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Estimation of Pungency of Green Chillies using UV Spectrophotometer

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Abstract: The major focus of this study is to estimate pungency and compare capsaicin of three varieties of green chillies bought from local market Bengaluru (India). Pungency in chilli fruit is caused by chemicals grouped under capsaicinoids. The quantity of capsaicinoids which determines the pungency of the pepper is measured in Scoville Heat Units (SHU) using organoleptic test. But organoleptic test is not accurate and palate dependent. At present the most dominant technique used to measure capsaicin accurately in chillies is (High Performance Liquid Chromatography) HPLC. But HPLC measurement is expensive, time consuming and experts are required to handle the instrument. HPLC need to be installed in a sophisticated laboratory for it use. So, a new method for measuring pungency of chillies or capsaicin in chilli is needed. UV (Ultraviolet) spectrometry has been proposed in this paper to estimate pungency of green chillies. It has been shown that capsaicin content in green chillies is proportional to SHU, so green chillies can be graded for their hotness in SHU. Measurement on three varieties of chillies has been presented.

Keywords: Chilli, Pungency, Capsaicin, SHU, Ultraviolet.

I. INTRODUCTION

Chillies are often described as the “king of spices” and it shares a place on most dinner tables along with salt. Chilli is used to produce pungency or hotness in foods. The chemical that causes hotness or burning sensation in chillies belong to a family of chemical compounds called capsaicinoids which include capsaicin (C), Dihydrocapsaicin (DHC), Nordihydrocapsaicin (NHDC), Homodihydrocapsaicin (HDHC), Homocapsaicin (HC) and Nonivamide [1]. Hotness is measured in scoville heat units which in general is proportional to the quantity of capsaicinoid per unit weight of the chilli. The concentration of capsaicinoids varies from species to species and acts differently. By nature capsaicin is hydrophobic; colourless, odourless and waxy but reported as volatile [2] [3]. Typical amount of different capsaicinoids generally found in chilli, as percentage of total capsaicinoids, and Scoville heat unit (SHU) are given in Table 1[4] [5].

Table 1: Chemical structure of common Capsaicinoids

Capsaicinoids Name	Abbrev.	Typical Relative Amount	SHU
Capsaicin	C	69%	16,000,000
Dihydrocapsaicin	DHC	22%	15,000,000
Nordihydrocapsaicin	NDHC	7 %	9,100,000
Homodihydrocapsaicin	HDHC	1%	8,600,000
Homocapsaicin	HDHC	1%	8,600,000
Nonivamide	PAVA		9,200,000

A lot of research has been carried out in measuring pungency of dry chillies using HPLC; but, less importance is given to pungency measurement of green chillies. Green chillies have high economic value and are used in applications like medicines, cosmetics etc. and mainly in Indian cuisine. Capsaicin in chilli is an irritant and produces a sensation of burning in any tissue with which it comes into contact. Capsaicin present in chilli is commonly used in food products to provide added spiciness or "heat”. Capsaicin is used in many pharmaceutical preparations for cold, bad throat, chest congestion and used externally as ointment for painful joints. Chillies are very rich in Vitamins like A, C, B6 and iron, copper and carbohydrates [6]. Capsaicin is also an active ingredient in riot control and personal defense pepper spray from attackers.

Scoville heat unit of chilli is traditionally measured by organoleptic method which is subjective and person dependent. Analytical methods available at present to measure the hotness of dry chillies are different chromatographic techniques like, High Performance Liquid Chromatography, GC-MS (Gas Chromatography Mass Spectrometry), Thin Layer Chromatography, UV/Visible/Near-Infrared spectrophotometric technique and Colorimetric method. These techniques measure the quantity of capsaicinoids in unit weight of the sample which is calibrated to give SHU. Chromatographic methods are very expensive and time consuming and a simpler and more cost effective method is use of

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UV/Visible/Near-Infrared Spectrophotometry to measure SHU. A few have tried spectrometric technique to measure hotness of dry chilli and this technique has not been tried for green chilli. In this paper quantity of capsaicinoids in three varieties of green chilli has been presented and has been related to SHU rating.

Physico-chemical properties of capsaicin play an important role in measurement of hotness and are given in Table 2 [7] [8].

Table 2 : Properties of Capsaicin

Properties	Values
Chemical formula	$C_{18}H_{27}NO_3$
Molecular weight	305.41 g/mole
Melting point	62 to 65 °C
Boiling point	210 to 220 °C
Flash point	113 °C
Solubility	In water 28.93 mg/L at 25 °C Freely soluble in alcohol, ether and benzene
UV – VIS λmax	280 nm
Vapor pressure	1.38 X 10^{-8} mmHg at 25 °C

Three varieties of fresh green chillies of different morphology are selected for experiments, which have different levels pungency. The local names of the three varieties of chillies are Akash, Haveri and Menasinakai which are shown in figure 1. These three chilli varieties are selected for study to span a fairly large range of SHU. Variation of absorbance vs. wave length is measured on chilli extract of different concentrations in methanol solvent.

Figure 1: Akash, Haveri and Menasinakai fresh green chillies (from left to right)

UV absorption spectroscopy is an instrument used to determine quantity of compounds that absorb UV radiation. Identification of compound is by comparing the absorption spectrum of the sample with absorption spectrum of known solvent. The response of UV spectrophotometer is a plot of absorbance versus wave length in nm. In this work, UV-1800 UV-Vis Spectrophotometer from Shimadzu with wavelength accuracy ±0.3nm (190nm – 1100nm) and resolution of 1 nm is used. Shimadzu spectrophotometer works with a Software called UV probe agent, to automatically transfer and store spectral or absorbance data in PC. The sample is kept in a Quartz cuvette of volume 3.5ml with two sides polished (Labsil Instruments, Bangalore, India) and the path length of the beam is 10mm.

Extraction of green chilli:

Capsaicin in green chilli is extracted in solvent methanol and diluted to required concentration for measuring the absorbance. The extraction procedure is as below.

- 10 gram Green chilli is ground to a paste.
- One gram of the paste is taken for extraction.
- 25 ml of methanol solvent is added to 1gram of paste and stirred for 10 minutes using magnetic stirrer at room temperature.

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- This solution is sonicated (Ana Matrix sonicator) at 50°C for 20. Sonication temperature of 50 °C is selected for two reasons; firstly methanol boiling point is 64.7°C and secondly higher temperature may evaporate some of the compounds [9].
- The resulting solution is filtered into a glass flask and finally made up to 20ml by adding methanol. A picture of stock solution of three varieties of green chilli is shown in figure 2.

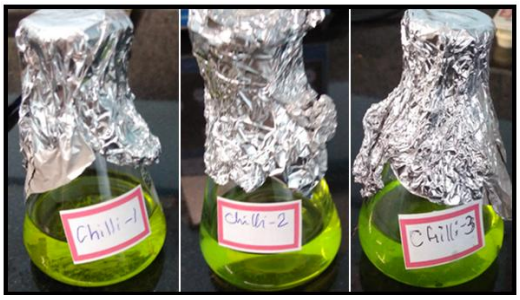

Figure 2: Extracted stock solution of Akash (chilli-1), Haveri (chilli-2) and Menasinakai

Measurement of spectrum:
UV spectrum of Akash, Haveri and Menasinakai are measured at different analyte concentrations; 3 ml methanol is taken in a cuvette to which different amounts of stock solution is added; like 20µL, 40µL, 60µL, 80µL, 100µL, 200µL and 300µL for UV absorbance analysis.

III. RESULT AND DISCUSSION

Table 3: Absorbance vs. Wavelength of Akash fresh green chilli

Wave length (nm)	Concentration in micro liter						
	20µL	40 µL	60 µL	80 µL	100 µL	200 µL	300 µL
	Absorbance Values						
664.5-665	0.003	0.008	0.008	0.011	0.013	0.028	0.038
468.5-469		0.011	0.013	0.019	0.023	0.047	0.067
437-439		0.014	0.017	0.024	0.03	0.061	0.087
318.5- 326.5		0.017		0.031	0.041		
269.5-270	**0.025**	**0.059**	**0.086**	**0.117**	**0.149**	**0.296**	**0.434**
200.5-204	0.231	0.482	0.712	0.945	1.175	2.021	2.598
Total	0.259	0.591	0.836	1.147	1.431	2.453	3.224

Table 3 shows absorbance peaks at different wavelengths for Akash chilli extract over a concentration range of 20µL to 300µL. All peaks do not appear at all concentrations except at 80µL and 100µL concentrations. However, two peaks one in the range 269.5 – 270nm and another in 200.5 - 204 nm range are seen at all concentrations. Also it may be observed that the peaks observed at wavelengths other than those mentioned above contribute to less than 10% of the total absorbance and less than 1% of saturation value of absorption. Hence, they have been neglected. It is reported that absorbance peak for capsaicin occurs at 280nm [10] and for benzene derivative at 204nm [11]. It has also been reported that the wave lengths at which absorbance peak occurs is affected by presence of other compounds [12]. Thus the peak at 270nm is most probably due to capsaicin and other due to presence of benzene derivative, an aromatic compound.

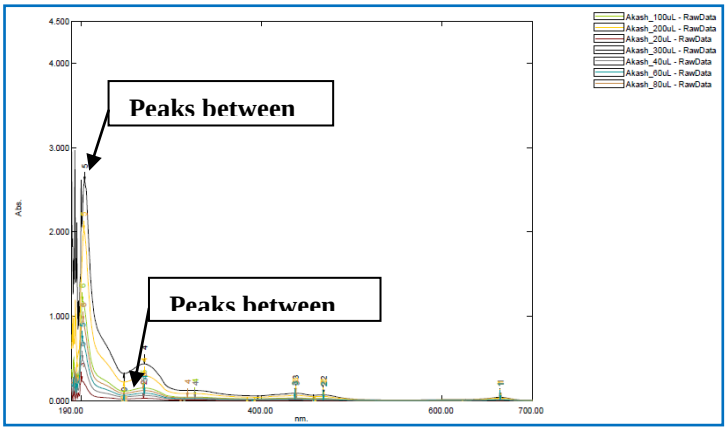

Figure 3: UV-Vis spectra for different concentrations (20µL, 40µL .etc) of Akash fresh green chilli stock solution

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Figure 3 shows UV visible spectra of Akash chilli stock solution for different concentrations (20µL, 40µL, 60µL, 80µL, 100µL, 200µ and 300 µL). In the UV spectral response, X-axis represents wave lengths (nm) and Y-axis represents Absorbance (Abs) in arbitrary units. Arrow indicates two prominent peaks occurring around 270 nm and (200.5 – 204) nm as discussed earlier. Figure 4 and 5 show, variation of absorbance with concentration of Akash fresh green chilli stock solution at two wavelengths.

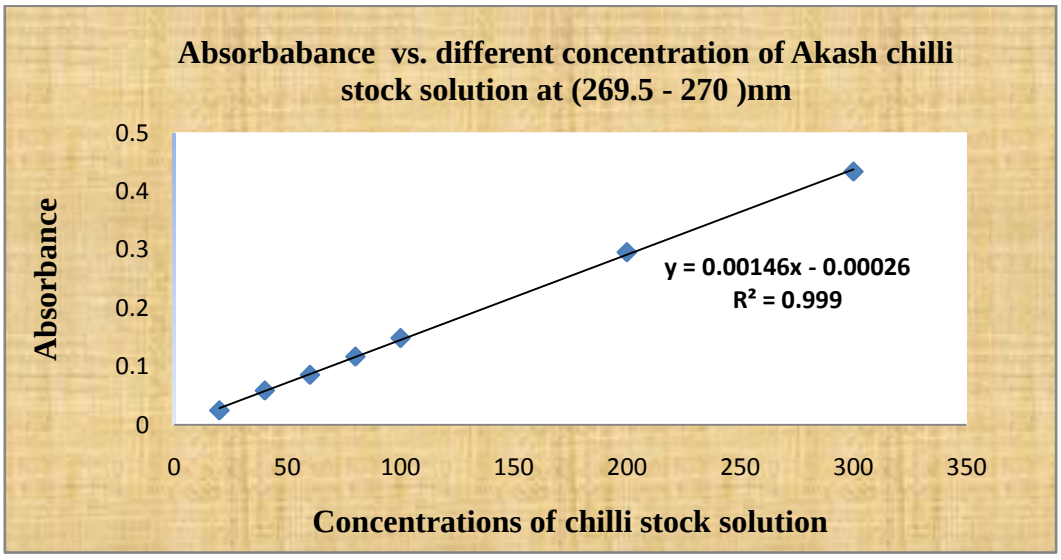

Figure 4: Variation of absorbance at (269.5 – 270) nm with concentration of extract for Akash fresh green chilli

It may be observed that absorbance is linearly related to concentration at both wavelengths with a regression coefficient greater than 0.98. This indicates that though the peaks occur over a range of wavelengths the absorbance causing component is same.

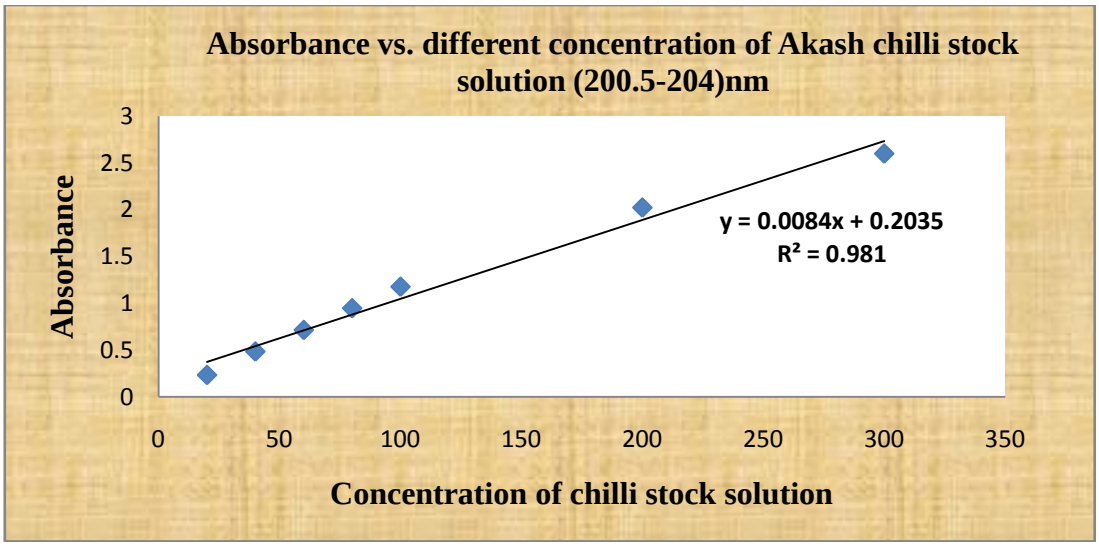

Figure 5: Variation of absorbance at (200.5 – 204) nm with concentration of extract for Akash fresh green chilli

Similar experiments are conducted for Haveri and Menasinakai green chillies for UV spectrum analysis and the results are given in tables 4 and 5 respectively.

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Table 4: Absorbance vs. Wavelength of Haveri fresh green chilli							
Wave length (nm)	**Concentration in micro liter**						
	20µL	40 µL	60 µL	80 µL	100 µL	200 µL	300 µL
	Absorbance Values						
664-666.5		-0.01	-0.008	-0.004	-0.004	0.01	0.022
468.5-471		-0.016	-0.014	-0.012	-0.009	0.007	0.023
436.5- 437		-0.015	-0.012	-0.008	-0.005	0.017	0.038
339.5- 340	0.006	-0.005	0.005	0.016	0.026	0.08	0.134
265-268.5	**0.001**	**0.006**	**0.026**	**0.041**	**0.061**	**0.157**	**0.251**
201.5-204	0.19		0.601	0.779	0.978	1.753	2.348

Table 5 : Absorbance Vs. Wavelength of Menasinakai fresh green chilli							
Wave length (nm)	**Concentration in micro liter**						
	20µL	40 µL	60 µL	80 µL	100 µL	200 µL	300 µL
	Absorbance Values						
667-665					-0.003	0.001	0.006
`583.5				-0.006			
468- 497.5				-0.008			0.005
435.5							0.009
322-340.5		-0.005	0.008	0.018	0.029	0.084	0.139
291-293.5				**0.015**	**0.025**	**0.078**	**0.13**
200-202	0.113	0.155		0.324	0.406	0.801	1.166

It is observe that the peak of capsaicin in Haveri and Menasinakai green chilli also does not occur at one single wavelength but over a range of wavelengths from (265 to 268.5) nm and (291 to 293.5) nm respectively. But, it may be observed that absorbance is linearly related to concentration for Haveri green chilli with a regression coefficient greater than 0.99 (figure 6) and for Menasinakai green chilli with a regression coefficient 1 (figure 7). It may also be observed that, in figure 7, Menasinakai stock solution concentrations does not have peak for 20µL, 40µL and 60µL concentrations. So the plot is considered from 80µL concentrations onwards till 300µL. This may be due to negligible amount of capsaicin at low concentrations of stock solution.

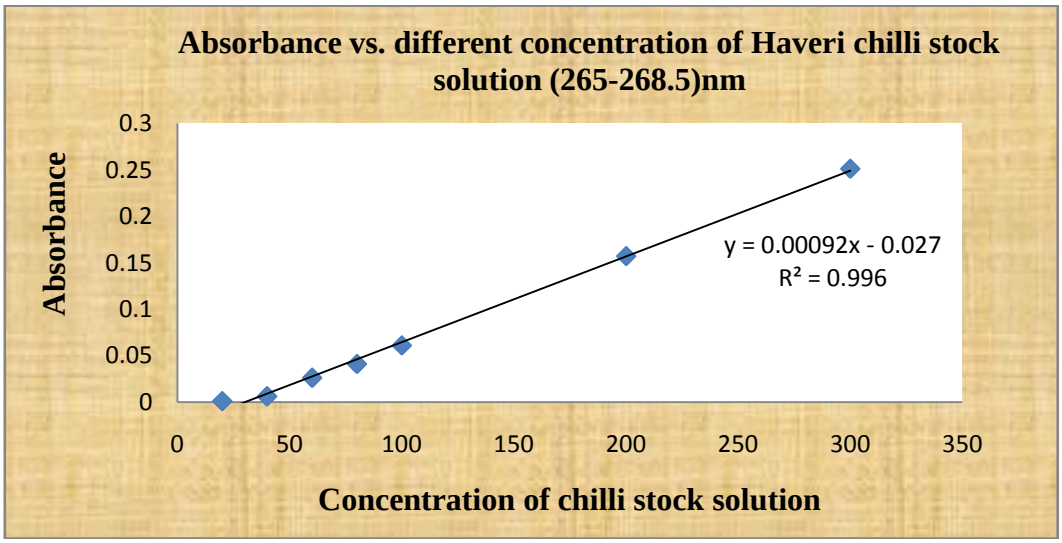

Figure 6: Variation of absorbance at (265 – 268.5) nm with concentration of extract for Haveri fresh green chilli

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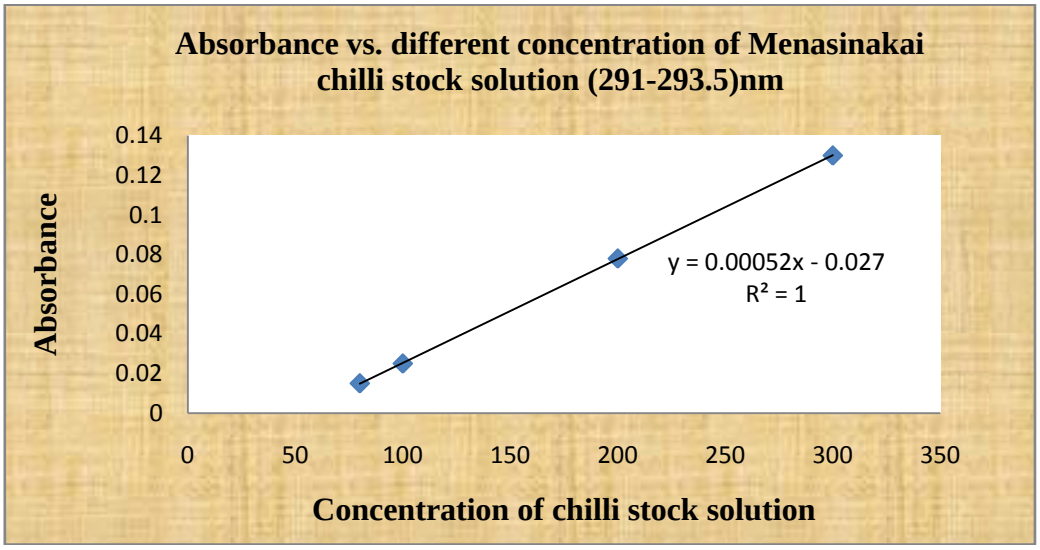

Figure 7: Variation of absorbance at (291 – 293.5) nm with concentration of extract for Menasinakai fresh green chilli

Table 6 shows comparison of absorbance values of green chillies at wave length range (265 to 293.5) nm for 300µl, 200µL and 100µL concentration of stock solution for three types of chillies. The SHU values of these three types of chillies were measured in a standard laboratory using HPLC method for calibrating the sensor and absorbance with SHU.

Table 6: Comparison of absorbance value of green chilli at (265 to 293.5) nm for 300µL, 200µL and 100µL stock solution

Green Chilli Type	Absorbance Values(300µL)	Absorbance Values(200µL)	Absorbance Values(100µL)	SHU in Kilo Units
Akash	0.434	0.296	0.149	32.7
Haveri	0.251	0.157	0.061	12.5
Menasinakai	0.13	0.078	0.025	1

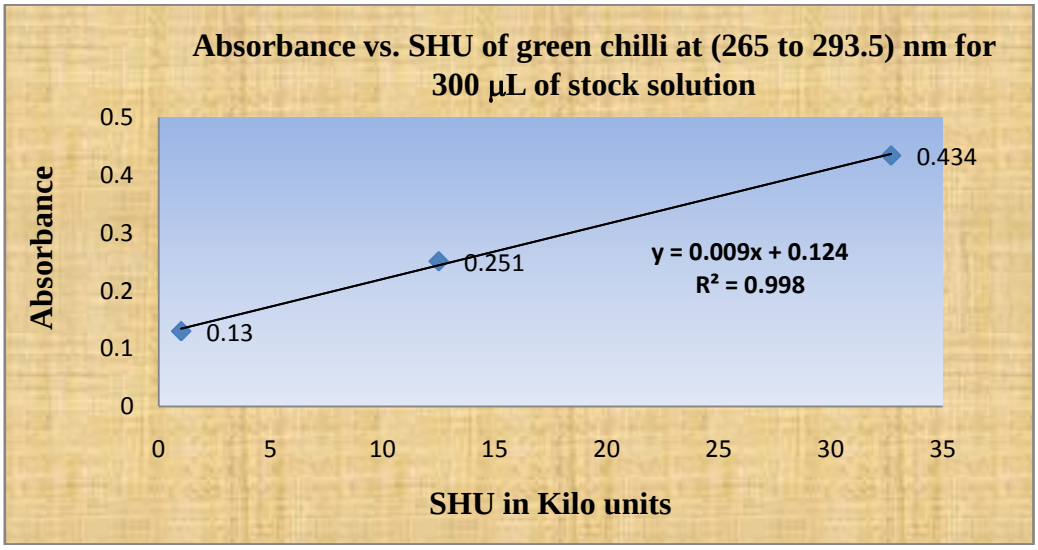

Figure 8: Plot of Absorbance vs. SHU of green chilli at (265 to 293.5) nm for 300 µL of stock solution

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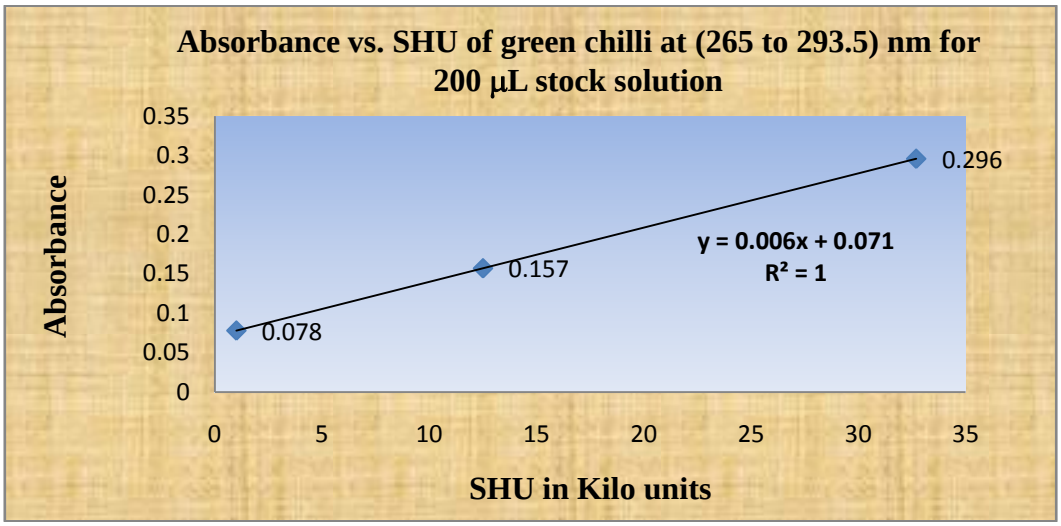

Figure 9: Plot of Absorbance vs. SHU of green chilli at (265 to 293.5) nm for 200 µL of stock

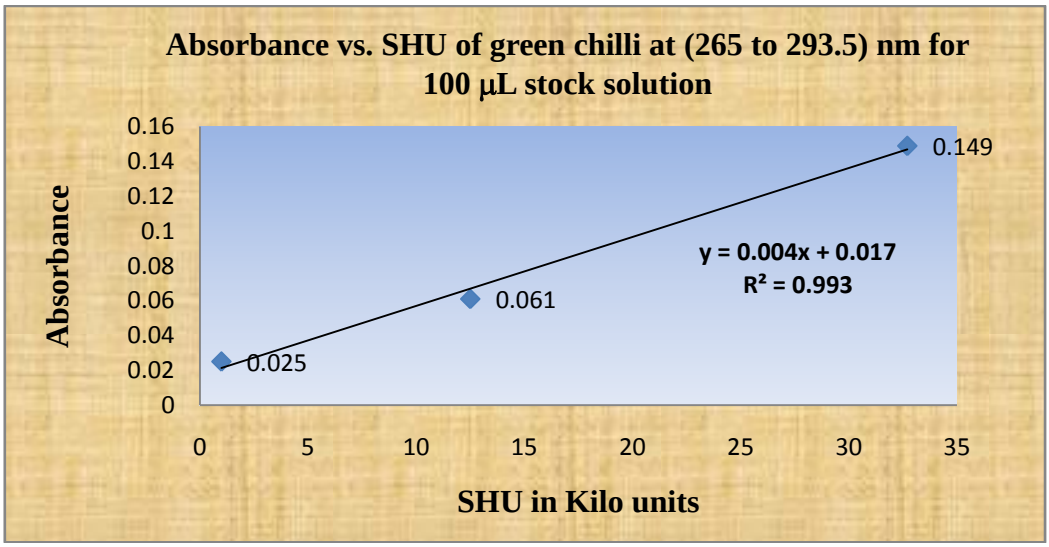

Figure 10: Plot of Absorbance vs. SHU of green chilli at (265 to 293.5) nm for 100 µL of stock solution

Figure 8, figure 9 and figure 10 shows that, the absorbance of green chilli correlates to SHU linearly in the range (265 – 293.5) nm, for 300µL, 200µL and 100µ stock solution.

Table 7 shows comparison of absorbance values of green chillies at wave length range (200 to 204) nm for 200µl concentration of stock solution for three types of chillies.

Table 7: Comparison of absorbance value of green chilli at (200 to 204) nm 200µL stock solution

Green Chilli Type	Absorbance Values	SHU in Kilo Units
Akash	2.021	32.7
Haveri	1.753	12.5
Menasinakai	0.801	1

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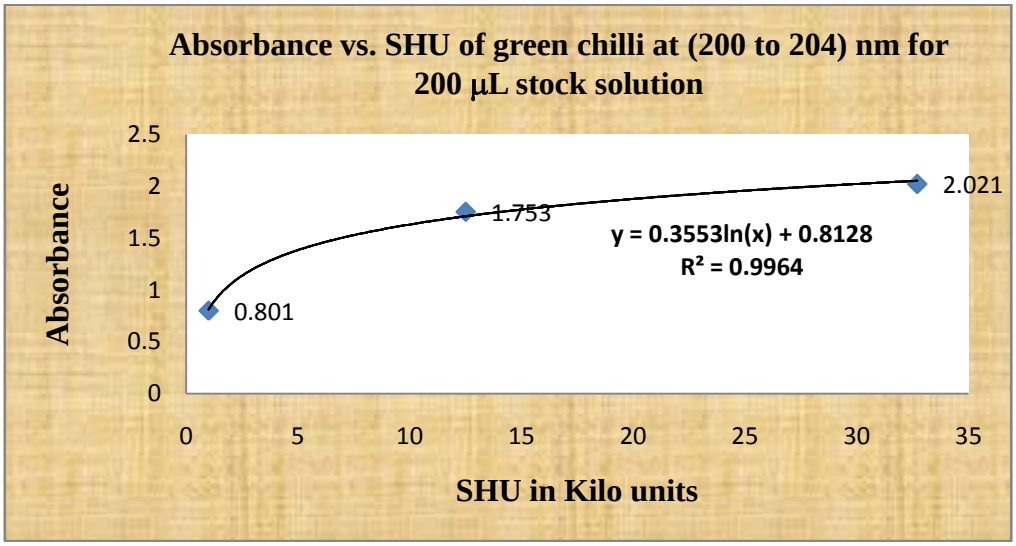

Figure 11: Plot of Absorbance vs. SHU of green chilli at (200 to 204) nm for 200 µL of stock solution

Figure 11 shows that the absorbance of green chilli, in the range (200 – 204) nm for 200µL stock solution, is not directly related to SHU. This is a significant finding which means benzene derivatives at (200 – 204) nm may not contribute to pungency of chilli.

VI. CONCLUSION

Scoville organoleptic test is not practical in market because of its inaccuracy, subjectivity and taster dependency. Spectra of three varieties of fresh green chillies stock (Akash, Haveri & Menasinakai) have been measured and presented. Absorbance peaks occur over a range of wave lengths in different chillies and not at one single wave lengths. Two major peaks for fresh chillies are around 280 nm and 204 nm; the first one is reported to be due to capsaicin and other due to other volatile compounds present in chilli. The relation between concentrations of stock solution vs. absorbance is linear for green chillies. In fresh chillies it is possible to calibrate absorbance value at (265 – 293.5) nm with SHU which is linear. Among three varieties of chilli used Akash is maximum hot compared to Haveri and Menasinakai. Menasinakai is least hot among three green chillies. It has been shown that the SHU values of green chilli can be measured using UV spectrometer.

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