Indian J. Pl. Genet. Resources **11**(2): 213-218, 1998

A COLORIMETRIC METHOD FOR ESTIMATION OF CAPSAICIN IN CHILLI FRUITS

S. Mandal, Poonam Suneja and D.K. Hore[1]

National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110 012

A simple colorimetric method involving sodium tungstate- phosphomolybdic acid reagent is described for determining the pungency, due to capsaicin present in oleoresin of chilli fruits. Capsaicin concentration of some locally available chilli fruits and its pericarp, after removal of seed were determined with reference to a calibration graph. Oleoresin nitrogen concentrations of some samples were also determined by conventional Kjeldahl's method and converted to capsaicin by suitable multiplication factor. The results are compared and discussed.

Key words : Capsaicin, chilli fruits, oleoresin, colorimetric method, kjeldahl nitrogen

The capsicums and chillies are used in food preparations in which the quality is of much importance. The quality is based on characteristics like pungency level, a bright red colour, a good flavour, a medium-sized fruit with a moderately thin pericarp, a smooth, glossy surface, less seeds in the fruit and a firm stalk. In pungent chillies that are to be used for the preparation of capsicum oleoresin, a high pungent flavour assumes more importance than the appearance. The pungent flavour being determined by the capsaicin content. Chemically capsaicin is N-3-methoxy-4-hydroxybenzyl-8- methylnon-trans-6-enamide. However, Benett and Kirby (1968), Kosuge and Furata (1970) and Masada *et al.* (1971) have reported that capsaicin is actually a mixture of atleast five closely related vanillyl amides as shown in Figure 1. Various methods are recorded in the literature for the quantitative estimation of capsaicin in capsicum fruits. Among these a gas-liquid chromatography method devised by Todd *et al.* (1977) also permits the resolution of individual capsicinoids. Gonzalez and Tamirano (1973) developed a procedure based on soxhlet extraction of green chilli with isopropanol and ultraviolet absorbance at 281nm. Paper chromatography technique was applied for the purification of sample. Colorimetric method of North (1949) based on the colour reactions

---

[1] National Bureau of Plant Genetic Resources, Regional Station, New Kench's Trace, Shillong 793013.

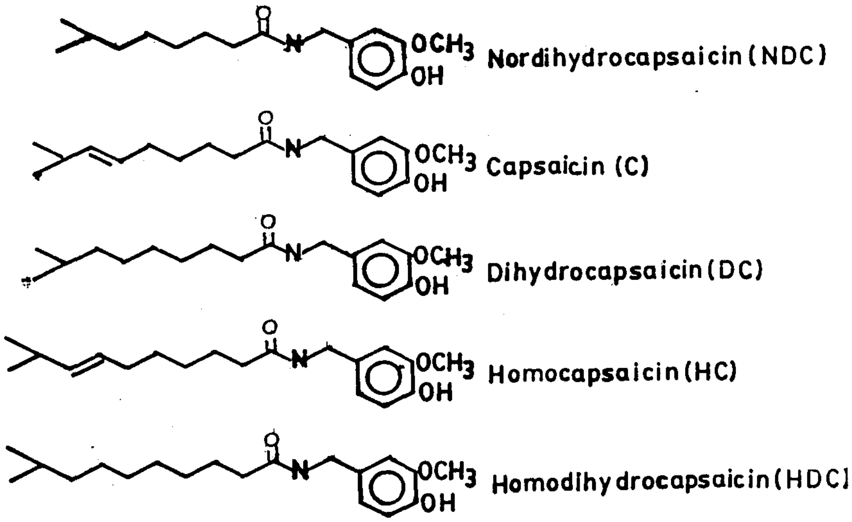

Fig. 1. Natural capsaicinoids

of capsaicin and phosphotungstic-phosphomolybdic acid. Selective solubility technique was followed in extraction procedure to reduce the phenolic interference. All these procedures are time consuming and is not suitable in germplasm evaluation programme. Moreover, Phosphotungsic-phosphomolylbdic acid reagent of North (1949) when applied in the oleoresin extract, colour development was not observed even after 2 h of reaction.

In this paper,a method is described for the determination of the total capsaicin content of samples of *Capsicum* species that have different degrees of pungency. This method is based on a extraction of capsicum oleoresin by acetone followed by the colorimetric determination procedure involving sodium tungstate and phosphomolybdic acid in presence of sodium hydroxide with reference to a standard solution of capsaicin.

MATERIALS AND METHODS

Plant Material

Among five chilli fruits, sample no. 1, 2 & 4 were obtained from the National Bureau of Plant Genetic Resources, Regional Station, Shillong and sample no. 3 and 5 were procured from the local markets based on their morphological variations. All chemicals used were of analytical reagent grade. Standard capsaicin (8- Methyl-N-vanillyl-6-nonenmide) was purchased from

Sigma Chemical, USA. Whole fruit (without stalk) and seedless pericarp parts were ground in a Remi Anupam blender. Evaporation was done in a fumehood. The absorbance at 650 nm was determined by a Beckmann spectropotometer Model 26. Nitrogen content of the samples were determined by the classical Kjeldahl method with kjeltec auto 1030 analyzer from Tecator, Sweden. The samples were digested in the presence of 1013 scrubber unit of Tecator, Sweden.

Extraction of oleoresin from chilli powder

Ground sample (0.2 g) was mixed with 10ml of dry acetone (25 g anhydrous sodium sulphate was added to 500 ml acetone atleast one day before use) for about 3 h by occasional shaking; filtered through Whatmann No. 1 filter paper to get the clear supernatant. Supernatant was devided in 3 parts of 0.5 ml each for colorimetric determination of capsaicin, and from the remaining supernatant 5.0 ml were taken for the estimation of oleoresin percentage.

Colorimetric determination of capsaicin

Supernatant containing oleoresion was dried on heating slab inside fume hood. To dry residue (oleoresin), 0.5 ml of 4% sodium tungstate was added and followed by 4.5 ml of 0.2% sodium hydroxide to dissolve the capsaicin present in oleoresin. In the above mixture 2 ml of 2% phosphomolybdic acid containing 1.2% sodium tungstate was added and kept for 2h, at room temperature to develop blue colour. The solution was filtered through Whatmann No. 42 filter paper and absorbance was recorded at 650 nm against a reagent blank. The relative concentrations of capsaicin present in ground sample and oleoresin are calculated by reference to calibration curve and oleoresin percentage, respectively.

Calibration of standard curve

Pure capsaicin (5 mg) was dissolved in 5 ml of 0.2% sodium hydroxide solution. This would serve as stock solution equivalent to 1000 ppm of capsaicin. Two ml of this stock solution was diluted to 10 ml with 0.2% sodium hydroxide to get standard capsaicin solution of 200 µg/gml. A series of concentrations containing 15, 20, 30, 40, 50, 60 and 70 µg/g capsaicin was prepared in 0.2% sodium hydroxide and colour was developed with sodium tungstate and phosphomolybdic acid as described earlier. From the absorbance values recorded at 650 nm, a standard curve plotting absorbance against capsaicin up to 70 µg was prepared and the quantity of capsaicin in the samples was determined from this callibration curve. One optical density at 650 nm was equivalent to 285.71 µg of pure capsaicin.

RESULTS AND DISCUSSION

Table 1 shows the relative concentrations of capsaicin and acetone extracted oleoresin percentages in five different chilli samples both in whole fruit and in pericarp.

Table 1. Quantitative determination of capsaicin in oleoresin of whole fruits and pericarp parts of some chilli samples with reference to pure capsaicin

Sample No.	Sample	Oleoresin (%)	Capsaicin (%)	
			Oleoresin	Dry powder
1	Whole fruit	11.97	2.842	0.3402
1(a)	Pulp	12.50	5.225	0.6532
2	Whole fruit	11.92	3.089	0.3683
2 (a)	Pulp	13.00	5.523	0.7181
3	Whole fruit	15.53	3.145	0.4880
3 (a)	Pulp	15.23	7.075	1.077
4	Whole fruit	15.45	3.719	0.5746
4 (a)	Pulp	16.00	6.802	1.090
5	Whole fruit	15.53	5.027	0.7810
5 (a)	Pulp	16.20	7.5405	1.221

Chilli contains up to 61 per cent seed in its fruit. In the present studies 11.92 to 15.53 per cent of oleoresin was recorded by using acetone as solvent for extraction. Naveș (1974) reported 8.7-16.5 per cent oleoresin and used dichloroenthane as solvent. Szabo (1970) asserted that acetone is an excellent solvent for capsaicin and is the most frequency used water miscible solvent. Capsaicin concentration was found to vary between 0.3402 to 0.7810 per cent on dry weight basis on the whole fruit. Govindarajan and Ananthakrishna (1970) reported 0.12 per cent capsaicin concentration in Mysore variety chilli and 0.7 per cent for Guntur Variety. Generally pulp part of the fruit gives little higher percentage of oleoresin than the whole fruit; even in some cases (sample no. 3) whole fruit gives higher percentage of oleoresin than the pulp. But capsaicin concentration in pulp oleoresin always in higher proportion nearly 1.5 to 2.2 times when compared with the whole fruit oleoresin. This is mainly because, capsaicin oleroresin of whole fruit contains considerable amount of fixed oil originating mainly from the seed. Pearson (1976) reported a range of 15.5 to 27.0 per cent fixed oil in whole chillies. Chilli fruit of sample no. 3 may have comparatively higher amount of oil percentage of the whole fruit as expected. It is well known that the seed of the fruit does not contain any capsaicin (Tice, 1933), therefore proportional increase in capsaicin concentration is not expected in the oleoresin extract of the whole fruit (sample No. 3).

Oleoresin of the whole fruit was found to contain 0.1444 to 0.2308% nitrogen. Nitrogen concentrations when converted to the capsaicin by multiplying a factor of 21.786 (Pure capsaicin contains 4.59% nitrogen), the calculated capsaicin percentages are very close to experimentally determined value of the presently described colorimetric method. This indicates capsaicin is the only nitrogenous substance present in the oleoresin of the fruit (whole as well as pulp) and reacts completely with the presently used colour reagent.

Non-capsaicin nitrogen of the residue (meal) obtained after oleoresin extraction with acetone, alongwith whole fruit nitrogen and seed nitrogen were also determined. The dry chilli fruit was found to contain an average of 2.1720 per cent nitrogen whereas 2.6508% nitrogen was present in seed. An average of 2.50 per cent nitrogen was found in residue after oleoresin extraction. This nitrogen concentration of the meal was found same, when ethyl acetate was used as the solvent for the extraction of oleoresin. Even further extraction with ethyl acetate does not change the nitrogen content value for the meal.

The present acetone extraction and colorimetric procedure has precision to satisfactory degree, accuracy and simplicity and suitable for germplasm evaluation programme for the capsaicin quantitation.

ACKNOWLEDGEMENTS

We are thankful to Director, NBPGR and Head, Division of Germplasm Evaluation, NBPGR, New Delhi, for providing necessary facilities during the course of present investigation.

REFERENCES

Bennett, D.J. and G.W. Kirby. 1968. Constitution biosynthesis of capsacin *J. Chem Soc.* (C) 442-446.

Gonzalez, A.T. and C.W. Tamirano. 1973. A new method for the determination of capsaicin in capsicum fruit. *J. Food sci.* **38** : 342-344.

Govindarajan, V.S. and S.M. Ananthakrishna. 1970. Observations on the separation of capsaicin from capsicum and its oleoresin. *J. Food Sci. Tech.* **7** : 212.

Kosuge, S. and M. Furata. 1970. Studies of the pungent principles of *Capsicum*. XIV chemical constitution of the pungent principle. *Agric. Biol. Chem.*, **34** : 248-258.

Masada, Y., K. Hashimoto., T. Incue, and M. Suzuchi. 1971. Analysis of pungent principles of *Capsicum annum* by combined gas chromatography and mass spectrometry. *J. Food Sci.,* **36** : 858-860.

Naves, Y.R. 1974. Technologie et Chimie des Parfums Naturels, Paris : masson et Cie.

North, H. 1949. Colorimetric determination of capsaicin in oleoresin of Capsicum. *Analytical Chemistry.* **21** : 934-936.

Pearson, D. 1976. The Chemical Analysis of Foods, 7th edn, London : J. and K. Churchill.

Szabo, P. 1970. Production of paprika oleoresin. *Amer Perfumer and Cosmetic.* **85** : 39-42.

Tice, L.F. 1933. 'A simplified and more efficient method for the extraction of capsaicin'. *Amer. J. Pharm.,* **105** : 320-325.

Todd, P.H., M.G. Bensinger and T. Biftu. 1977. Determination of pungency due to capsicum by gas-liquid chromatography. *J. Food Sci.,* **42** : 660-665, 680.