CHAPTER 8

Extraction of Oleoresins and Pungency Analysis in Fruits of *Capsicum chinense*

8.1 Introduction

The term "oleoresin" has been used for desolventized total extracts by a specified solvent. It sometimes refers to a product obtained by benefication of one or more functional components in the total extracts by some amount of fractionation (Prakash and Eipeson, 2003). In its use as a food additive, the best oleoresin of *Capsicum* is that which contains those components that constitute colour and flavour (pungency, aroma and sensory factors) and that which truly recreates, when appropriately diluted in food formulations, the sensory qualities of fresh materials (Govindarajan, 1986). Oleoresins are essentially divided into three types. (1) Oleoresins paprika is used as a food colouring agent in processed meats, dairy products, soups, sauces and snacks. (2) Oleoresin red pepper is a source of both colour and pungency, essentially used in canned meats, sausages, in some snacks and in a dispersed form in some drinks, such as gingerale. (3) Oleoresin *Capsicum* (African) is the most pungent and used for the counter-irritant property in plasters and pharmaceutical preparations (Govindarajan, 1986; Prakash and Eipeson, 2003). The use of the oleoresins from *Capsicum* species in therapeutic applications is increasing day by day. This is leading to a high demand of oleoresins and active principles in the pharmaceutical industry. Despite the advantages in the use of
oleoresin over the ground spices, the world production and use of spice oleoresins is only around 1,600-1,800 tons and represents only 10% of total international spice trade. Oleoresin chillies with a pungency range of 0.25-1.0 x 106 Scoville units (1.66-6.66% capsaicinoids) and colour up to 20,000 units are used by larger food industries in the USA and the UK (Johnson, 1993; Ravishankar et al., 2003).

8.1.1 Pungency and its measurement

Several pungent compounds found in nature are derivatives of O-methoxyphenol, the major principles naturally present in Capsicum are capsaicin and dihydrocapsaicin (Kulka, 1967). The degree of pungency and the character of taste sensation vary markedly with different varieties of chillies. Kobayashi (1925) revealed that the vanillylamide moiety and acyl residues with appropriate chain lengths were required for exerting pungency. Nelson (1919) reported that pungency depends on chain length. The alkyl residue with CSH, showed the strongest pungency. Decrease in pungency was found to be associated with both longer and shorter alkyl chain lengths (Nelson, 1919). With regard to the pungency of this group of compounds, an aromatic ring having a phenolic hydroxyl group and an ether group such as methoxy in ortho position to each other is a basic prerequisite. A side-chain is also necessary. The length and composition of this side-chain are important. The pungency is greatly enhanced by an acid amide group, in this instance vanillylamide, as found in the capsaicinoid molecule (Kulka, 1967). The pungency principle capsaicinoids is located in the placenta of the fruit. The basic principle of pungency evaluation using an organoleptic method was established by Scoville (1912). The Scoville scale is a measurement of pungency, the "spicy heat" of chili peppers. The number of Scoville heat units indicates the amount of capsaicin present
(Peter, 2001). The scale is named after its creator, American pharmacist Wilbur Scoville. His method, devised in 1912, is known as the Scoville Organoleptic Test. This method is based on sensory evaluation. Oleoresin solutions variably diluted with sugar solution are tasted in increasing concentration. The highest dilution at which pungency is just detected is taken as a measure of the heat value. SHU for pure capsaicin are reported as 15-17 X 10^6 (Suzuki et al., 1957; Todd, 1958). Suzuki et al. (1957) compared the pungency determined by the orgalloleptic method to that of chemical methods. Hartman (1970) correlated SHU to the proportion of capsaicinoid content measured using Gas liquid chromatography. Johnson et al. (1993) developed the HPLC method for the separation of capsaicin and other phenyl propanoids. Govindarajan et al. (1977) proposed a standardized procedure for the evaluation of the pungency of SHU by which a linear regression was obtained between SHU and the capsaicin content of samples. The pungency of various chilli pepper varieties of the world are presented in Table 8.1.

8.1.2 Colour of oleoresins capsicum

Oleoresin is the product obtained by solvent extraction of the powdered dried ripe red pods of Capsicum, with the subsequent removal of the solvent. Oleoresin is evaluated strictly on a unit colour basis. The colour is the principal criterion for assessing its quality. The pigment content can range from 0.1 to 0.8% (Pruthi, 2003). The major colouring pigments in Capsicum are capsanthin and capsorubin, comprising 60% of the total carotenoids. Other pigments are betacarotene, zeaxanthin, violaxanthin, neoxanthin and lutein (Anu and Peter, 2000). In the present study, solvent extraction of oleoresins and its quality assessment from fruits of both in vivo and in vitro raised plants were investigated.
8.2 Materials and methods

8.2.1 Extraction of oleoresin

Fruits of *C. chinense* were collected from Rüzaphema village, Nagaland and Plant Biotechnology Laboratory glasshouse, North Eastern Hill University, Shillong. The crisp dry chilli was crushed into fine powder. Oleoresin was extracted from this powder with a solvent mixture of acetone and hexane (60:40, v/v) following the method of Sampathu *et al.* (2006). After the solvent addition, a contact time of 15 h was given and about 100 ml was drained out while simultaneously adding fresh solvent mixture on to the material to keep it soaked. Totally 4 more such extracts were taken after a contact time of 1 h every time. The extract were pooled and desolventized in a Büchi Rotavopor R-205 at 40°C. Further desolventisation was carried out by passing N₂ gas to the extract till solvent was removed.

8.2.2 Determination of colour value

To make the estimation completely objective, instrumental analysis was employed. Colour of specified dilution was estimated at 458 nm, the absorbance reading multiplied by a dilution factor and the result expressed as Nesslerimetric colour value. About 1.0 g of oleoresin was weighed and the volume was made to 100 ml with acetone in a volumetric flask. From this solution, 1.0 ml was pipetted out and added into a second 100 ml volumetric flask and the total volume was made with acetone. The absorbance of the 0.01% solution of oleoresin was read at 458 nm. The value was multiplied by 61,000 to obtain the colour value as described by Pruthi (2003).
8.2.3 High performance liquid chromatography

Quantitation of capsaicin content using HPLC was carried out as described earlier in Chapter 7 (7.2.3-4). Capsaicin content was expressed in percentage and capsaicinoids concentrations were converted to SHU by multiplying the capsaicin content in percentage coefficient corresponding to the heat value for pure capsaicin, which is $1.6 \times 10^5$.

8.2.4 Determination of SHU (Organoleptic evaluation)

Oleoresin (0.4 g) was weighed and 2 drops of ethyl alcohol was added and the volume was made to 100ml with water. A stock (A) solution was prepared by adding 1ml from this solution and making the volume to 50 ml with water (12,500 SHU or $12.5 \times 10^3$ SHU). Further dilutions from stock (A) were prepared using 3% sucrose solution. Samples for sensory analysis were prepared in geometric series and arithmetic series as shown below.

8.2.5 Geometric series

a) Blank- 250ml of 3% sugar solution + 1 drop of ‘A’

b) 2.5 ml of A – made up to 250 ml with 3% sugar solution (pungency 250/2.5) x 12,500=1250x $10^3$ SHU)

c) 5 ml of A- made up to 250 ml with 3% sugar solution (pungency 625x$10^3$ SHU)

d) 10 ml of A- made up to 250 ml with 3% sugar solution (pungency 312x$10^3$ SHU)

e) 20 ml of A- made up to 250 ml with 3% sugar solution (pungency 156x$10^3$ SHU)

f) 40 ml of A- made up to 250 with 3% sugar solution (pungency 78x$10^3$ SHU)
8.2.6 Arithmetic series

a) Blank- 250ml of 3% sugar solution + 1 drop of ‘A’

b) 2.5 ml of A – made up to 250 ml with 3% sugar solution (pungency 250/2.5) x 12,500 = 1250x10³ SHU)

c) 5 ml of A- made up to 250 ml with 3% sugar solution (pungency 625x10³ SHU)

d) 7.5 ml of A- made up to 250 ml with 3% sugar solution (pungency 416x10³ SHU)

e) 10 ml of A- made up to 250 ml with 3% sugar solution (pungency 312x10³ SHU)

f) 12.5 ml of A- made up to 250 ml with 3% sugar solution (pungency 250x10³ SHU)

8.2.7 Sample preparation

One set of samples (geometric series/arithmetic series) containing 6/7 dilutions coded with 3-digits random numbers, were presented to the panellists. Samples were arranged in a sequence of increasing order of intensity of stimulus. Panellists were asked not to disturb the sequence and taste them one by one in the same sequence (ascending order). A glass of water and a palate cleansing agent (puffed rice/ bland crackers/ biscuits) were also served along with the samples.

8.2.8 Calculations

The format of the score sheet is presented in Table 8.2. Pungency value for SHU was scored and the mean was calculated as follows:

Geometric mean = \((x_1 \times x_2 \times x_3 \ldots \times x_n)^{1/n}\)
8.2.9 Statistical analysis

Data was analyzed using one-way analysis of variance (ANOVA) at the 0.05 significance level in JMP® version 7.0.1 (SAS Institute, Cary NC). The significant differences among the means were assessed by Tukey’s Honestly Significant Difference (HSD) test used post-hoc on significant findings.

8.3 Results

In the present investigation, oleoresin was obtained by solvent extraction of powdered dried ripe fruits of *C. chinense* (Fig. 8.1a, b). Oleoresin content in fruits of *C. chinense* from both *in vivo* and *in vitro* raised plants were analyzed and expressed in percentage. Fruits collected from *in vivo* grown plants yielded 11.38 % oleoresins with 3.88 % total capsaicin content where as in the case of *in vitro* propagated plants, oleoresins yield was found to be 11.2 % with 3.75% total capsaicin content (Table 8.3). The amount of oleoresins and capsaicin present in the fruits of *in vitro* and *in vivo* grown plants were significantly not different according to Tukey’s HSD.

8.3.1 Appearance and odour

Desolventized extracts of *C. chinense* fruit oleoresins appeared dark red, somewhat viscid liquid with a pungent odour and a very high bite (Fig. 8.1b).

8.3.2 HPLC and organoleptic evaluation of SHU

Comparison of modern techniques using HPLC and organoleptic evaluation of pungency is presented in Table 8.3 (Fig. 8.1c). The results indicated that the Scoville Heat Unit using HPLC method (620800 SHU) was slightly lower than that of organoleptic evaluation method (625000).
8.3.3 Colour value

A maximum of 157380 colour value was obtained from fruits of in vitro propagated plants. However, slightly lower colour value of 1150670 was obtained from fruits of in vivo raised plants (Table 8.3) (Fig. 8.1d).

8.4 Discussion

Good quantities of chillies are exported in the form of oils and oleoresins. Of all the countries, India is the major producer and only 10% of the total production is exported. The total estimated production in India is 9,500,000 tonnes of which the exports account for only 65,000 tonnes. Other than exports in bulk, exports are in the form of oil, oleoresins, powders, pastes and mixtures (Thampi, 2003). There is a need for the development of improved high yielding varieties of chili that are rich in attractive red pigments and which have higher drying yield and disease resistance. There is also a need to develop better, simpler and quicker methods of quality evaluation of chillies and chilli oleoresin in conjunction with better sensory evaluation techniques (Pruthi, 2003). In the present study, high yield of oleoresins (11.38±0.74%) was obtained using acetone and hexane (60:40, v/v). The oleoresins produced from Indian chillies are not suitable for pharmaceutical purposes and export since their pungency is very low (Pruthi, 2003). However, in the present study, the capsaicin content in oleoresins was also found to be considerably high (3.88±0.92%) as compared to other chilli varieties. Hence, oleoresins from this species may be suitable for pharmaceutical purposes. The heat value, colour value, oleoresins yields from fruits of in vitro propagated plants were found to be statistically not significant as compared to in vivo raised plants; this indicated that, in vitro propagation in spite of offering advantages over conventional propagation
method retained the quality of the fruits of *C. chinense*. Chili oleoresin is one of the most celebrated and most popular natural colourants for different categories of processed foods. Hence there is a greater need for emphasis on intensive R&D work, notably in developing countries like India, which produces the largest quantity (about 900,000 tonnes) of chillies annually (Pruthi, 2003).