CAPSAICIN AND COLOUR EXTRACTION FROM DIFFERENT VERITIES OF GREEN AND RED CHILLI PEPPERS OF ANDHRAPRADESH

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ABSTRACT

Twelve different chilli varieties at three different stages (green, red ripe, red dry) were collected. The chillies were subjected for the estimation and extraction of capsaicin and colour. The estimation of capsaicin was done in the laboratory to determine the pungency for each chilli variety collected. The pungency level was given in Scoville units. The chilli sample which has the highest pungency was subjected for the extraction of capsaicin. From the pericarp of the chilli the colour was extracted and the colour intensity was found according to the ASTA (American Spice Trade Association) and CU (Colour Units). The large scale industrial extraction procedure for the extraction of capsaicin and colour was collected.

Key words: Green and Red Chilli Samples, Capsaicin, Colour Oleoresin.

INTRODUCTION

In India, Pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colors and antioxidant compounds (Nevarro *et al.*, 2006). Peppers are classified by fruit characteristics, i.e. pungency, color, shape, flavor, size, and their use (Smith *et al.* 1987; Bosland 1992). Hot peppers are popular food in many parts of the world for their

sensory attributes pungency and aroma. Most parts of the world, pungency increases the acceptance of the insipid basic nutrient foods (Bosland 1999). In, Tunisia Hot peppers (*Capsicum annuum* L.) are widely produced and consumed as raw, cooked, or processed products. The consumption of hot peppers is due mainly to their very pungent flavor. Pungency is a key characteristic associated with members of the genus Capsicum and is also an important fruit quality attribute (Jarret *et al.,* 2007). The pungency is caused by capsaicinoids, and among the most abundant of these components are capsaicin (trans-8 methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8 methyl- Nvanillylnonanamide), which are responsible for about 90% of total pungency (Ravishankar *et al.* 2003; De Masi *et al.*, 2007).

In addition to capsaicin and dihydrocapsaicin, many less abundant capsaicinoids have been detected in Capsicum extracts, including nordihydrocapsaicin, norcapsaicin, homocapsaicin, homodihydrocapsaicin , etc. (Constant & Cordell, 1996, 1995). An accurate determination of the levels of various capsaicinoids has become important because of the increasing demand by consumers for spicy foods, and the increasing use in pharmaceuticals (Kaale *et al.*, 2002). In addition to being widely utilized to give a peppery flavor to meals, capsaicinoids are molecules that have various other properties and applications that make these compounds very interesting to study. All of these capsaicinoides have a notable antimutagenic and antitumoral property (Surh et *al.*, 1995; Surh and Seoul, 2002; Antonius *et al.*, 2009) they also present a high antioxidant activity (Henderson & Slickman, 1999; Antonious *et al*., 2009).

It is a genus of plants from the nightshade family (Solanaceae) native to the America, where it was cultivated for thousands of years by the people of the tropical America, and is now cultivated worldwide. Some of the members of *Capsicum* are used as spices, vegetables, and medicines. The fruit of *Capsicum* plants have a variety of names depending on place and type. They are commonly called chilli pepper, red or green pepper, or sweet pepper in Britain, and typically just capsicum in Australia and Indian English. The large mild form is called bell pepper in the US. They are called paprika in some other countries (although paprika can also refer to the powdered spice made from various capsicum fruit). The original Mexican term, chilli (now chile in Spanish) came from the Nahuatl word chilli or xilli, referring to a huge capsicum variety cultivated at least since 3000 BC, according to remains found in pottery from Puebla and Oaxaca.

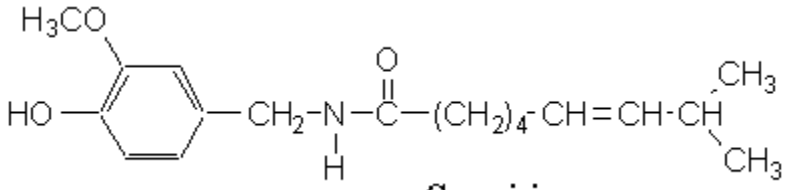
Capsaicin (8-methyl-N- Vanillyl-6-nonenamide) is the active component of chilli peppers. It is a volatile phenolic chemical similar in structure to vanillin, present in the placenta that bears the seeds in the chilli pepper. It is an irritant for mammals, including humans, and produces a sensation of burning in any tissue with which it comes into contact. Capsaicin and its analogs are called capsaicinoids and are produced by chilli peppers, probably as deterrents

against herbivores perhaps through an evolutionary process. Capsaicin is a flavorless, odorless chemical concentrated in the veins of chillies. Capsaicin and dihydrocapsaicin constitute 80-90 percent of the total capsaicinoids in a chilli pepper. The secretion of capsaicin is an adaptation to protect the fruit from consumption by mammals while the bright colors attract birds that will spread the seeds. The amount of capsaicin in capsicums is highly variable and dependent on genetics, giving almost all types of capsicum varied amount of perceived heat. The only capsicum without capsaicin is the bell pepper, a cultivar of *Capsicum annuum*, which has a zero rating on the scoville scale. Chilli peppers are of great importance in native American medicine, and capsaicin is used in modern medicine mainly in topical medications as a circulatory stimulant and pain reliever.

Most of the capsaicin in a pungent (hot) pepper is concentrated in blisters on the epidermis of the interior ribs (septa) that divide the chambers of the fruit to which the seeds are attached. A study on capsaicin production in fruits of *Capsicum chinense* showed that capsaicinoids are produced only in the epidermal cells of the interlocular septa of pungent fruits, that blister formation only occurs as a result of capsaicinoid accumulation, and that pungency and blister formation are controlled by a single locus.

Molecular formula - $C_{18}H_{27}NO_3$

Chemical Structure of Capsaicin (8-methyl-N- Vanillyl-6-nonenamide)



Capsaicin

The amount of capsaicin in hot peppers varies very significantly between varieties, and is measured in Scoville Heat Units (SHU).

Colour is the first notable characteristic of food and often predetermines or "colours" our expectation. We use colour as a way to identify food and a way to judge the quality of food. Studies demonstrate that colour predetermines our expectations of flavor and taste. The colour of chillies is no guide to the intensity of their flavour. All chillies begin life green and turn yellow or red as they ripen, although there is no rule that green or red is hotter.

Colour of ground paprika represents its main quality attribute. It is a kind of dark-red colourful oil liquid, a fine food colour with good mobility. It is light-resistant, heat-resistant, acid-resistant, and alkali-resistant and will not be affected by metal ion. It will be soluble in oil and ethanol. With special processing, it can be soluble in water. More than 20 different pigments from paprika fruits have been identified (Deli *et al*., 2001) green chlorophylls, yellowish orange lutein, zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin and β-carotene. Red capsanthin, capsorubin and cryptoxanthin are characteristic exclusively for the genus *Capsicum* and are the main pigments that determine the colour of red pepper. β-, α-, γ-carotene and β-cryptoxanthin have provitamin activity. A number of investigations revealed that carotenoid rich diet lowers the risk from some degenerative disorders, including different kinds of cancer, cardiovascular and ophthalmologic disorders. Red paprika is a fruit rich in antioxidants. (Daood *et al*., 1996; Deepa *et al*., 2006; Howard *et al*., 2000; Marin *et al*., 2004)

The colour imparted by the oleoresin ranges from red to orange, depending upon the concentration used. Commercial oleoresins are available in strengths ranging from 40,000 to 100,000 ASTA (American Spice Trade Association) colour units. Chillies come in all shapes, sizes and colours ranging from tiny pointed extremely hot, bird's eye chilli to the large mild fleshy peppers like the anaheim. Indigenous to Central and South America and the West Indies, they have been cultivated there for thousands of years before the Spanish conquest, which eventually introduced them to the rest of the world. Mexican cooking is one of the world's oldest cuisines, the explorers of the new world brought back the tomatoes and peppers, red hot chillies, avocados, various beans, vanilla and chocolate, these flavours were to change the flavour of Europe. Today there are probably 400 different chillies are grown, and are one of the most widely cultivated crops today, grown from the Far East, China, Japan, Thailand and Indonesia to India to Mexico.

Chilli peppers are the fruits of the genus *Capsicum*. There are several chilli pepper varieties and they have been domesticated for years. Chilli peppers are used to season foods in many cultures and they have become a staple in the diets of Italians, Spaniards, Hungarians, Indians, Chinese, Indonesians, and Africans. When we eat pepper the capsaicin irritates the lining of our mouth causing that familiar burning sensation. Our brain responds to this stimulus

by producing endorphins, our body's own natural pain reliever. The production of endorphins in response to eating chilli peppers may explain why they can be addicting.

Byadagi chilli, which is less spicy and is well-known for their deep red colour, is in great demand now. Since the preparation of oleoresin, its demand from foreign countries has shot up. Byadagi village, which was earlier famous for its sale of chillies, has now seen a sudden spurt in the growth of cold storage units, wherein these chillies are stored so that they don't lose their colour. Also, several industries that produce oleoresin have been started in the district. On a visit to a cold storage unit, one can experience shivering cold even in scorching summer, as the temperature within the unit is from 4 to 6 degree celsius. Storing Byadagi chillies in the cold storage not only preserves their natural colour, but also results in the extraction of more oleoresin from these chillies. Only the required amount of chillies is taken out from the unit and sent to the industry for the preparation of oleoresin. Nearly 30 to 40 per cent more oleoresin can be extracted from the chillies stored in the cold unit. At present, chilli growers and traders can store their chillies at a cost of Rs 60 to 80 per bag.

MATERIALS AND METHODS

Procedure for organoleptic estimation of Capsaicin

- Obtain the necessary materials required for the estimation process.
- Label the test tubes 1:10, 1: 100, 1: 1,000, 1: 10,000, 1:100,000.
- Fill each test tube with 4.5 ml of 5% sucrose solution.
- Place the weighing boat on the electronic balance. Zero the balance by pushing the "zero" button.
- Cut off a small piece of the pepper and place it in the weighing boat. Weigh 5gm sample of pepper. Cut off or add more pepper until the balance reads 5gm (re-zero the balance if necessary).
- Place the pepper sample in the glass mortar and add 5ml of 95% ethanol. Use the pestle to grind up the pepper in the ethanol.
- Use a clean pipette to transfer 0.5ml of the extract into the test tube marked 1:10. Shake the test tube well.

- Use a clean pipette to transfer 0.5ml of the extract in the 1:10 test tube into the test tube marked 1:100. Shake the test tube well.
- Use a clean pipette to transfer 0.5ml of the extract in the 1:100 test tube into the test tube marked 1:1,000. Shake the test tube well.
- Use a clean pipette to transfer 0.5ml of the extract in the 1:1,000 test tube into the test tube marked 1:10,000. Shake the test tube well.
- Use a clean pipette to transfer 0.5ml of the extract in the 1:10,000 test tube into the test tube marked 1:100,000. Shake the test tube well.
- Place a clean pipette in the 1:100,000 test tube.
- Taste test is performed with the other four members of our group. Using the clean pipette in the 1:100,000 test tube to place a drop of the solution on each person's stirrer. All members placed the drop on their tongues and determined if they can detect the capsaicin.
- Then we moved the same pipette to the next test tube (1:10,000) and repeated the taste test. We continued until at least three people in our group can detect the capsaicin in the solution, and then mark the appropriate box in the data table.
- We repeated steps 12-14 until all chilli samples have been tested.
- We recorded the Scoville units in the data table. If capsaicin is detected at the 1:10,000 dilution, then the Scoville unit for that pepper is 10,000.

Procedure for colorimetric estimation of Capsaicin

- Weigh 0.5gm chilli powder into a glass-stoppered test tube of volumetric flask.
- Pipette out 10ml of dry acetone into the flask and shake it for 3hr in a mechanical shaker. Let the contents settle down or centrifuge (10000rpm for 10min).
- Pipette out 1ml of the clear supernatant into a test tube and evaporate to dryness in a hot water bath.
- Dissolve the residue in 5ml of 0.4% sodium hydroxide solution.
- Add 3ml of 3% phosphomolybdic acid.
- Shake the contents and let stand for 1hr.
- Filter the solution quickly into centrifuge tubes to remove any floating debris.
- Centrifuge at about 5000rpm for 10-15min.

- Transfer the clear blue coloured solution directly into the cuvette and read the absorbance at 650nm.
- Run a reagent blank along with the test samples.
- Prepare a standard graph using 0-200µg capsaicin simultaneously i.e. pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of working standard solution and proceed as above.

Procedure for chemical extraction of Capsaicin

- A sample of finely powdered chilli pepper is obtained.
- A soxhlet extraction apparatus is setup for the extraction process.
- 125ml of round bottom flask with a ground glass top is used for the extraction process.
- 5gm of chilli powdered sample is placed in to the cellulose thimble.
- 85ml of methylene chloride solvent is taken in to the round bottom flask.
- The thimble with sample is placed into the recycler, and then this is fixed on round bottom flask containing methylene chloride solvent.
- The assembly is then placed on to the heater with a ring stand and clamp.
- The cold tap water is circulated through the condenser.
- The solvent inside the round bottom flask is gently made to boil by adjusting the regulator of the heater.
- The filling or the siphoning cycle is made to occur for several times.
- The process is observed to ensure smooth operation so that we can confidently let it run unattended.
- The process is allowed to run for a period of time determined by consulting our instructor. Several hours is recommended.
- After the extraction, the capsaicin from the pepper sample is present in the methylene chloride "extract" in the round bottom flask.

Procedure for chemical extraction of colour

The extraction is done by measuring the absorbance of chillies alcoholic extract at 450nm.

- 100mg of finely powdered chilli sample is taken in a 250ml conical flask.
- 100ml of isopropanol is added and the flask is stoppered tightly.
- The contents are swirled and kept for at least 3 hours at 70^0C or over night for 16 hrs at room temperature.
- In the former case (3 hours) the extract is cooled to room temperature and filtered through whatman No.12 filter paper.
- The first 10ml of the extract is discarded.
- 25ml of ensuing extract is transferred to a 50ml volumetric flask and the volume made up with isopropanol.
- The thoroughly mixed solution is transferred to the cell and its absorbance is determined at 450nm.

Calculation:

Absorptivity of standard=

$$\frac{Absorption\ of\ std.potassiumdichromate\ solution\ at\ 450nm}{Cell\ length\ (cm)\ \times\ Conc.(\frac{mg}{ml})}$$

It is denoted by 'A'

Extractable colour as per ASTA units=

$$\frac{Absorption\ of\ extract\ at\ 450nm * 200}{Cell\ length\ (cm) \times A}$$

If the concentration of $K_2Cr_2O_7$ and the sample is 0.5mg/ml and the same length cells are used, the extractable colour as per ASTA units is calculated as:

$$\frac{Absorbance\ of\ chilli\ extract\ at\ 450nm \times 200}{Absorbance\ of\ potassiumdichromate\ solution\ at\ 450nm}$$

Bureau of Indian standards IS: 9345-1979 method estimates objectively by measuring colour at 485nm.

10mg of oleoresin is diluted to 100ml with acetone using a tungsten lamp source and an acetone blank, absorbance of the above oleoresin solution is measured and multiplied with 61,000 to obtain the colour value.

We have selected different verities of Green chilli and red chilli samples in order to find out in which verities will get more capsaicin. The verities are BSS-304, DAVANUR DELUX, INDAM-5, INDAM-10, INDAM-42, INDAM-48, INDAM-51, INDAM-54, INDAM-67, INDAM-2006-1, NS-17O1, US-341 of both green and red ripe chillies and also red dry chilli samples.

RESULTS AND DISCUSSION

ORGANOLEPTIC ESTIMATION

Table-1: THE RESULT TABLE FOR GREEN CHILLIES:

SL.NO	NAME OF	DILUTION					SCOVILLE
1.	BSS-304					Y	100,000
2.	DAVANUR					Y	100,000
3.	INDAM-5			Y			1,000
4.	INDAM-6			Y			1,000
5.	INDAM-42			Y			1,000
6.	INDAM-48			Y			1,000
7.	INDAM-51			Y			1,000
8.	INDAM-54			Y			1,000
9.	INDAM-67			Y			1,000
10.	INDAM-			Y			1,000
11.	NS-17O1				Y		10,000
12.	US-341			Y			1,000

Y-Yes (detection of hotness)

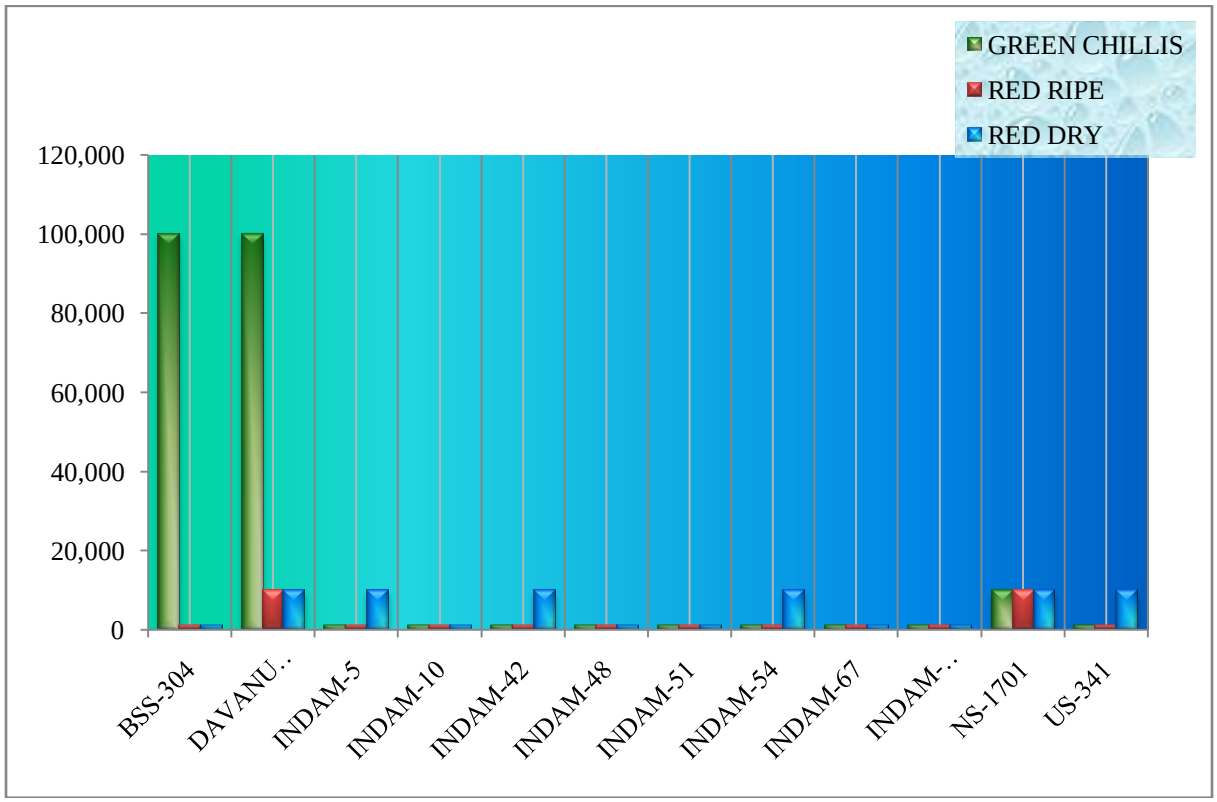
Table-2: THE RESULT TABLE FOR RED RIPE CHILLIES:

SL.NO	NAME OF PEPPER	DILUTION					SCOVILLE UNIT
		1:10	1:100	1:1,000	1:10,000	1:1,00,000	
1.	BSS-304			Y			1,000
2.	DAVANUR DELUX				Y		10,000
3.	INDAM-5			Y			1,000
4.	INDAM-10			Y			1,000
5.	INDAM-42			Y			1,000
6.	INDAM-48			Y			1,000
7.	INDAM-51			Y			1,000
8.	INDAM-54			Y			1,000
9.	INDAM-67			Y			1,000
10.	INDAM-2006-1			Y			1,000
11.	NS-17O1				Y		10,000
12.	US-341			Y			1,000

Table-3: THE RESULT TABLE FOR RED DRY CHILLIES:

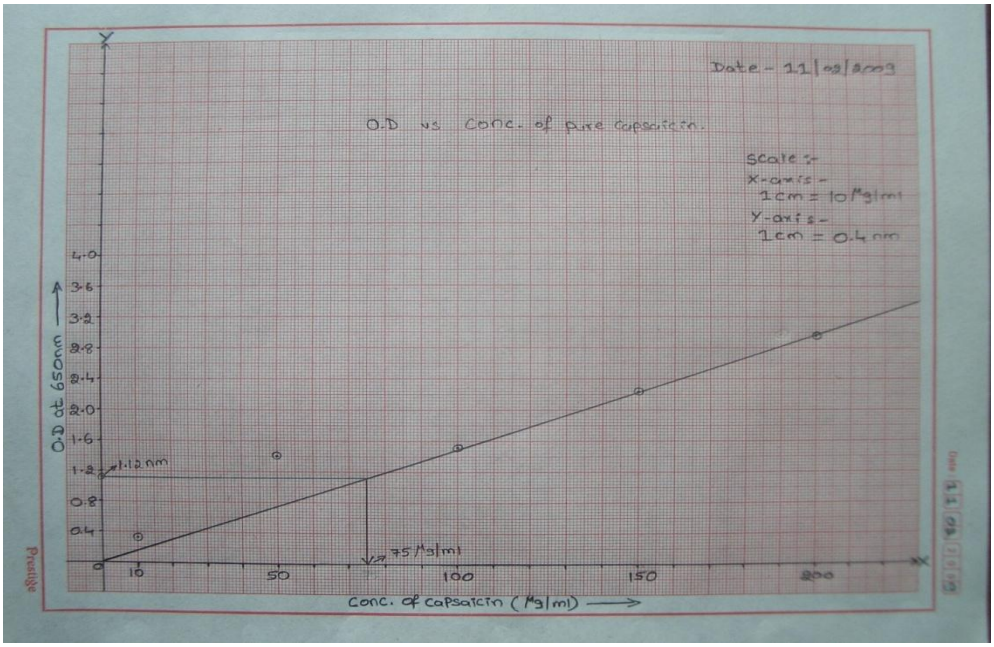
SL.NO	NAME OF PEPPER	DILUTION					SCOVILLE UNIT
		1:10	1:100	1:1,000	1:10,000	1:1,00,000	
1.	BSS-304			Y			1,000
2.	DAVANUR DELUX				Y		10,000
3.	INDAM-5				Y		10,000
4.	INDAM-10			Y			1,000
5.	INDAM-42				Y		10,000
6.	INDAM-48			Y			1,000
7.	INDAM-51			Y			1,000
8.	INDAM-54				Y		10,000
9.	INDAM-67			Y			1,000
10.	INDAM-2006-1			Y			1,000
11.	NS-17O1				Y		10,000
12.	US-341				Y		10,000

Representation of hotness in chilli samples at three different stages



COLORIMETRIC ESTIMATION:

GRAPH



Calculation:

At 650nm the O.D obtained was 1.12nm.

The standard graph of O.D vs. Pure capsaicin was plotted.

Through standard graph, the obtained O.D 1.12nm was plotted and concentration of capsaicin was determined.

The concentration of capsaicin in DAVANUR DELUX is 75µg/ml as shown in above graph.

As per the procedure, thick red extract was obtained which contains both capsaicin and colour as shown in the figure below .

CHEMICAL EXTRACTION OF COLOUR

Calculation:

If the concentration of $K_2Cr_2O_7$ and the sample is 0.5mg/ml and the same length cells are used, the extractable colour as per ASTA units is calculated as:

$$\frac{Absorbance\ of\ chilli\ extract\ at\ 450nm \times 200}{Absorbance\ of\ potassiumdichromate\ solution\ at\ 450nm}$$

Absorbance of chilli extract at 450nm = 1.83nm.

Absorbance of potassiumdichromate solution at 450nm = 0.39nm.

Substituting these in the above equation, we get as below

$\frac{1.83 \times 200}{0.39}$ = 938.46 colour units.

The colour value for DAVANUR DELUX obtained as per above calculation is 938.46 colour units.

Bureau of Indian standards IS: 9345-1979 method estimates objectively by measuring colour at 485nm.

10mg of oleoresin is diluted to 100ml with acetone using a tungsten lamp source and an acetone blank, absorbance of the above oleoresin solution is measured and multiplied with 61,000 to obtain the colour value.

As per Bureau of Indian standards we obtained result for DAVANUR DELUX as,
O.D at 485nm, absorbance is 0.58nm.
So the colour value is given by: O.D at 485nm × 61,000 colour units.
0.58 × 61,000 = 35,380 colour units.

Although there are several reports on extraction of capsaicin and colour from chilli peppers (Attuquayefio *et al.*, 1987; Kanner *et al.*, 1977), Chilli peppers, like the habanero (*Capsicum chinense*), are a rich source of valuable phytochemicals such as capsaicin. The approaches followed by other groups have not resulted in detail about different varieties of green chilli and red chilli (BSS-304, DAVANUR DELUX, INDAM-5, INDAM-6, INDAM-42, INDAM-48, INDAM-51, INDAM-54, INDAM-67, INDAM-2006-1, NS-17O1, US-341) which is a committed step in the production of capsaicin, the pungent principle in pepper and colour. The extraction efficiency of capsaicinoids and colour components from chilli pepper (variety Byadagi) was studied, but for other varieties not yet reported till date. Capsaicinoid content and colour value were determined in raw material and residue material after extraction. The colour intensities of residue material and obtained extracts were given by ASTA (American Spice Trade Association) and CU (Colour Unit) value, respectively. In our approach, we started with BSS-304, DAVANUR DELUX and continued for all varieties as mentioned above. We have started with the following methods.

1. Estimation of capsaicin content by following methods:
    a. Organoleptic method.
    b. Colorimetric method.

2. Extraction of capsaicin content from the high **SHV** (Scoville Heat Value) estimated chilli sample.
3. Extraction of colour from chilli sample.

Chilli samples were collected at different stages (green stage, red ripe stage, and red dry stage from Indo American Hybrid Seed Company). Capsaicin content was estimated from different chilli samples collected. Capsaicin content was extracted.
Interestingly, the high pungency level correlated with high levels of capsaicin content was found in BSS-304, DAVANUR DELUX i.e. 100,000 Scoville units (Table-1).

These results showed that the level of capsaicin is high in BSS-304, DAVANUR DELUX varieties compared to other varieties.

As per our experiment, we have gone through the extraction process for colour extraction and followed the above procedure and got the results. The colour unit obtained for our sample DAVANUR DELUX is 35,380 colour units (as per Bureau of Indian Standards) and 938.46 colour units (as per the procedure calculation).

CONCLUSION

Now a day's natural colour has great value. People are very conscious about health hazards caused by chemical colours, so natural colours would help overcome these. It produces great value in market for its usage in food, textile, and cosmetics. Capsaicin is also used in food industry and pharmaceutical industry. Capsaicin overcomes the storage of chillies and it can be stored for longer periods. It can be made available in low growing chilli areas. Many experiments are carried out on chillies to get better yield. Better colour and capsaicin yielding varieties of chillies would help extraction industries and in turn farmers to get better market.

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