1 466	8	58 146	198
4	98% C-40 + 2% β-carotene	73 261	245	1 931	12	76 758	264
5	97% C-40 + 3% β-carotene	83 448	283	2 204	16	87 252	271
6	Capsanthin	1 415 200	6 100	37 720	328	1 557 600	5 640
7	99% C-40 + 1% capsanthin	54 290	244	1 459	11	58 905	187
8	98% C-40 + 2% capsanthin	68 015	198	1 824	9	74 250	215
9	97% C-40 + 3% capsanthin	81 282	311	2 205	13	89 265	245

$\bar{x}$ mean value of 4 measurements

±s standard deviation of 4 measurements

Table 2 summarises the pigment concentrations obtained by the different spectrophotometric methods. Column 3 gives the total pigment content by BENEDEK's procedure, without shedding any light on the quantum of red and yellow principles, either individually or collectively. Each 1% increment in ß-carotene/capsanthin is reflected by a corresponding projection of ca. 10 g/kg to the pigment pool. Thus, BENEDEK's method is sensitive and is useful for rapid objective measurement of total pigments in capsicum extracts.

Columns 5, 7 and 9 of Table 2 display the distribution of red, yellow and total pigments by the method of FEKETE & co-workers. As compared to the value obtained by BENEDEK's method, here the total colour matter is distinctly higher. What is puzzling, however, is the twist in the distribution pattern of red and yellow pigment groups. ß-Carotene resurrects partly in the red and partly in the yellow pigment pool, though the total hike of 10 g/kg for 1% increment is almost in harmony with the expected theoretical equivalent. For each 1% increment in capsanthin, the red pigment concentration increases by 13 g/kg, ie., 3 g/kg in excess of the theoretical figure. However, the yellow pigments show a fall by ca. 3 g/kg, keeping the net hike at ca. 10 g/kg. Hence, it is concluded that this method

Table 2

Pigment distribution by different spectrophotometric methods, g/kg

Run No.	Substrate	Method: BENEDEK Total pigments $\bar{x}$	BENEDEK Total pigments ±s	FEKETE & co-workers Red pigments $\bar{x}$	FEKETE & co-workers Red pigments ±s	FEKETE & co-workers Yellow pigments $\bar{x}$	FEKETE & co-workers Yellow pigments ±s	FEKETE & co-workers Total pigments	HASPEL & HORICKOVA Red pigments $\bar{x}$	HASPEL & HORICKOVA Red pigments ±s	HASPEL & HORICKOVA Yellow pigments $\bar{x}$	HASPEL & HORICKOVA Yellow pigments ±s	HASPEL & HORICKOVA Total pigments
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	C-40	21.97	0.37	14.30	0.31	12.25	0.24	26.55	15.80	0.27	27.57	0.35	43.37
2	99% C-40 + 1% β-carotene	31.69	0.46	21.15	0.51	15.60	0.33	36.75	21.13	0.40	36.44	0.45	57.57
3	98% C-40 + 2% β-carotene	40.56	0.39	27 74	0.44	18.08	0.31	45.82	25.30	0.28	42.40	0.41	67 70
4	97% C-40 + 3% β-carotene	49.99	0.62	33.82	0.55	22.04	0.42	55.86	27.23	0.33	50.40	0.51	77.63
5	99% C-40 + 1% capsanthin	30.59	0.47	27 70	0.31	10.23	0.22	37.93	27.12	0.50	23.33	0.54	50.45
6	98% C-40 + 2% capsanthin	40.49	0.38	40.59	0.46	7.69	0.30	48.28	39.72	0.33	14.34	0.29	54.06
7	97% C-40 + 3% capsanthin	49.33	0.58	53.01	0.75	4.79	0.35	57.80	51.99	0 61	7.12	0.42	59.11

$\bar{x}$ mean value of 4 measurements

±s standard deviation of 4 measurements

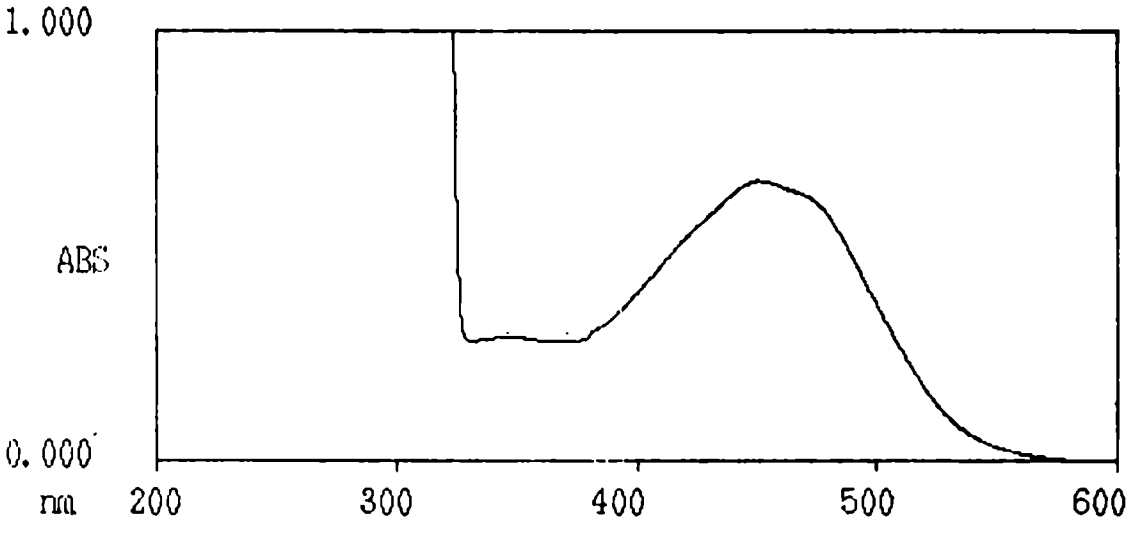
is unlikely to ensure correct quantification of red and yellow pigments.

Finally, in columns 10, 12 and 14 of Table 2 are assembled the red, yellow and total pigment concentrations as determined by the method of HASPEL & HORICKOVA. The estimated total pigment content is higher than that by the other methods. As in the above case, ß-carotene is partitioned between red and yellow pigments. For incremental addition of 1% capsanthin, the red pigments increase by ca. 12 g/kg; on the other hand, the yellow pigments diminish by 4-9 g/kg so that the net hike in the total pigment content is less than the calculated value.

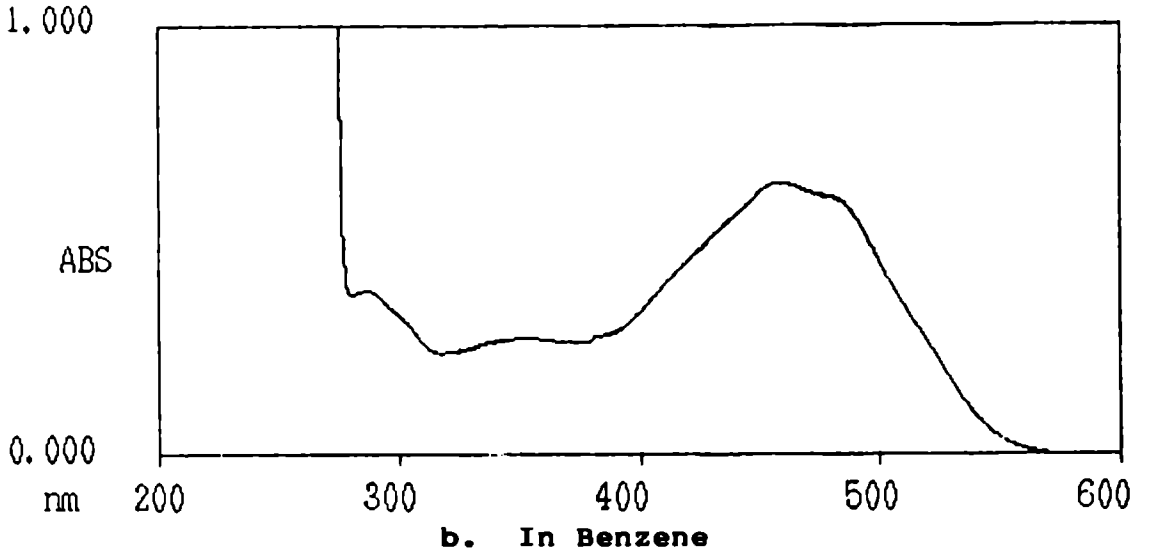
Typical VIS Spectra of chilli colour in the three solvent systems are displayed in Fig 1.

In Table 3 are assembled the CV and HPLC distribution of pigments in 8 samples of capsicum extracts. Samples 1-8 display colour values which increase in that order. Clearly then, there is no correlation between the percentage of red or yellow components and the total pigment concentration; this conclusion is contrary to that of earlier workers (VINKLER & RICHTER, 1972) The results of Table 1 and Table 2 also back the above conclusion.

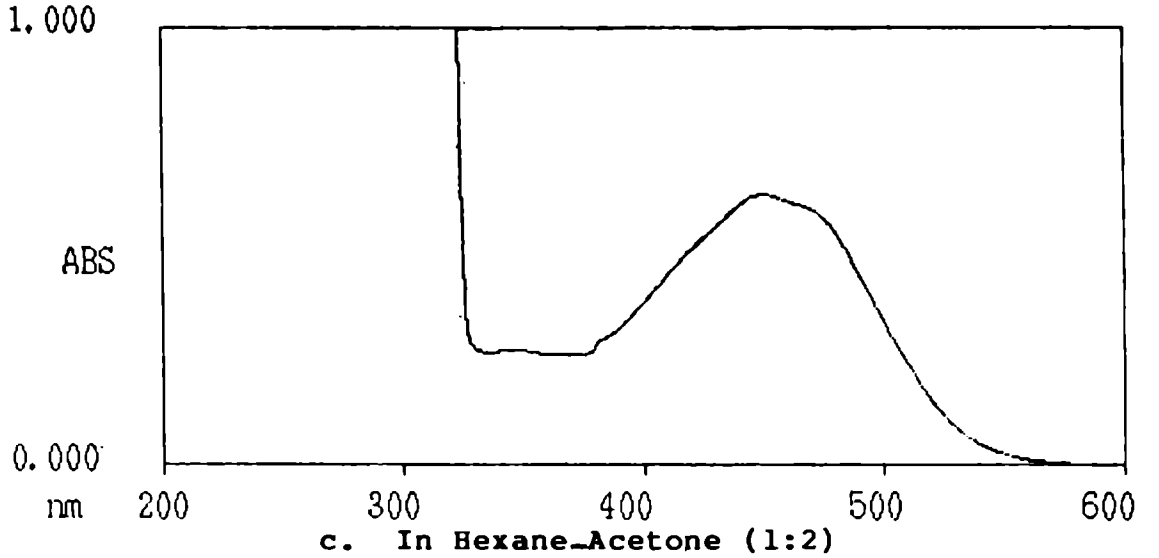
Table 3 also gives the EOA CV/1600 value and the



a. In Acetone



b. In Benzene



c. In Hexane-Acetone (1:2)

Fig 1. VIS Spectra of chilli colour

Table 3

HPLC evaluation of carotenoids distribution in different capsicum extracts

Sample	1	2	3	4	5	6	7	8
Colour Value								
EOA	4 770	14 030	21 838	35 868	54 290	76 128	97 112	104 188
MSD-10	5 042	14 982	23 364	38 280	57 948	81 576	104 280	111 408
ASTA	127	381	561	952	1 490	1 974	2 590	2 738
EOA CV/1600	2.98	8.76	13.64	22.41	33.93	47.58	60.69	65.11
Pigment conc. by BENEDEK's method,g/kg	2.49	7.98	11.81	20.00	29.96	41.50	55.18	58.82
Components	%	%	%	%	%	%	%	%
β -Carotene	3.33	8.56	6.33	12.25	12.60	16.47	9.33	15.24
Non Identified	5.04	2.31	1.55	1.06	0.64	0.35	0.22	0.27
Cryptoxanthin	7.23	6.52	8.38	7.84	7.39	8.14	8.04	8.04
like-Lutein	14.51	8.20	12.36	9.63	8.20	8.07	8.57	8.64
trans-Lutein	0.98	1.08	0.77	0.46	0.44	0.51	0.53	0.43
trans-Zeaxanthin	7.90	8.40	8.41	8.05	9.09	9.05	7.05	8.58
Yellows	8.21	5.96	7.28	5.64	5.17	5.48	6.00	5.14
trans-Capsanthin	33.44	38.80	31.69	33.60	35.54	32.08	36.46	33.41
Violaxanthin	4.34	5.39	5.76	5.44	5.22	5.27	5.81	5.16
cis-Capsanthin	12.46	11.97	14.99	13.43	13.08	12.32	14.96	12.52
cis-trans-Capsorubin	2.56	2.81	2.48	2.60	2.63	2.26	3.03	2.57
% Red Pigments	52.80	58.97	54.92	55.07	56.47	51.93	60.26	53.66

corresponding pigment concentration by BENEDEK's procedure for the 8 samples. The EOA CV/1600 value is higher than the pigment content through BENEDEK's procedure by 10-20% as against the earlier observation (SZABO, 1970)

HPLC profile of a typical run under the specified run conditions is presented in Fig 2. Here, the column and solvent system have been changed compared to that of Chapter III for better resolution.

In Table 4 are presented the CV and total pigments by HPLC in various samples of capsicum extracts. It is interesting to note that the total pigment concentration by HPLC and the corresponding MSD-10 CV /1600 value are almost in harmony The marginal difference between the two values, as listed in column 11 may be attributed to the loss of pigments during saponification (GOVINDARAJAN et al., 1987) and analysis. It may also be noted that the MSD-10 CV/1600 value agrees fairly well with ASTA CV/40 value. Thus, the pigment concentrations calculated by these two conversions approximate the HPLC value.

4.4 CONCLUSIONS

Colour value determined by EOA, ASTA or MSD-10 method hitherto taken for granted as the quality determinant in judging capsicum extracts is now shown to be in error. Also,

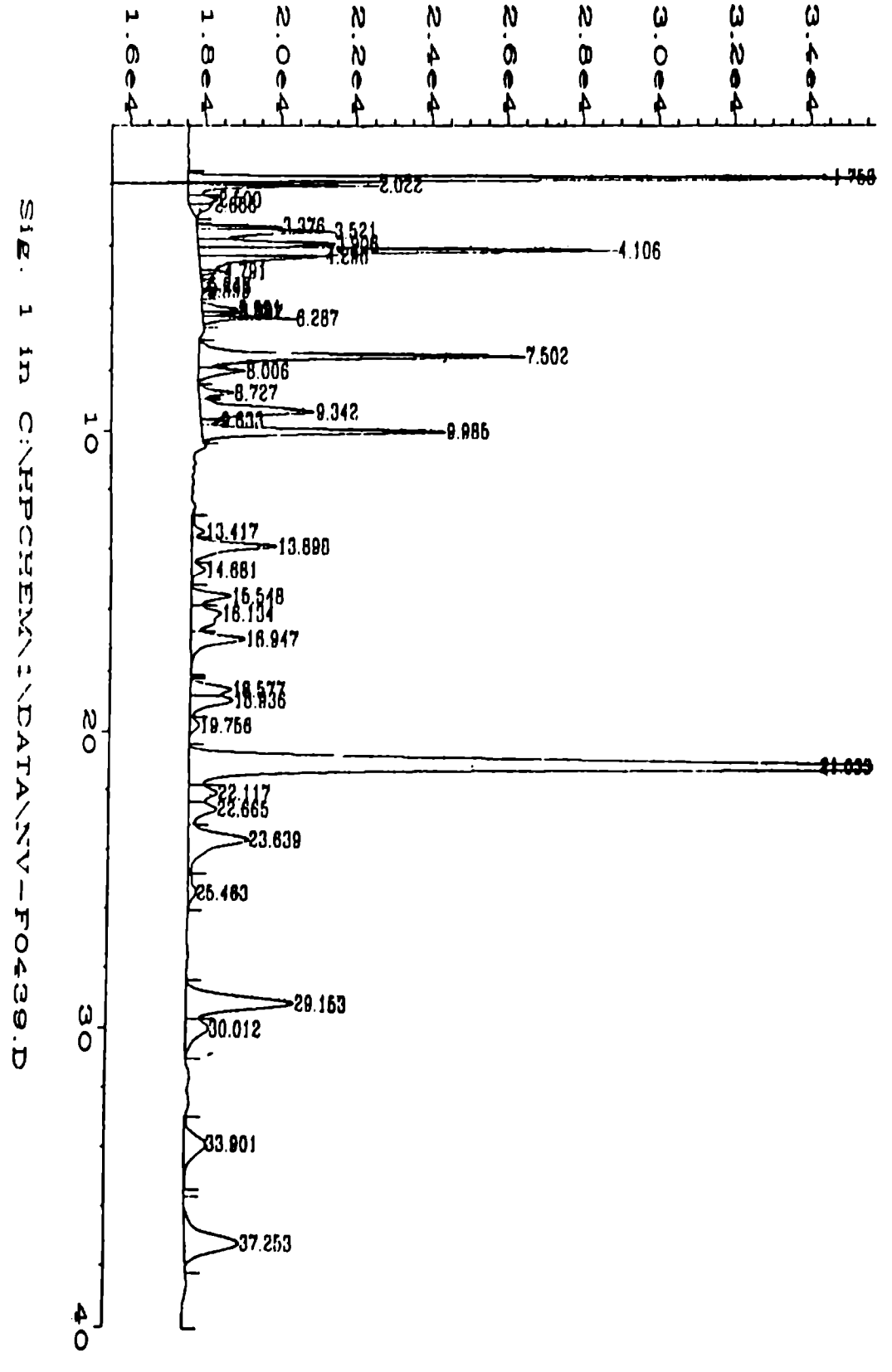


Fig 2. Typical HPLC profile of chilli oleoresin

Table 4

CV and HPLC concentration of pigments in capsicum extracts

Sample No.	EOA CV	ASTA CV	MSD-10 CV	EOA CV / 1600	ASTA CV / 40	MSD-10 CV / 1600	HPLC Conc., g/kg	(8 - 5)	(6 - 8)	(7 - 8)
1	2	3	4	5	6	7	8	9	10	11
1	118 900	3 188.16	128 000	74.31	79.70	80.00	78.52	4.21	1.18	1.48
2	113 200	3 034.00	121 800	70.75	75.85	76.12	74.85	4.10	1.00	1.27
3	109 400	2 935.60	117 700	68.37	73.39	73.56	72.46	4.09	0.93	1.10
4	95 600	2 568.24	102 900	59.75	64.20	64.31	62.38	2.63	1.82	1.93
5	58 200	1 561.28	62 600	36.37	39.03	39.12	38.07	1.70	0.96	1.05
6	39 600	1 061.08	42 900	24.75	26.52	26.81	25.24	0.49	1.28	1.57

the spectrophotometric methods used in the investigation do not guarantee accurate quantification of red and yellow pigments.

The MSD-10 CV divided by 1600 or ASTA CV by 40 provides rapid measurement of total pigment concentration in capsicum extracts.

For quantification of red and yellow pigment groups, one has to resort to HPLC.

No equilibrium is found to exist between the red and yellow pigment components of the spice.

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CHAPTER V

SUMMARY AND CONCLUSIONS

This thesis is essentially concerned with a study of the recovery of pungency-free colour matter from capsicum spice of Indian origin.

A comprehensive survey of the available literature on capsicum pigments is integrated in Chapter I.

The work presented herein are in the following phases

Phase 1 (Chapter II)	Extraction of the oleoresin from the spice.
Phase 2 (Chapter III)	Fractionation of oleoresin recover pungency-free colour concentrate.
Phase 3 (Chapter IV)	Examination of the popular analytical methods for the evaluation of the colour concentrate.

In Phase 1, we screened different varieties of chilli available to the oleoresin industry with a view to evaluating the yield, pungency and colour strength of the oleoresins derived therefrom. On the basis of the investigations, we grouped these varieties into (1) high colour, low pungent, (2) medium colour, high pungent, (3) low colour, high pungent, (4) medium colour, medium pungent

and (5) low colour, medium pungent varieties. For the recovery of colour principles, the spice belonging to Group (1) viz. Byadgi and Tomato are the best.

Having established the excellence of Byadgi and Tomato varieties as sources for the retrieval of pigments, we examined the performance of different extraction media for the recovery of colour and pungency factors. In view of the fact that Byadgi and Tomato chilli contain, in addition to high concentration of colour matter, some amount of capsaicinoids, the extraction solvent should be efficient enough to retrieve both these factors from the spice. Though acetone is solvent par excellence, it suffers from water miscibility, thus necessitating frequent rectification during processing. Hexane is good for colour, but does not extract capsaicinoids efficiently Ethylene dichloride is found to be the ideal extraction medium. Since the use of ethylene dichloride, being a chlorinated hydrocarbon, is facing resistance, ethyl acetate is proposed as a viable alternative.

Next, we examined the influence of spice particle size on extractor capacity, oleoresin yield, pigment recovery, percolation time and solvent requirement. The first four factors are found to increase with fall in particle size whereas the solvent requirement decreased. Except for high

percolation rate, all these factors enhance process economy For extraction, a particle size of 1 mm is recommended.

Liquid-liquid fractionation of capsicum oleoresin for its resolution into colour and pungency-rich fractions is presented in Phase (2) It is concluded that fractionation with methanol or acetone upto 30% water content can be conveniently used for the retrieval of colour matter from chilli oleoresin. Lower solvent concentrations can be employed in conjunction with a second solvent. Yield of colour matter and pigment recovery increase with water content of the solvent system, whereas the recovery of pungent principles decreases. It is also observed that in a given solvent system, the variation of water content has little effect on the distribution pattern of red and yellow pigment groups in the pigment phase. However, in the pungency fraction, fall in the concentration of red pigments with increase in water content was noticed when derived in the absence of hexane. Concentration of red pigments in the colour fraction is optimum when the oleoresin is partitioned with aqueous acetone in presence of hexane. Highest yield of colour fraction is obtained when the oleoresin is resolved between hexane and aqueous acetic acid. However, the recovery of total colour and red pigments in the colour

fraction are lower. Partitioning of the oleoresin between hexane and aqueous ethanol yields results almost in line with runs incorporating aqueous methanol.

Phase 3 discloses the inadequacy of the currently popular spectrophotometric methods in evaluating capsicum concentrates. Extensive investigation has led to the conclusion that the colour value determined by the EOA, ASTA or MSD-10 Method hitherto taken for granted as the quality determinant in judging capsicum extracts, does not reflect the true tinctorial profile of the substrate. Also, the spectrophotometric methods used in the quantification of red and yellow pigment groups are not dependable. In view of these findings, the best approach for evaluating capsicum colour is HPLC. MSD-10 Colour value divided by 1600 or ASTA Colour value by 40 provides rapid measurement of the total pigment concentration of capsicum extracts. Thus, this study provides the key to the hitherto presumed relationships between these factors.

Finally, it is established that no equilibrium exists between the red and yellow pigment components of the spice.

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